The Analysis of Food Samples for the Presence of Genetically Modified Organisms

Session 8

Characteristics of the Qualitative PCR Systems Described in the Manual

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During this course different detection systems will be used: 1) plant-specific primers will be used to confirm the presence and quality (amplifiability) of the DNA extracted from the samples; 2) the so called “screening method”, based on the specific detection of the most common regulatory sequences, the 35S promoter and nos terminator. These two methods will be performed following a simple PCR protocol. Finally, GMO-specific primers will be used in “nested PCR” for the selective detection and identification of the different transgenic lines. This chapter contains a brief introduction to the different systems used for the detection and characterization of Roundup Ready® soybean, MON810 maize and the \textit{cry}I(b) gene contained in Bt-176 maize. For further information on primer sequences and composition see Session 9.

Plant specific PCR

Detection of the \textit{lectin} gene

For the identification of soybean DNA, primers GMO3 and GMO4 (Meyer \textit{et al.}, 1996), which amplify a fragment of the \textit{lectin} gene (\textit{Le1}), specific to soybean, will be used. As indicated above, the purpose is to confirm the presence and quality of the DNA extracted from soybean containing samples, where DNA quality is intended here as amplifiability by PCR. The primers GMO3 and GMO4 are used as a nested PCR for the second soybean-PCR reported by Meyer and Jaccaud (1997) on the DNA extracted from processed foods. The expected product is an amplicon of 118 bp.

Detection of the \textit{zein} gene

The primers ZEIN3 and ZEIN4 (Studer \textit{et al.}, 1997) specific to the maize \textit{zein} gene (\textit{Ze1}, coding for a 10-kb protein) will be used to confirm the presence and quality of DNA extracted from maize-containing samples. As for the \textit{lectin} gene, the primers ZEIN3 and ZEIN4 have originally been designed as internal primers (second round PCR) in a nested PCR system for the detection of maize DNA extracted from
processed foods. If the extracted target DNA is present, intact and amplifiable, we expect the amplification of a band of 277 bp.

**Screening method: Detection of the CaMV 35S promoter and nos terminator**

The detection of the 35S promoter and nos terminator by PCR constitutes the so-called “screening method” for the identification of genetically modified plant-derived foodstuffs. The use of the 35S promoter and nos terminator as target sequences allows the detection of most genetically modified foodstuff since they are up-to-now present in nearly all EU approved genetically modified plants (Hemmer, 1997). The characteristics of some maize lines approved for market introduction in the EU are listed in Table 1 as an example. The 35S promoter and nos terminator specific primers used during the course were used to validate a PCR method for the detection of Roundup Ready® soybean and Maximizer maize (Bt-176) in processed food fractions (Lipp et al., 2001).

**Table 1. Characteristics of some transgenic maize lines authorised for market introduction in the EU**

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Character</th>
<th>Promoter</th>
<th>Gene(s) introduced</th>
<th>Terminator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event 176</td>
<td>Ciba-Geigy</td>
<td>Bt, bar</td>
<td>35S</td>
<td>bar</td>
<td>35S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PEPC</td>
<td>cryIA(b)/int.9 PEPC</td>
<td>35S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CDPK</td>
<td>cryIA(b)/int.9 PEPC</td>
<td>35S</td>
</tr>
<tr>
<td>Line Bt-11</td>
<td>Novartis</td>
<td>Bt, pat</td>
<td>35S</td>
<td>cryIA(b)/int. IVS6</td>
<td>nos</td>
</tr>
<tr>
<td>Line T25</td>
<td>AgrEvo</td>
<td>pat</td>
<td>35S</td>
<td>pat</td>
<td>35S</td>
</tr>
<tr>
<td>Line MON810</td>
<td>Monsanto</td>
<td>Bt</td>
<td>E35S</td>
<td>cryIA(b)/int. hsp70</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E35S</td>
<td>cryIA(b)/int. hsp70</td>
<td>-</td>
</tr>
</tbody>
</table>

**Detection of the CaMV 35S promoter**

This promoter regulates the gene expression of many transgenic plants such as Roundup Ready® soybean and maize line Bt-176. For its specific detection, primers p35S-cf3 and p35S-cr4 will be used (Lipp et al., 2001). The expected amplicon is a
123 bp fragment as indicated below in Figure 1, where primers p35S-cf3 and p35S-cr4 have been positioned in the corresponding region of the CaMV 35S promoter sequence.

```plaintext
ID   A18053_3; parent: A18053
AC   A18053;
FT   promoter 396..1779
FT   /note="35S3 promoter sequence derived from cauliflower mosaic virus isolate CabbB-JI"
SQ   Sequence 1384 BP;

ctgccgacag tggtcccaaa gatggacccc cacccacgag gagcatcgtg gaaaaagaag
acgttccaa cacgtcttca aagcaagtgg attgatgtga catctccact gacgtaaggg
atgacgcaca atcccactat ccttcgcaag acccttcctc tatataa

Figure 1. Partial sequence of the 35S promoter from Cauliflower Mosaic Virus (CaMV) and hybridisation sites of primers p35S-cf3 and p35S-cr4

Detection of the nos terminator

Primers HA-nos118-f and HA-nos118-r (Lipp et al., 2001) are used for the detection of the nos terminator. The nos terminator is present in the Roundup Ready® soybean and other lines of transgenic plants (e.g. maize line Bt-11). Amplification of the nos terminator will result in the production of a DNA fragment of 118 bp. In Figure 2, primers HA-nos118-f and HA-nos118-r have been positioned inside the sequence of the transgenic part of Roundup Ready® soybean.
Roundup Ready® soybean from Monsanto
Sequence of the transgenic part according to Patent WO 92/04449

HA-nos118-r >
151 nnnnnnnnn nnnnnnnnaga tccecgatct agtaacatag atgacaccgc
201 gcgcgaatt ttatccagtg ttgcgcgctta tattttgttct tacatccgcgt < HA-nos118-f
251 attaaatgta taattgcggg actctaatca taaaaaccccc ttcctaaat
301 aacgtcatgc aaattacagtt aattatataca tgcctaatgcgt aattcaacag
351 aaattatatg ataatcatcgg caagagcgggc aacaggtacc aatcttaaga

/ / 

1601 gatccaggtg tcgcctttct taccggtcctt ggccggcctgt gcctgccttt
1651 ccttgccgcgc atttgacgct gcctgccttt caagagcgggc ggtgatgcgc < GM09 >
1701 gtttcacgcc tcgcgagacc gcgcgaacatg aaggacccgt gggagatcga
1751 cttgctgcgg ggaatgcgga cggctgcgg gcggctgccag gatttcgcgg
1801 cgggctcggcg cgggcggctc gcgtcgcgtg cgtgcgcggc tgcgcgcgtcact
1851 gatgcgtgaa tccataaagga caaaacatttt ttgcaaaaaa ttgaatcttt
1901 tttcctgacc aacatatgatt ttgctgaatttt tttgaccaaa < GM08 >
1951 aaacaagaaa acttgaagat ttagggagtctt ggggatttgt gtaatggaaga
2001 ttgggtgataa aagagttttctt cccctctgcag atgtgtttaa ttgctgctcat
2051 tcgctagag tcacgctgtc tgcgtgtcgc cggcgcggctt aatgtgtcag < GM07
2101 aatgaaccto ctatatataga gcggggtttata tcgcgagagt tgggtgatttt < p35S-cr4
2151 tggatccttc tgggtgatag tggatggaagat gcataattt ccttacctctt tctctcgcgtt < p35S-cr3
2201 aagacgctgg tgcggcgttct tctttttgca cgggcgtctct cgggcgtttgg
2251 gcgctacgctt cggcgcgcgct tgggtgcgcgc gcgtcgcggttc ctgcgcgcctt
cgggttggg tgggctgcgg gcggctgcgg gcggccgtcgg gcgggtcgggc
tcccctcagt gattgtggc atctgtgtaga cgcgcgttcc ttttccacta
2301 tcccataaat gcggatcgc gatgtgcgtggtc cagaggtggttc cgcgcgttcagt
tctcagctt cagaggtggtg cagaggtggtg cagaggtggtg cagaggtggtg
2351 ccggtattata cccctttgggtt aaaagtcttcg catcg

Figure 2. Sequence of the transgenic part of Roundup Ready® soybean from Monsanto according to Patent WO 92/04449
GMO specific PCR

The amplification primers that are used for the identification of Roundup Ready® soybean, Bt-176 maize and MON810 maize have been chosen for their capacity to detect, in a specific way, the genetic structure inserted into the Roundup Ready® soybean, Bt-176 and MON810 maize genomes, respectively.

Specific detection of the CTP/EPSPS gene cassette in Roundup Ready® soybean

The primer pairs GMO9/GMO5 and GMO8/GMO7 were designed for the specific detection of the transgene of Roundup Ready® soybean by nested PCR (Meyer and Jaccaud, 1997). The external primers GMO9 and GMO5 are complementary to the DNA sequence corresponding to the CP4 EPSPS gene and to the CaMV 35S promoter. The amplification of DNA with these two primers results in an amplicon of 447 bp. The internal primers, GMO8 and GMO7, are complementary to the epsps petunia gene and to the CaMV 35S promoter. The amplification of DNA with these internal primers results in a fragment of 169 bp, as shown in Figure 2.

Detection of the synthetic cryIA(b) gene of maize Bt-176

The primer pairs CRYIA1/CRYIA2 and CRYIA3/CRYIA4 were designed for the specific detection of the synthetic cryIA(b) gene by nested PCR (Studer et al., 1997). The external primers, CRYIA1 and CRYIA2, and the internal ones, CRYIA3 and CRYIA4, are complementary to the DNA sequence of the cryIA(b) gene. As shown in Figure 3, the two external primers (CRYIA1/CRYIA2) delimit a fragment of 420 bp while internal primers CRYIA3/CRYIA4 produce a fragment of 189 bp.
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ID     I41419  standard; DNA; UNC; 1947 BP.
AC     I41419;
DE     Sequence 3 from patent US 5625136.
RA     Koziel M.G., Desai N.M., Lewis K.S., Kramer V.C., Warren G.W., Evola S.V.,
RA     Crossland L.D., Wright M.S., Merlin E.J., Launis K.L., Rothstein S.J.,
RT     "Synthetic DNA sequence having enhanced insecticidal activity in maize";
SQ     Sequence 1947 BP; 412 A; 729 C; 528 G; 278 T; 0 other;
       atggacaaca acacccccacat caacgagttgc atcccccctaca actgccctgag caaccccgag
60    gttggaggagct cggccgccgcga ggcagccagcg acggctctaca ccccccctaca caatcgacctg
120   agcctgaccc atgttctgctg gagccagggcg ctgggctctac gctgctgcccg gctgctgaggct
180   gtcggacacta tctaagggcctc ctggggaatg gatcggagct gcagccgctac gcagcctacg
240   caggaagctg ctcggccctc tgcagtctgct gcagcctgagc gctgctgcccg gctgctgaggct
300   cccacaaacc cggcctctcgcc cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
360   ctgaccaacc ccacccccct tcgggctctac gagaactacg cgcagcctac gcagccgctac gcagcctacg
420   tacgccggct cggccgccggc caggcaacgc cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
480   cctgcacggc cctggccgagc cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
540   ggccacccacc cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
600   cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
660   cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
720   cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
780   cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
840   cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
900   cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
960   cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
1020  CRYIA1 forward primer outer PCR atgggcaacgc ctgggctctac gctgctgcccg gctgctgaggct
1080  CCRYIA1 forward primer outer PCR
1140  CRYIA3 forward primer inner (nested) PCR agcctgagcc ccgagcccccg cctgagccccagc
taccgcaaga cgccgaggtctc cctgagcccccg cctgagccccagc
cggccgagttc ctgggctctac gctgctgcccg gctgctgaggct
1200  CCRYIA4 reverse primer inner (nested) PCR
Figure 3. Optimised cryIA(b) gene inserted in maize Bt-176: Sequence of the cryIA(b) gene SEQ ID No. 3 according to US Patent No 5625136 and Genebank Accession No 141419

Specific detection of the E35S promoter/hsp70 exon-intron cassette of maize MON810

The primer pairs mg1/mg2 and mg3/mg4 were designed for the specific detection of the E35S/hsp70 exon-intron 1 cassette by nested PCR (Zimmermann et al., 1998). This gene construction is specific of maize MON810. The external primers, mg1 and mg2 anneal to the E35S promoter sequence and to the hsp70 intron 1 region, respectively, while the internal primers are complementary to the DNA sequence of the E35S promoter and the hsp70 exon 1 region, respectively. As shown in Figure 4, the two external primers (mg1/mg2) produce a fragment of 401 bp while mg3/mg4 produce a fragment of 149 bp.
Figure 4. Schematic representation of part of the MON810 maize cassette including the enhanced CaMV 35S-promoter and the maize hsp70 intron, and relative position of primers mg1, mg2, mg3, and mg4 (modified from Zimmermann et al., 1998)
References


