Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.12

DEVELOPMENT OF A REAL-TIME PCR BASED METHOD FOR IDENTIFICATION OF GENETICALLY MODIFIED WHEAT WITH LUX PRIMERS

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Real-Time, LUX primer, GMO, wheat

Current E.U. legislation limits for the accidental presence of Genetically Modified Organism (GMO) derived products in foodstuff is 0.9 % w/w. To quantitatively determine the level of GMO derived products found in foodstuff, the most sensitive method currently available is Real-Time Polymerase Chain Reaction (PCR). All quantitative PCR (qPCR) strategies involve using interchelating dyes or hybridizing probes.

Common qPCR probes used are TaqMan probes, molecular beacons, FRET probes and Scorpion probes. A new innovation of the hybridizing probe method are the Light Upon extension (LUX) primers from Invitrogen (www.invitrogen.com). This technique is very sensitive and requires a labelled and unlabelled primer without the addition of an internal probe of the amplified product. This use of only two primers without the necessity of a probe allows a greater choice in selecting the amplicon for the sequence of interest.

One critical point in the development of quantitative PCR methods is DNA extraction method, different sources may require different extraction protocols. A modified CTAB protocol for the extraction of DNA from wheat has proven effective for good DNA yields and quality.

Accurate quantification of source DNA for amplification is also critical. We have found that quantification of DNA is best performed with appropriate standards on agarose electrophoresis gels, confirmed by spectrophotometry and analytical PCR to be the most reliable.

As no standards for the detection of GMO wheat flour are currently available on the market, it is necessary preparing standards containing mixtures of control and GM wheat flour.

The aim of this work is to develop a quantitative method for detecting the presence of GMO derived products in durum wheat with Real-Time PCR in combination with LUX primers. Critical points of the method were identified and modified in order to maximize the sensitively of the protocol.

Results from the Real-Time PCR experiments shows the high degree of sensitivity for the derived protocol while accurate reference standards or alternative systems are required.