Validation of a multiplex single PCR system amplifying 5 markers in a single tube

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Abstract
With the upcoming of genetically modified organismes (**GMO**), PCR technology has become an important tool in food analysis. Many protocols have been developed and published and validated. However, only a few multiplex systems are present. To fulfill the requirements of quality control and economic GMO testing a multiplex PCR system, detecting **maize**, **soya,** the **NOS-terminator**, the **35S-promoter** and an **internal control** was validated. This validation makes a reliable GMO screening possible and guarantees a highly reproducible sensitivity of 0,03% (>95%) for the 35S-promoter and 0,01% (>95%) for the NOS-terminator. The validation data correspond with international guidelines and allows to run this method in the ISO 17025 accredited scope.

Keywords: GMO, screening, PCR, multiplex, validation, international guidelines, ISO 17025

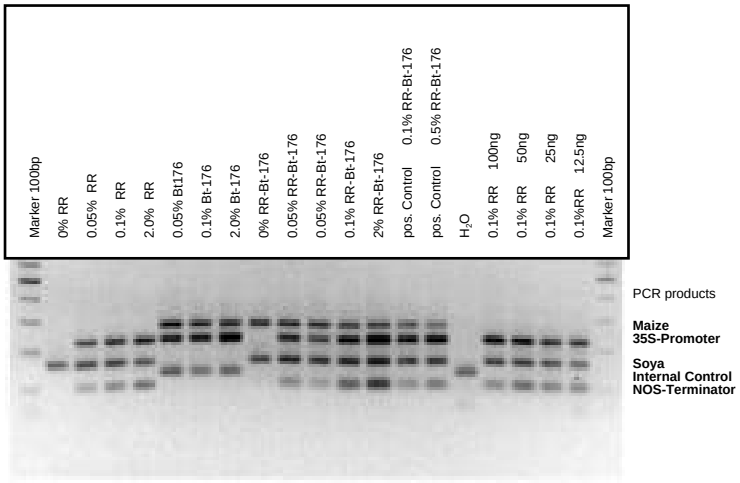

Figure 1

RR = Roundup-Ready Soya
Bt-176 = Maximizer Maize

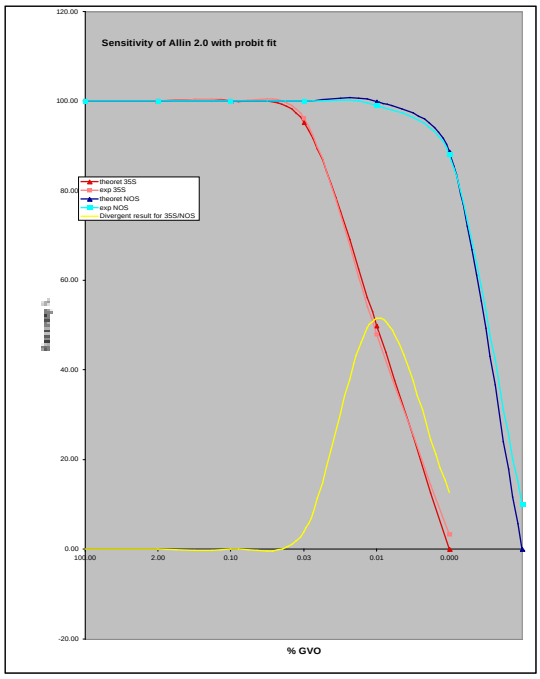

Figure 2

Materials and Methods

DNA-isolation
DNA-isolation was done applying the Wizard protocol publicated in "Schweiz. Lebensmittelbuch" chapter 52B (Wizard protocol, Promega). After RNase digestion, the content of DNA was measured photometrically (Genequant II, Pharmacia).

Amplification and Detection
160ng of DNA-template was added to the mastermix of Allin 2.0 multiplex screeningsystem, according to the manufacturers protocoll (full manual see www.biosmart.ch). The PCR was run on a Hybaid thermocycler (OMN-E).
The amplicons were detected on a 2% agarose gel after 40 minutes of electrophoresis at 200V (Biosmart Gelelectrophoresis, Maxi-system). The result was photographed using a digital camera (Sony).
Interpretation of the nested PCR-system was clear **(Figure 1)**.

Standards
For the validation and as a run control certified standards (Fluka) were used. Total DNA content was kept to 160ng per reaction.

Discussion

The screening system **Allin 2.0** exhibit a **detection limit** (>95%) for GMO of **0.03% (35S)** and **0.01% (NOS)** which is far enough sensitive to fulfill the requirements of the legislation **(Figure 1+2)**.
A negative result indicates clearly that the product has not to be labeled as GMO. Due to the **internal control** false negative results are detected clearly. Therefore no second analysis with spiked samples has to be performed. Additionally the detection of soya and maize in the same tube is very helpful, detecting unexpected components of maize in soya and vice versa.The intermediate precision which was assayed over 4 months was satisfactory. This is a basic necessity of a high and constant quality of an analysis-system.
The use of **Hot-Start Taq** DNA polymerase increased the sensitivity and specificity of the analyses. No unspecific Amplicons were observed. The generation of 4 markers in one tube makes the interpretation clear and easy. We expect that even more markers may be amplified and presenting a fast and cost effective alternative to chip assays.