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

Outcomes of Co-Extra

Non-PCR based Alternative Analytical Methods

Jim Murray

Lumora Ltd, UK

Although polymerase chain reaction (PCR) has to date been the overwhelming method of choice for the laboratory based detection of GMOs because of its sensitivity, familiarity of methodology, well developed standard operating procedures and availability of suitable equipment in testing laboratories, PCR nevertheless suffers from a number of distinct disadvantages. These include the relatively high cost of equipment and of the assays themselves, potential for contamination and the sensitivity to certain classes of contaminants and inhibitors, leading to a requirement for reliable DNA purification strategies. As a result, assays need to be carried out in a laboratory and the need to accumulate batches of samples further slows the total time required for the assay chain from sampling to the eventual result in the laboratory. These issues, together with the difficulty in designing cost-effective portable devices for PCR, have driven the search for alternatives to PCR, a number of which are now becoming available that seek to overcome some of the perceived limitations of the PCR approach.

Within WP5 of the Co-Extra project, a number of new alternatives to PCR based methods have been evaluated, which offer potential advantages over PCR for speed, cost, scale or portability. The purpose of this presentation will be to review these methods and report on their suitability and potential for GMO screening applications. For more details on non-PCR nucleic acid amplification methods, the publication by partner NIB may be consulted (1), and for a review on novel analytical approaches to GMO testing see (2). The methods reported comprise two main classes. The first are true alternatives to PCR, i.e. molecular tests that, like PCR, also test for the presence of specific DNA sequences, but which employ an alternative non-PCR method of nucleic acid amplification. These include the use of strand-displacing polymerases at a constant temperature (e.g. LAMP and RDC), or the use of transcription-mediated amplification (e.g. NASBA). All these methods do not require temperature cycling, operate at a constant temperature, and offer potential advantages including cost, speed, portability and reduced sensitivity to inhibitors over PCR. The technical advantages of these approaches include the possibility to combine their use with novel reporter systems, and the use of a new bioluminescent output known as BART has been evaluated in conjunction with LAMP and RDC. The second type of method does not seek to detect DNA sequences but employs spectroscopic techniques to distinguish GM and non-GMO material. Method evaluation for their suitability for GMO detection was carried out by the partners NIB, INRA, CSL, Lumora, NIAB and CRA-W within WP5 of the EU Co-Extra Project.

In the first class, techniques known as NASBA, LAMP and RDC were evaluated, either alone or in conjunction with the BART bioluminescent reporter system. In the second, near-infrared spectroscopy was evaluated. The main characteristics of these techniques is summarised.

Loop-mediated Isothermal Amplification (LAMP), developed by the Eiken Chemical Company is a simple, rapid, specific and cost-effective nucleic acid amplification technology. Details are described on http://loopamp.eiken.co.jp/e/lamp/index.html. It is characterized by the use of 4 different primers, specifically designed to recognize 6 distinct regions on the target DNA template, in a process that proceeds at a constant temperature driven by a strand displacement reaction. Amplification and detection of target genes can be completed in a single step, by incubating the mixture of DNA template, primers and a strand displacement DNA polymerase, at a constant temperature. It provides high amplification efficiency, with replication of the original template copy, occurring 10^9-10^{10} times during a 15-60 min reaction. The primer pairs used in this amplification can be designed using a web tool at http://primerexplorer.jp/e/.

RDC (*Reaction déplacement chimeric*) is an isothermal DNA amplification procedure developed by Biomerieux, and is based on the use of chimeric primers consisting of an RNA stretch embedded within flanking DNA sequences. Cleavage of the hybrid duplex between the RNA region formed when the primer is hybridized to its DNA target provides the initiation for a strand-displacing polymerase. For details see US Patent 5824517 (Cleuziat and Mandrand; (http://www.patentstorm.us/patents/5824517.html).

Both RDC and LAMP are among isothermal amplification technologies that can be interfaced with a unique reporter system known as BART (bioluminescent assay for real-time). BART is a bioluminescence real time assay developed by Lumora [http://www.lumora.co.uk/] that allows the quantitative analysis of DNA amplification in real time. In BART, PPi produced during DNA amplification is converted to ATP by the action of ATP sulphurylase. This ATP is then used in a coupled simultaneous reaction by thermsotable firefly luciferase and luciferin to produce a light output permitting real-time analysis of amplification kinetics. A unique feature of BART is an initial burst of light, associated with the on-set of exponential amplification, followed by a rapid decrease, as pyrophosphate reaches a critical threshold. The time to reach this light peak is therefore a function of the amount of target DNA in the sample at the beginning of the reaction (time to maximum; T_{max}), and a unique feature of the BART reporter. Quantification of BART is based on time to peak and not absolute light intensity, making it less prone to inhibition simplifying data interpretation and the hardware requirements. LAMP in conjunction with BART provides a robust, sensitive and reliable method for qualitative detection of GMOs at low levels of presence (0.1%) and has the potential for quantitative or semi-quantitative manifestations. It is also suitable and demonstrated in small, low cost devices that can be used in the field or other low-resource settings, both because of the equipment requirements and its ability to function with very simple and rapid DNA preparations, even from fresh leaf tissue.

NASBA is an isothermal nucleic acid amplification method that mimics retroviral replication and was originally applied to detection and quantification of RNA targets, but has also been adapted for DNA detection, and it was evaluated in this manifestation. Amplification occurs because the target is transcribed into RNA, which is then reverse-transcribed back into DNA, thereby providing more template copies for RNA transcription. The transcription is carried out by T7 RNA polymerase and requires the incorporation of the appropriate promoter sequence onto the template, which is achieved by appropriate primer design. This method was modified to allow DNA amplification using a two step procedure: first step with tailed primers, second step with universal primers. NASBA was developed well with performance characteristics similar to PCR, and adaptation to real-time detection using Molecular Beacons has been reported. However integration with the BART system is not straightforward due to the high concentration of ATP present in the NASBA reaction.

Near Infrared spectroscopy uses spectral properties of sample in IR to detect GMO's. The method was developed for Roundup Ready soybean (GTS-40-3-2) due to its specific characteristics. The

method is non-invasive and can be applied on-site, therefore suitable for analysis of large sample lots of more expensive material, e.g. seeds.

References:

1. Morisset, D., et al . (2008) . Eur Food Res Technol **227**: 1287-1297.
2. Michelini, E., et al . (2008) Anal Bioanal Chem **392**: 355–367.