

**Table 14: Primer sequences and amplicon length in PCR-assays to detect GMOs**

| Target sequence                                  | Primer 1 (5' => 3')<br>Primer 2 (5' => 3')           | Amplicon length (bp) | Application (ID) | Cycling parameter | Ref.  |
|--|--|----------------------|------------------|-------------------|-------|
| <b>I.) Across interfaces</b>                     |  |                      |                  |                   |       |
| <b>Structural gene / regulatory sequence</b>     |  |                      |                  |                   |       |
| antisense PG P-35S                               | AGGGGAAAGTGGAAAACCATC<br>CCACTGACGTAAGGGATGACG       | 427                  | Tomato (1)       | x                 | 12    |
| antisense PG P-35S                               | TTTGGAGCTAAGGGTGTATGGA<br>AGTTCATTTTCATTTGGAGAGGACA  | 472                  | Tomato (12)      |                   | 17    |
| sense PG P-35S                                   | GAAGATCTGCATGGACCTGAAAA<br>AGTTCATTTTCATTTGGAGAGGACA | 478                  | Tomato (12)      |                   | 17    |
| nptII P-nos                                      | GAACTCGTCAAGAAGGCGATA<br>GTTCAAATGCGCCTAAGGTCA       | 943                  | Tomato (12)      | x                 | 17    |
| gene IIIA nos 3'                                 | CTACTGATTACGGTGTCTGCTA<br>TGAATCCTGTTGCCGGTCTTG      | 658                  | Tomato (12)      | x                 | 17    |
| P-35S PVX cp                                     | CCACTGACGTAAGGGATGACG<br>CCAGTTCATACCCTGAGC          | 502                  | Potato           | x                 | 5     |
| P-35S CTP (of CP4 epsps)                         | TGATGTGATATCTCCACTGACG<br>TGTATCCCTTGAGCCATGTTGT     | 172                  | Soybean (8)      | x                 | 24    |
| P-35S dhfr                                       | ATCATTGCGATAAAGGAAAGGC<br>CTGCCTCCGACTATCCAAACCA     | 540                  | Corn             | x                 | 4     |
| P-35S dhfr                                       | ATCATTGCGATAAAGGAAAGGC<br>AAAGCCACAAAAGTCCCAT        | 840                  | Corn             | x                 | 4     |
| CP4 epsps nos 3'                                 | nda<br>nda   | 213/314              | Soybean (8)      | x                 | 13    |
| <b>Regulatory sequence / regulatory sequence</b> |  |                      |                  |                   |       |
| P-35S nos 3'                                     | CAATCCCCTACTATCCTTCGC<br>CATCGCAAGACCGGCAACAG        | 890                  | Tomato (12)      | x                 | 17    |
| <b>Structural gene / structural gene</b>         |  |                      |                  |                   |       |
| cat lacZ   | TTTGTATTCTGAGCATAGTGA<br>ATAGCGACGAGAGTTAG           | 623                  | Microorganism    | x                 | 10    |
| kat A cat  | CAGCGACTTGAGAAAAACGAGTG<br>TGTCAGATAGGCCTAATGACTG    | 1321                 | Microorganism    | x                 | 9, 20 |
| nptII ocd  | TATCGCCTTCTTGACGAGTTC<br>CTGTGGCGGGAACCTCCACGA       | 401                  | Tomato (12)      | x                 | 17    |
| ocd gene IIIA                                    | CGATCCTGAGCGACAATATGA<br>TAGCAGCACCGTAATCAGTAG       | 660                  | Tomato (12)      | x                 | 17    |
| PVY-cp nptII                                     | nda<br>nda   | ca. 1800*            | Potato§          | x *               | 22    |
| <b>II.) Within single structural genes</b>       |  |                      |                  |                   |       |
| als  | CAGGTCAAGTGGCACGTAGGATG<br>GGCTGCTTGTCTTTCCAATCT     | 642                  | Cotton           |                   | 14    |
| aphIV (hygromycin-phosphotransferase)            | CGCCGATGGTTTCTACAA<br>GGCGTCGGTTTCCACTAT             | 839                  | Potato           | x                 | 8, 20 |
| barnase  | CTGGGTGGCATCAAAGGGAACC<br>TCCGGTCTGAATTTCTGAAGCCTG   | 160                  | Corn (21)        | x                 | 16    |
| barstar  | TCAGAAGTATCAGCGACCTCCACC<br>AAGTATGATGGTGATGTCGCAGCC | 235                  | Corn (21)        | x                 | 16    |
| gus  | TCCGTAGAAACCCAACC<br>GCTAGCCTTGTCATTG                | 674                  | Papaya (26?)     | x                 | 25    |
| gus  | ACGTCCTGTAGAAACCCCAA<br>CCCGCTTCGAAACCAATGCC         | 1097                 | Alfalfa          | x                 | 1     |

|   |   |                   |  |   |       |
|---|---|-------------------|--|---|-------|
| nptII                                   | GAACAAGATGGATTGCACGC<br>GAAGAACTCGTCAAGAAGGC              | 785               | Alfalfa                                | x | 1     |
| nptII                                   | nda<br>nda  | 506               | Tom.paste (51, 54)                     | x | 11    |
| nptII                                   | GGATCTCCTGTCATCT<br>GATCATCCTGATCGAC                      | 173               | Tom (1), Pot, SuB                      | x | 12,18 |
| nptII                                   | nda<br>nda  | 512               | Tomato (1)                             | x | 19    |
| nptII                                   | GGTGCCCTGAATGAACTG<br>TAGCCAACGCTATGTCCT                  | ca. 500           | Microorganism§                         | x | 21    |
| nptII                                   | CTCGACGTTGTCACCTGAAGCGGGAAG<br>AAAGCACGAGGAAGCGGTCAGCCCAT | 489b              | Microorganism§                         | x | 23    |
| nptII                                   | CCGACCTGTCCGGTGCCC<br>CCGCCACACCCAGCCGGCC                 | 475               | Soybean (8)                            | x | 15    |
| pat (synthetic?)                        | nda<br>nda  | nda               | Rapeseed§ (66?)                        |   | 3     |
| pat (synthetic?)                        | nda<br>nda  | 552               | Corn (15?)                             |   | 19    |
| pat (synthetic)                         | nda<br>nda  | nda               | Rapeseed§ (66?)                        |   | 2     |
| pat (synthetic)                         | nda<br>nda  | 532               | Corn/Rapeseed§                         |   | 6     |
| PG<br>(polygalacturonase)               | CGTTGGTGCATCCCTGCATGG<br>GGATCCTTAGAAGCATCTAGT            | 180 (380)a        | Tomato (12)                            |   | 17    |
| PG<br>(polygalacturonase)               | nda<br>nda  | 427               | Tomato (12)                            |   | 19    |
| II.) Within single regulatory sequences |   |                   |  |   |       |
| P-35S                                   | GCTCCTACAAATGCCATCA                                       | 195 or 390<br>195 | Tom (1), Soy (8), Corn,<br>SuB, Potato | x | 18,19 |
| P-35S                                   | nda<br>nda  | nda               | Rapeseed§ (66?)                        |   | 3     |
| P-TA29                                  | CTTTTGGTTAGCGAATGC<br>CTACCATGGTAGCTAATTTT                | 880               | Tobacco                                | x | 7     |
| T-nos (= nos 3')                        | GAATCCTGTTGCCGGTCTTG<br>TTATCCTAGTTTGCGCGCTA              | 180               | Soybean (8), Pot.                      | x | 18    |

Tom, Tomato; Pot, Potato; SuB, sugarbeet.

\* cycling parameters had to be adjusted to the unusual amplicon size.

a) use of these primers resulted in a 380 bp amplicon in control lines, and an additional 180 bp product in transgenic lines (no intron).

b) This amplicon was the result of the second set of primers in a nested PCR; the size of the first product was 753 bp.

§ DNA was extracted from the soil (degraded plant material, or microorganisms).

x Cycling parameter described

**References:** 1, Blake et al. (1991); 2, Ernst et al. (1996); 3, Feldmann et al. (1996); 4, Golovkin et al. (1993); 5, Jongedijk et al. (1992); 6, Kirchhof et al. (1996); 7, Kriete et al. (1996); 8, LMBG-Methodensammlung (1996); 9, LMBG-Methodensammlung (in press); 10, LMBG-Methodensammlung (in preparation); 11, Meyer, G.; Hanse Analytik, Bremen (personal communication); 12, Meyer (1995a); 13, Padgett et al. (1995); 14, Petition from DuPont for genetically modified cotton (1995); 15, Petition from Monsanto for herbicide-tolerant soybean (1993); 16, Petition from PGS for SeedLink® corn (1995); 17, Petition from Zeneca for genetically modified tomatoes (1995); 18, Pietsch et al. (1997); 19, Pietsch and Waiblinger, (1996); 20, Schulze et al. (1996); 21, Smalla et al. (1993); 22, Stax et al. (1994); 23, Tsushima et al. (1995); 24, Wurz and Willmund, (1997); 25, Yang et al. (1996)