



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

**Outcomes of Co-Extra**

# *New real-time PCR methods available for routine GMO detection labs - applicability and performance*

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Reliable and cost-effective methods for GMO detection are essential for establishing an efficient system for traceability as well as for monitoring different aspects of GMO coexistence with conventional crops. After several years of primarily gel-based PCR analyses, real-time PCR methods have become state-of-the-art for qualitative detection as well as for quantification of genetically modified components in food and feed. Within the framework of Co-Extra, several new real-time PCR-based methods have been developed in order to enhance efficiency and cost-effectiveness of GMO analysis, further improve reliability of GMO analysis, overcome certain limitations of current real-time PCR methods and finally complement the existing method portfolio with methods for identification and quantification of non-EU approved GMOs formerly not detectable or not quantifiable.

Enhancing efficiency and cost-effectiveness is of major importance as GMO analysis is getting increasingly complex due to the growing number of approved and commercialized GMOs. Whereas in the beginning of GMO analysis a screening for 35S promoter sequences was common practice, the situation has become much more difficult. This is also due to the fact that more and more GMOs are lacking the typical screening elements. Consequently there is an urgent need for multiplex screening and identification methods in order to avoid an increase in cost for traceability to an economically unbearable level. Within Co-Extra several multiplex real-time PCR assays ranging from duplex to pentaplex format have been developed and thoroughly validated providing improved tools for screening for traditional screening elements like 35S promoter and nos terminator as well as tools

targeting additional screening targets. Some of the newly developed assays will be presented. Furthermore multiplex-specific requirements for method validation will be addressed.

A second objective of PCR method development within Co-Extra was further improving reliability of GMO analysis. In this context real-time PCR control assays have been developed detecting important donor-organisms of building blocks frequently used in GM plants. In case of positive testing results for screening targets originally derived from *Agrobacterium*, *Bacillus* and figwort mosaic virus the newly developed control assays detecting these donor-organisms can be used in order to confirm that the positive screening results are true indicators of GMOs - and are not due to the presence of bacterial (*Agrobacterium*, *Bacillus*) or viral DNA (FMV) in the food or feed sample. Another means of enhancing reliability of GMO analysis was the development and validation of an improved IPC (Internal Positive Control) which can be used for cost-efficient and sensitive verification of absence of PCR inhibition. Examples of assays including this IPC will be presented.

Another objective of the developmental work within Co-Extra was to overcome limitations of current real-time PCR methods such as to enable GMO quantification in samples with very low DNA content or to make GMO analysis portable for on-site testing. As an example the method for on-site real-time PCR quantification of GT73 *Brassica napus* will be presented.

Finally real-time PCR assays detecting two transgenic potato events which are certified for food consumption in Russia will be highlighted which complement the portfolio of event-specific detection methods available for EU approved GMOs.