New techniques and methods developed by the Belgian NRL-GMO to identify unauthorized GMOs in the UGMMONITOR project.

Fraiture Marie-Alice[1,3,4,] Herman Philippe[1], Berben Gilbert[2], Debode Frédéric[2], Janssen Eric[2], Taverniers Isabel[3], De Loose Marc[3] and Roosens Nancy[1]

[1] WIV-ISP (Platform of Biotechnology and Molecular Biology; Biosafety and Biotechnology Unit); [2] CRA-W; [3] ILVO (Institute for Agricultural and Fisheries Research); [4] UGent (Faculty of Pharmaceutical Sciences; Laboratory of Pharmaceutical Biotechnology)

Genetically modified organisms (GMOs) are defined as organisms "in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination" (Directive 2001/18/EC). The introduction and the control of GMOs in the food and feed chains in the European market are submitted to the European legislation in order to guarantee the freedom of choice to the consumers (Reg. EC n°1829/2003 and 1830/2003). However, the enforcement of this legislation is complex for several reasons. First of all, the number and the diversity of commercialised GMOs will increase significantly in the 5 next years. Second, in addition to genes conferring insect resistance or herbicide tolerance, a larger range of traits will be developed (*e.g.* abiotic stress tolerance, disease resistance and nutritional allegations). Third, the present commercialised GMOs are principally produced by American and European companies which have a major interest to be authorized to commercialise their products on the European market. Nevertheless, in 2015, more and more GMOs will be developed by Asian technological centres intended for the local consumption. These GM crops will be very probably not submitted to European approbation. Thus, the frequency of unauthorised GMOs (UGMs) on the European market should significantly increase by their accidental (or adventitious) presence in raw material and processed food (Stein and Rodriguez-Cerezo, 2009). In this context, efficient and innovative methods and techniques must be developed to: (i) improve UGM detection in the food/feed chain and (ii) collect data enabling their safety risk assessment and the potential risk they could pose to human health.
The project entitled "UGMMONITOR" consists in the development of a platform in molecular biology and a database (biosafety information) for the detection of UGMs in food and feed. It is financed by the Federal Public Service Health, Food Chain Safety and Environment (convention RF 11/6242) (starting date: 01 May 2012) and will allow to develop high-tech methods for a better management of UGMs control. One of the main goals of this project is to provide a database of UGMs containing information useful for risk assessment and also an integrated high-tech approach to detect UGM in food/feed chain.

Hereafter, a small overview of the new methods and techniques developed by the different partners are given.

The Scientific Institute of Public Heath (WIV-ISP)

On the one hand, in order to create the "UGMMONITOR BIOSAFETY DATABASE", a large inventory of UGMs and their characterisation will be carried out, with a particular attention on rice. To this end, information will be collected in a publicly available database such as the commercialised GMOs in the next coming years, the type of food/feed that can be contaminated by UGMs, the available data to conduct a risk assessment etc. By this way, this database will represent a precious tool to provide necessary data for the enforcement of food/feed chain control in particular in case of crises.

On the other hand, according the information obtained in the database, new SYBR®Green screening methods targeting unauthorized GM-rice will be developed. In a second step, a strategy coupling DNA walking methods and nested PCR will be integrated to the SYBR®Green detection methods developed at WIV-ISP in order to prove the presence of UGMs/GMOs in a food matrix by the isolation of the transgenic-endogenic junction(s). The obtained amplicons will be sequenced to confirm/infirm the presence of transgenic crop from the analysed sample. This strategy will be adapted, first, on rice as crop model, and will be evaluated for its sensitivity in regards to the problem of "low level presence".

Instituut voor Landbouw- en Visserijonderzoek (ILVO)

A novel anchor PCR protocol with automated fluorescent detection through capillary gel electrophoresis (CGE), developed within ILVO in the FOD-RF project GMODETEC, will be tested in this project on rice DNA. First, a selection of flanking unique sequences – that have to be known – will be made, based on a screening of the gDNA library for rice. For each selected target element, 3 different "anchor primers" will be developed (primers 2 and 3 thereby nested for primer 1) and combined with difference restriction enzymes (and primers) in anchor PCR tests. A database of unique anchor PCR fingerprint patterns will be developed for a set of known GM rice events. For the optimized protocol, in a later stage the sensitivity, detection limit and specificity will be determined.

Besides development of novel high-tech methods and technologies, existing techniques will be evaluated for their suitability for UGM detection on the one hand, and applied for GM rice as a model plant on the other hand. To these belong: generic DNA quality or integrity tests, PCR inhibition tests, PCR-based screening tests and combination of screening elements in a matrix model, PCR element hopping, and finally event-specific PCR analysis. A combination of these tools will be used to assess in how far the nature of an "unexplainable signal" can be explained, and possibly present UGM(s) can be detected.

Centre Wallon de Recherches Agronomiques (CRA-W)

The contribution of CRA-W to the project mainly includes the assessment of new DNA analytical techniques belonging to the so-called "Next Generation Sequencing" (NGS) category in detection of a genetic modification on rice as model. In recent years sequencing has indeed become more and more efficient. It is now possible to sequence a whole genome in a short time at an affordable price. The limits of the approach are more and more the handling of the so-generated big data sets. Based on the results obtained with rice as model, it will be possible to state when such techniques might be of interest within a global strategy of detection of UGMs. The technique to be applied consists in the implementation of a NGS approach but where only a fraction of the total DNA extract is sequenced after an enrichment step that selects fragments in which a piece of a known screening element is present. A transfer of some real-time PCR targets to analytical pyrosequencing where targets are sequenced in only 2 hours at a price similar to that of real-time PCR will also be attempted.

Next to that, involvement of CRA-W also includes the development of new screening elements with a special focus on transgenic animals (GM fish).

nancy.roosens@wiv-isp.be

Corrigendum:

In the introduction of the article "GMOseek research project (2009 - 2011) for GMO detection" published in Labinfo No. 7 of January 2012, the phrase:

"The GMOseek project (SAFEFOODERA: "Food Safety – forming a European platform for protecting consumers against health risks") was financed by the European Commission under the ERA-NET platform for protecting consumers against health risks and was running from 1/06/2009 till 31/05/2011."

should be read as:

"The GMOseek project (SAFEFOODERA: "Food Safety – forming a European platform for protecting consumers against health risks") was financed by the **Food Standards Agency (FSA, UK)** and the **Bundesamt für Verbraucherschutz und Lebensmiddelsicherheit (BVL, Germany)** under the ERA-NET platform for protecting consumers against health risks and was running from 1/06/2009 till 31/05/2011."