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(54) **Development of phytophthora resistant potato with increased yield**

(57) The present invention relates to transgenic potato plants having an increased resistance against *Phytophthora infestans* and a comparable yield of potato tubers compared with the wildtype potato plants, wherein

the blb1-gen and blb2-gen are integrated within a specific genetic background into the potato plant.

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**Description**

**[0001]** The present invention relates to transgenic potato plants having an increased resistance against *Phytophthora infestans* and a comparable yield of potato tubers compared with the wildtype potato plants, wherein the blb1-gene and blb2-gene are integrated within a specific genetic background into the potato plant.

**[0002]** Late blight caused by the oomycete *Phytophthora infestans* is one of the most severe threats to potato production worldwide. Despite many years of resistance breeding, the only effective way to prevent crop failures or reduced yields is the application of fungicides that prevent or cure an infection by *P. infestans*. As the disease development of late blight is extremely fast, it is necessary to run a tight fungicide regime, which has to start before first symptoms occur. Furthermore, *P. infestans* seems to have a high potential to adapt to specific fungicides and to develop resistance, as already seen in the case of metalaxyl-fungicides (Gisi U, Cohen Y (1996) Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure Annual Rev Phytopathol. 34: 549-572).

**[0003]** In several Western European countries, legislation on the use of plant protection products is becoming more restrictive regarding the application of specific fungicides, making chemical control of the disease and the prevention of resistance development more difficult.

**[0004]** An alternative and/or complementary approach to the use of fungicides is the development of potato cultivars that harbour improved resistance to *P. infestans*.

**[0005]** In recent years, two potato varieties containing *S. bulbocastanum* derived resistance were developed via conventional breeding. Both varieties, Toluca and Bionica, contain a single *S. bulbocastanum* resistance gene that confers full resistance against *P. infestans*. But from an agronomical point of view the two potato varieties do not match modern potato varieties in terms of yield potential.

**[0006]** As the introgression of the *S. bulbocastanum* derived resistance into modern potato varieties turned out to be difficult and time consuming, a much more efficient approach is the isolation of the genes that code for *Phytophthora* resistance in *S. bulbocastanum* and their transfer into current potato cultivars by biotechnological methods.

**[0007]** To generate the durably resistant potato plants, the Rpi-blb1 and the Rpi-blb2 genes were combined under control of their native regulation elements. The resultant vector construct contained the genomic sequence of the Rpi-blb1 gene under control of the native Rpi-blb1 promoter and Rpi-blb1 terminator, all derived from *S. bulbocastanum*, in combination with the genomic sequence of the Rpi-blb2 gene under control of the native Rpi-blb2 promoter and Rpi-blb2 terminator all from *S. bulbocastanum* (WO 2008/034876). The obtained transgenic potatoes expressing blb1-protein and blb2-protein showed increased resistance to *Phytophthora infestans*. However, it was found that the yield of the developed potato plants was decreased.

**[0008]** It is an object of the present invention to provide potato plants having an improved resistance to *Phytophthora infestans* and a comparable yield with the wildtype potato.

**[0009]** The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the examples included herein. Unless otherwise noted, the terms used herein are to be understood according to conventional usage by those of ordinary skill in the relevant art. In addition to the definitions of terms provided herein, definitions of common terms in molecular biology may also be found in Rieger et al., 1991 Glossary of genetics: classical and molecular, 5th Ed., Berlin: Springer-Verlag; and in Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1998 Supplement). It is to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized. It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

**[0010]** Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al., 1989 Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, N.Y.; Maniatis et al., 1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, N.Y.; Wu (Ed.) 1993 Meth. Enzymol. 218, Part I; Wu (Ed.) 1979 Meth Enzymol. 68; Wu et al., (Eds.) 1983 Meth. Enzymol. 100 and 101; Grossman and Moldave (Eds.) 1980 Meth. Enzymol. 65; Miller (Ed.) 1972 Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; Old and Primrose, 1981 Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink, 1982 Practical Methods in Molecular Biology; Glover (Ed.) 1985 DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (Eds.) 1985 Nucleic Acid Hybridization, IRL Press, Oxford, UK; and Setlow and Hollaender 1979 Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

**[0011]** The object of the present invention is solved by the provision of Phytophthora-resistant-transgenic potato plant, seed, tuber, plant cell or tissue thereof having a specific integration site for the blb1-gene and blb2-gene.

**[0012]** One embodiment according to the present invention provides a Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue, preferably comprising a nucleotide sequence having at least 80 % identity with SEQ-ID-No. 1 (cf. Figure 2a).

**[0013]** One embodiment according to the present invention provides a Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue comprising a nucleotide sequence having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 flanked by flanking regions having at least 80% identity with SEQ-ID-No. 20 and/or SEQ-ID-No. 21 (cf. Figs 2b,c ,e).

**[0014]** SEQ-ID-No. 1 refers to the part of the recombinant construct inserted into the plant genome including blb1-gene, blb2-gene and ahas-gene, wherein SEQ-ID-No. 1 further includes the flanking genomic sequences of the plant (cf. Fig.2a).

**[0015]** SEQ-ID-No.2 refers to the part of the recombinant construct inserted into the plant genome including blb1-gene, blb2-gene and the ahas-gene. Preferably, blb1-gene, blb2-gene and optionally the ahas-gene are expressed, if a sequence having at least 80 % identity to SEQ-ID-No. 2 is inserted into the plant genome. Preferably, blb1-gene, blb2-gene and or ahas-gene are expressed, if a sequence having at least 80 % identity to SEQ-ID-No. 2 is inserted into the plant genome (cf. Fig. 2b).

**[0016]** SEQ-ID-No. 3 refers to the part of the recombinant construct inserted into the plant genome including blb1-gene, blb2-gene but without the ahas-marker-gene. Preferably, blb1-gene, blb2-gene are expressed, if a sequence having at least 80 % identity to SEQ-ID-No. 3 is inserted into the plant genome (cf. Fig. 2c).

**[0017]** SEQ-ID-Nos. 2 and/or 3 may be referred to herein later as insert.

**[0018]** SEQ-ID-No. 20 refers to the left flanking region of the insert having SEQ-ID-Nos. 2 or 3.

**[0019]** SEQ-ID-No. 21 refers to the right flanking region of the insert having SEQ-ID-Nos. 2 or 3.

**[0020]** The term "flanking region" refers the region of the plant genome flanking either the right or left site of the insert which is integrated into the plant genome.

**[0021]** One embodiment according to the present invention provides a Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue thereof comprising a) a recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No.3 and

**[0022]** further comprising a junction sequence selected from the group consisting of b)

i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 126 and 136 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,

ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 505 and 515 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,

iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 625 and 635 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or

iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 4752 and 4762 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,

and/or

further comprising a junction sequence selected from the group consisting of

v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 282 and 292 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,

vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 877 and 887 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,

vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 827 and 837 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or

viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 9905 and 9915 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

**[0023]** A preferred embodiment of the present invention provides a Phytophthora-resistant transgenic potato, seed, tuber, plant cell or tissue thereof

- a) a recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No.3 and further comprising a junction sequence selected from the group consisting of
- b)

- i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 131 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
- ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment 510 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
- iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 630 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or
- iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 4757 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11

and/or

further comprising a junction sequence selected from the group consisting of

- v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 287 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 882 basepairs using a polymerase chain reaction with two primers having the nucleotide sequence of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 832 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or
- viii) nucleic acid sequence that can be used to amplify a nucleotide fragment of 9910 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

**[0024]** A nucleic acid sequence that can be used to amplify a nucleotide fragment of a certain length using a polymerase chain reaction with two primers means the product of said polymerase chain reaction with two primers. In particular, this means a nucleic acid sequence that is amplified to a nucleotide fragment of a certain length using a polymerase chain reaction with two primers.

**[0025]** A junction sequence includes either a right or left part of the recombinant construct inserted into the plant genome and partially includes plant genomic sequences of the flanking region of said right or left part of the recombinant construct. In particular, the junction sequence comprises either a left part of the recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or 3 and a part of the flanking region having at least 80% identity to SEQ-ID-No. 20 or a right part of the recombinant construct having at least 80 % identity to SEQ-ID-No. 2 or 3 and a part of the flanking region having at least 80 % identity with SEQ-ID-No. 21. Identity with respect to partial sequences of the recombinant construct means in this case identity over the entire length of said left or right part of the recombinant construct (Fig. 2e).

**[0026]** In particular, there is at least a partial overlap of either a part of the left part of the recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or 3 and the junction sequence having at least 80 % identity with SEQ-ID-No. 22 or a part of the right part of the recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or 3 and the junction sequence having at least 80 % identity with SEQ-ID-No. 23. Identity with respect to partial sequences of the recombinant construct means in this case identity over the entire length of said left or right part of the recombinant construct (Fig. 2e).

**[0027]** PCR means polymerase chain reaction, i.e. the selective enrichment of nucleic acids of defined length within a mixture of nucleic acids with primers specific for said nucleic acid by using Taq-polymerase or the like (US 5,656,493; Sambrook et al. 1989, Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, N.Y.).

**[0028]** An alternative embodiment provides a Phytophthora-resistant transgenic potato, seed, tuber, plant cell or tissue comprising

- a) a recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No.3 and further comprising a junction sequence selected from the group consisting of
- a sequence having at least 80 % identity to SEQ-ID-No. 22 and/or SEQ-ID-No. 23.

**[0029]** One embodiment according to the present invention provides a Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue obtainable from the seeds as deposited under Accession-No. NCIMB 41841 (Solanum

tuberosum) at NCIMB (NCIMB Ltd, Ferguson Building, Craibstone Estate Bucksburn, Aberdeen AB21 9YA, Scotland, Great Britain) on May 12, 2011. A transgenic potato plant, seed, tuber, plant cell or tissue according to the present invention comprising the recombinant construct which is amplifiable as defined above may be obtained by propagation or crossing a potato plant with a potato plant obtained from the seeds deposited under Accession-No. NCIMB 41841 (of elite-event D) and subsequent selection of the plants carrying the recombinant construct by detection with PCR using the above defined primer pairs. The berries containing the seeds have been hand-harvested, extracted and dried recently. The seeds may be stored at room temperature. Seed may be treated with 0,04% GA (giberellic acid) in order to break dormancy and enhance germination.

**[0030]** In one embodiment for crossing the pollen of the father plant is transferred from its stamen to the isolated carpel of the mother plant. The true seed bearing berries are harvested and the seeds are separated from the peel and flesh of the berries. The seeds are replanted, grown to plants and subsequently the plants carrying the recombinant construct are selected by detection with PCR using the above defined primer pairs.

**[0031]** The mother plant and/or the father plants may be the phytophthora-resistant transgenic potato plant according to the present invention. In one embodiment the mother plant is phytophthora-resistant transgenic potato plant according to the present invention and the father plant may be a non-transgenic plant, e.g. selected from the group consisting of Agria, Sarpo Mira, Cara, Valor, Innovator, Diamant and Bintje. In an alternative embodiment the father plant is the phytophthora-resistant transgenic potato plant according to the present invention and the mother plant may be a non-transgenic plant, e.g. selected from the group consisting of Agria, Sarpo Mira, Cara, Valor, Innovator, Diamant and Bintje.

**[0032]** One embodiment of the present invention provides a method for providing a Phytophthora-resistant transgenic potato plant or part thereof comprising the following steps:

- a) introducing a recombinant nucleic acid having at least 80 % identity with SEQ-ID- No. 2 or SEQ-ID-No. 3 into the genome of potato plant cells,
- b) integrating said recombinant nucleic acid into the genome,
- c) regenerating plant from said plant cells,
- d) selecting plant comprising a nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and a junction sequence selected from the group consisting of a sequence having at least 80 % identity to SEQ-ID-No. 22 and/or SEQ-ID-No. 23.

**[0033]** One embodiment of the present invention provides a method for providing a Phytophthora-resistant transgenic potato plant or part thereof comprising the following steps:

- a) introducing a recombinant nucleic acid having at least 80 % identity with SEQ-ID- No. 2 or SEQ-ID-No. 3 into the genome of potato plant cells,
- b) integrating said recombinant nucleic acid into the genome,
- c) regenerating plant from said plant cells,
- d) selecting plant comprising a nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and a junction sequence selected from the group consisting of

- i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 126 and 136 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,

- ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 505 and 515 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,

- iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 625 and 635 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or

- iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 4752 and 4762 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,

and/or

further comprising a junction sequence selected from the group consisting of

- v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 282 and 292 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,

- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 877 and 887 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 827 and 837 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17

and/or

- viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 9905 and 9915 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequence of SEQ-ID-No. 18 and SEQ-ID-No. 19.

**[0034]** One method of the present invention provides a method for providing a Phytophthora-resistant transgenic potato plant or part thereof, wherein in step d) plants are selected comprising a nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and a junction sequence selected from the group consisting of

- i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 131 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
- ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment 510 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
- iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 630 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or
- iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 4757 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,

and/or

- v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 287 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 882 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 832 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or
- viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9910 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

**[0035]** One embodiment of the present invention provides a kit for the detection of the specific integration place, in particular for the detection of elite-event D as deposited under Accession-No. NCIMB 41841, comprising the primer pairs

SEQ-ID-No. 4 and 5,  
 SEQ-ID-No. 6 and 7,  
 SEQ-ID-No. 8 and 9,  
 SEQ-ID-No. 10 and 11,  
 SEQ-ID-No. 12 and 13,  
 SEQ-ID-No. 14 and 15,  
 SEQ-ID-No. 16 and 17, and/or  
 SEQ-ID-No. 18 and 19.

**[0036]** The kit disclosed can be used for purposes of quality control (e.g., purity of seed lots), detection of the specific integration place, in particular elite-event D as deposited at NCIMB having deposition-No. NCIMB 41841 on May 12, 2011, in plant material or material comprising or derived from plant material, such as french fries, potato meal, mash potatoes etc. but not limited to food or feed products.

**[0037]** Briefly, genomic DNA is amplified by PCR using a primer which specifically recognizes the 5' or 3' flanking sequence of the insertion site in the elite event D, in particular the above mentioned primer pairs, e.g. primers SEQ-ID-No. 4 and SEQ-ID-No. 5, SEQ-ID-No. 6 and SEQ-ID-No. 7, SEQ-ID-No. 8 and SEQ-ID-No. 9, SEQ-ID-No. 10 and SEQ-ID-No. 11, SEQ-ID-No. 12 and SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO:

17, SEQ-ID-No. 18 and SEQ-ID-No. 19, respectively. If PCR using above mentioned primer combinations on the plant material yields a fragment of

126 to 136 bp (131 bp),  
505 to 515 bp (510 bp),  
625 to 536 bp (630 bp),  
282 to 292 bp (287 bp),  
877 to 887 bp (882 bp),  
827 to 837 bp (832 bp),  
4752 to 4762 bp (4757 bp)  
9905 to 9915 bp (9910), respectively,

the transgenic plant is determined to have the herein defined specific integration place, e.g. the selected elite-event D. The fragment length can be determined by gel electrophoresis using markers Sambrook et al., 1989 Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, plainview, N.Y.).

**[0038]** One embodiment of the present invention provides a detection method for a specific integration place, preferably for the identification of elite event D, comprising the steps of

a) isolating DNA from a potato plant as a test sample  
b) exposing the test sample, a positive and a negative sample a primer pair as defined above under PCR-conditions, and  
c) evaluating the amplification of a DNA-fragment

i) of between 126 and 136 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,  
ii) of between 505 and 515 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,  
iii) of 625 and 635 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or  
iv) of 4752 and 4762 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,

and/or

v) of between 282 and 292 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequence of SEQ-ID-No. 12 and SEQ-ID-No. 13,  
vi) of 877 and 292 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,  
vii) of 827 and 837 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or  
viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9905 and 9915 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19

compared with said positive and negative control.

**[0039]** In a preferred embodiment in step e)

evaluating means the amplification of a nucleotide fragment selected from the group consisting of a nucleotide fragment

i) of 131 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,  
ii) of 510 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,  
iii) of 630 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or  
iv) of 4757 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11

and/or

of a nucleotide fragment selected from the group consisting of a nucleotide fragment

- i) of 287 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- ii) of 882 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- iii) of 832 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or
- iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9910 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19

compared with said positive and negative control.

**[0040]** The test sample comprises genomic DNA isolated from transformed plant material, e.g. from plants, seed, tuber, plant cells or an tissue thereof. The positive sample is a sample comprising genomic DNA isolated from plants including SEQ-ID-No. 1, e.g. genomic DNA isolated from plants grown from seeds deposited under Accession-No. NCIMB 41841. The negative sample is a sample comprising genomic DNA isolated from the non-transgenic original variety used for transformation, e.g. the Fontane variety.

**[0041]** The PCR reaction can be run with various DNA polymerases, such as the Pfu Ultra, Pfu Turbo or Herculanase DNA Polymerase (Agilent Technologies, Santa Clara, CA, US). The composition for the protocol of the Pfu Ultra, Pfu Turbo or Herculanase DNA polymerase may be as follows: 1x PCR buffer, 0.2 mM of each dNTP, 1µg genomic DNA of Sample, 50 pmol forward primer, 50 pmol reverse primer, 1 u Pfu Ultra, Pfu Turbo or Herculanase DNA polymerase.

**[0042]** The amplification cycles may be as follows:

1 cycle of 60 seconds at 98°C, followed by 35 cycles of in each case 10 seconds at 98°C, 30 seconds at the annealing temperature given in the table below and 60 seconds per 1000bp product length (see table below) at 72°C, followed by 1 cycle of 10 minutes at 72°C, then 4°C.



Table 1

Event D	Border	Sense primer	SEQ-ID No.	Antisense primer	SEQ ID No	Annealing temperature [°C]
	LB	TCAAAACGGATGTTAATTCAGTACATT	4	CCAGTTCCCAATTGACTACTAGAAA	5	52
	LB	TCTGTTGAATTACGTTAAGC	6	CTCAGAAGAAAGAATTGTTC	7	48
	LB	GTTTCTTAAGATTGAATCCTGTTGC	8	GCCCATCTCTATTTTACTCACTAA	9	51
	R B	CCAAGATAGTGTTTCAGGAAAGTTATT	12	AAATTCATGGTAGAACTGGAGGAG	13	52
	R B	AACTGAATTTTGGGATTGAG	14	GAGTCAGTTAAATTAAGTCTTCAG	15	49
	R B	ACAAGAATAGCAAGGATTATCC	16	GAAGTTCGAACAACATTCTT	17	49
Left flanking region to blb1	LB	GTGAACTAGGAAACCTAAATC	10	CAACTAATAAAACCAAGGAC	11	52
Right flanking region to blb1	R B	AACTGAATTTTGGGATTGAG	18	ATGTAGCAGCATTGAGTTTT	19	50

**[0043]** One embodiment according to the present invention provides a plant, tuber, seed, detectable by the above defined kit or by the above defined detection method.

**[0044]** One embodiment of the present invention provides a polynucleotide comprising

- a) a recombinant nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and
- b) further comprising a junction sequence selected from the group consisting of
  - i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 126 and 136 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
  - ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 505 and 515 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
  - iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 625 and 635 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or
  - iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 4752 and 4762 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,

and/or

further comprising a junction sequence selected from the group consisting of

- v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 282 and 292 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13
- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 877 and 887 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15
- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 827 and 837 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17 and/or
- viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 9905 and 9915 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

**[0045]** One preferred embodiment of the present invention provides a polynucleotide comprising

- a) a recombinant nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and
- b) further comprising a junction sequence selected from the group consisting of
  - i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 131 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
  - ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment 510 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
  - iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 630 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or
  - iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 4757 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11

and/or

- v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 287 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 882 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 832 basepairs, using a

polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or

viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9910 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19

stably integrated into a potato plant cell nucleus.

**[0046]** One embodiment of the present invention provides a polynucleotide having at least 80% identity with SEQ-ID-Nos. 22 or 23.

**[0047]** A preferred embodiment according to the present invention is a polynucleotide comprising a nucleotide sequence having at least 80 % identity with SEQ-No. 1 preferably stably integrated into a potato plant cell nucleus.

**[0048]** In a further embodiment, the polynucleotide comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 91 %, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, 2, 3, 44 and/or 45, preferably stably integrated into the genome of a potato plant cell.

**[0049]** In yet another embodiment, the polynucleotide comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 91 %, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, 2, 3, 44 and/or 45 and comprising the blb1 and the blb2 genes, stably integrated into the genome of a potato plant cell. The polynucleotide can also further comprise one or more of SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, and/or 19 in the regions flanking the inserts.

**[0050]** "Polynucleotides" according to the present invention may be isolated polynucleotides and/or recombinant polynucleotides. Recombinant polynucleotides or recombinant construct mean any polynucleotide produced by gene technology modification e.g. by man. The gene technology modification may be transforming a plant cell with a nucleotide sequence preferably using agrobacteria.

**[0051]** "Identity" between two nucleic acids and/or refers in each case over the entire length of the nucleic acids.

**[0052]** For example the identity may be calculated by means of the Vector NTI Suite 7.1 program of the company Informax (USA) employing the Clustal Method (Higgins DG, Sharp PM. Fast and sensitive multiple sequence alignments on a microcomputer. Comput Appl. Biosci. 1989 Apr; 5(2):151-1) with the following settings:

**[0053]** Multiple alignment parameter:

Gap opening penalty	10
Gap extension penalty	10
Gap separation penalty range	8
Gap separation penalty	off
% identity for alignment delay	40
Residue specific gaps	off
Hydrophilic residue gap	off
Transition weighing	0

**[0054]** Pairwise alignment parameter:

FAST algorithm	on
K-tuple size	1
Gap penalty	3
Window size	5
Number of best diagonals	5

**[0055]** Alternatively the identity may be determined according to Chenna, Ramu, Sugawara, Hideaki, Koike, Tadashi, Lopez, Rodrigo, Gibson, Toby J, Higgins, Desmond G, Thompson, Julie D. Multiple sequence alignment with the Clustal series of programs. (2003) Nucleic Acids Res 31 (13):3497-500, the web page: <http://www.ebi.ac.uk/Tools/clustalw/index.html> and the following settings

DNA Gap Open Penalty	15.0
DNA Gap Extension Penalty	6.66

(continued)

DNA Matrix	Identity
Protein Gap Open Penalty	10.0
Protein Gap Extension Penalty	0.2
Protein matrix	Gonnet
Protein/DNA ENDGAP	-1
Protein/DNA GAPDIST	4

**[0056]** All the nucleic acid sequences mentioned herein can be produced in a known way by chemical synthesis from the nucleotide building blocks, e.g. by fragment condensation of individual overlapping, complementary nucleic acid building blocks of the double helix. Chemical synthesis of oligonucleotides can, for example, be performed in a known way, by the phosphoamidite method (Voet, Voet, 2nd edition, Wiley Press, New York, pages 896-897). The accumulation of synthetic oligonucleotides and filling of gaps by means of the Klenow fragment of DNA polymerase and ligation reactions as well as general cloning techniques are described in Sambrook et al., 1989 Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory.

**[0057]** Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). At least 80% sequence identity, preferably at least 85% sequence identity, especially preferred at least 90%, at least 95 %, at least 98%, at least 99% sequence identity, or even 100% sequence identity, with the nucleic acid having SEQ-ID-Nos. 1, 2, 3, 20, 21, 22 and/or 23 is preferred.

**[0058]** The recombinant construct may encompass nucleotides having nucleic acid substitutions, deletions and/or insertions relative to the unmodified nucleic acid in question, wherein the protein coded by such nucleic acids has similar or higher functional activity as the unmodified protein coded by the unmodified nucleic acid from which they are derived. In the substitutions may be based on the degenerative amino acid code.

**[0059]** A "deletion" refers to removal of one or more amino acids from a protein or to the removal of one or more nucleic acids from DNA.

**[0060]** An "insertion" refers to one or more nucleic acid residues being introduced into a predetermined site in the nucleic acid.

**[0061]** Methods for the manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gene in vitro mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

**[0062]** As used herein, the term "recombinant construct" preferably refers to an expression cassette having at least 80 % identity with SEQ-ID-No. 2 and/or 3. In one embodiment homologues of the expression cassette have at the DNA level at least 80%, preferably of at least 90%, especially preferably of at least 95%, quite especially preferably of at least 98%, at least 99% or 100% identity over the entire DNA region of SEQ-No. 2 and/or 3. Preferably, the recombinant construct comprises the blb1-gene including the blb1-promotor and the blb1-1-terminator as well as the blb2-gene including the blb2-promotor and the blb2-2-terminator as defined below as well as a mutated ahas-gene including the p-nos-promotor and the t-nos-promotor, which are preferably capable to express the blb1 and blb2 gene and optionally the ahas gene. Said recombinant construct may be introduced in a plant cell by gene technological methods e.g. agrobacteria transformation.

**[0063]** In one embodiment, the recombinant construct or expression cassette or transgenic plant comprises the nucleotide sequence of SEQ ID NO: 1, 2, 3, 44 and/or 45.

**[0064]** As used herein, the term "blb1-gene" refers to a gene having at least 80 % identity with SEQ-ID-No. 46. In one embodiment homologues of the blb1-gene have at the DNA level at least 90%, preferably of at least 95%, especially preferably of at least 98%, at least 99% or 100% identity over the entire DNA region of SEQ-ID-No. 46.

**[0065]** As used herein, the term "blb2-gene" refers to a gene having at least 80 % identity with SEQ-ID-No. 47. In one embodiment homologues of the blb2-gene have at the DNA level at least 90%, preferably of at least 95%, especially preferably of at least 98%, at least 99% or 100% identity over the entire DNA region of SEQ-ID.No. 47.

**[0066]** As used herein, the term "mutated ahas-gene" refers to a gene having at least 80 % identity with SEQ-ID-No. 48. In one embodiment homologues of the mutated ahas-gene have at the DNA level at least 90%, preferably of at least 95%, especially preferably of at least 98%, at least 99% or 100% identity over the entire DNA region of SEQ-ID-No. 48.

**[0067]** Preferably, the Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue thereof ex-

presses a functional protein corresponding to the blb1- and blb2-genes and optionally the ahas gene. Preferably, the Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue thereof expresses SEQ-No. 46 and 47 and optionally SEQ-ID-No. 48 and/or the corresponding protein (cf. Fig. 2h).

**[0068]** The transgenic plant, seed, tuber, plants cell or tissue according to the present invention have a Phytophthora-resistance compared to the wildtype plant.

**[0069]** The wild type plant is a plant of a similar, more preferably identical, genotype as the transgenic plant having increased resistance to the Phytophthora-resistance, but does not comprise a recombinant nucleic acid comprising the blb1-gene and blb2-gene preferably regulated by their respective natural promoters and terminators.

**[0070]** As used herein the term "Phytophthora-resistance" or "Phytophthora-resistant", means reducing or preventing an infection with Phytophthora infestans. Phytophthora-resistance does not imply that the plant necessarily has 100% resistance to said infection. In preferred embodiments, the resistance to infection Phytophthora infestans in a resistant plant is greater than 10%, 15%, 20%, 25 %, 30%, 35 %, 40%, 45 %, 50%, 55 %, 60%, 65 %, 70%, 75 %, 80%, 85 %, 90%, or 95% in comparison to a wild type plant that is not resistant to Phytophthora infestans.

**[0071]** The term "Phytophthora-resistance" as used herein refers to the ability of a plant, as compared to a wild type plant, to avoid infection by Phytophthora infestans, to be killed by Phytophthora infestans, to hamper, to reduce, to delay, to stop the development, growth and/or multiplication of Phytophthora infestans. The level of Phytophthora infestans resistance of a plant can be determined in various ways, e.g. by scoring/measuring the infected leaf area in relation to the overall leaf area. Another possibility to determine the level of resistance is to count the number of Phytophthora infestans colonies on the plant or to measure the amount of spores produced by these colonies. Another way to resolve the degree of fungal infestation is to specifically measure the amount of Phytophthora infestans by quantitative (q) PCR. (e.g. Llorente et al (2010) A quantitative real-time PCR method for in planta monitoring of Phytophthora infestans growth. Lett Appl Microbiol. 51 (6):603-10.)

**[0072]** Furthermore, the transgenic plant, seed, tuber, plants cell or tissue according to the present invention provides a "comparable yield" compared to the wildtype plant, seed, tuber, plants cell or tissue.

**[0073]** The term "comparable yield" as used herein refers to the ability of the transgenic potato plant compared to wildtype plant to provide a similar amount of tubers. A similar amount of tubers means that the relative yield of transgenic tubers based on the yield/ha of wildtype tubers (kg/ha) is at least 95 % , preferably at least 96%, at least 97%, at least 98%, at least 99% of the yield/ha of the wildtype tubers or more preferably the same or more than the yield/ha of the wildtype tubers.

**[0074]** The %-relative yield is calculated as follows:

$$\% = \text{transgenic tubers (kg/ha)} \times 100\% / \text{wildtype tubers (kg/ha)}.$$

**[0075]** The term "plant" is intended to encompass plants at any stage of maturity or development, as well as any tissues or organs (plant parts) taken or derived from any such plant unless otherwise clearly indicated by context. Plant parts include, but are not limited to, plant cells, stems, roots, flowers, ovules, stamens, seeds, leaves, embryos, meristematic regions, callus tissue, anther cultures, gametophytes, sporophytes, pollen, microspores, protoplasts, hairy root cultures, and/or the like. As used herein, a "plant cell" includes, but is not limited to, a protoplast, gamete producing cell, and a cell that regenerates into a whole plant. Tissue culture of various tissues of plants and regeneration of plants therefrom is well known in the art and is widely.

**[0076]** The present invention also includes seeds produced by the plants of the present invention. Preferably, the seeds comprise a nucleic acid sequence having at least 80 % identity with SEQ-ID-No.1. The generated transformed plants may be propagated by clonal propagation or classical breeding techniques.

**[0077]** For the purposes of the invention, "recombinant construct" or "recombinant nucleic acid" means an expression cassette or a vector construct comprising the blb1-gene and the blb2-gene in combination with their natural promoters and terminators. Said expression cassette comprising the blb1-gene, blb2-gene, blb1-promotor, blb2-promotor, blb1-terminator and blb2-terminator are defined above.

**[0078]** As used herein, the term "transgenic" preferably refers to any plant, plant cell, tuber, callus, plant tissue, or plant part that contains the recombinant construct or a part thereof which is preferably introduced by non-essentially biological processes, preferably agrobacteria transformation. The recombinant construct or a part thereof is stably integrated into a chromosome, so that it is passed on to successive generations by clonal propagation, vegetative propagation or sexual propagation. Said successive generations are also transgenic. Essentially biological processes may be crossing of plants and/or natural recombination.

**[0079]** A transgenic potato plant, seed tuber, plants cell or tissue for the purposes of the invention is thus understood as meaning that the recombinant cassette integrated into the genome. Preferably, the blb1-gene and/or the blb2-gene and/or the ahas-gene are not present in the genome of the original plant and preferably are present in the genome of

the transgenic plant not at their natural locus of the genome of the original plant.

**[0080]** Natural locus means the location on a specific chromosome, preferably the location between certain genes, more preferably the same sequence background as in the original plant which is transformed.

**[0081]** Preferably, the transgenic potato plant, seed tuber, plants cell or tissue thereof expresses the blb1-gene and the blb2-gene. The term "expression" or "express" means the transcription of a specific gene or specific genes or specific genetic vector construct. The term "expression" or "express" in particular means the transcription of a gene or genes or genetic vector construct into structural RNA (rRNA, tRNA) or mRNA with preferably a subsequent translation of the latter into a protein.

**[0082]** The term "increased expression" or "overexpression" or "increase of content" as used herein means any form of expression that is additional to the original wild-type expression level. For the purposes of this invention, the original wild-type expression level might also be zero (absence of expression or absence of respective gene(s)).

**[0083]** The wildtype plant cells may be transformed with one of the above described recombinant construct. Suitable methods for transforming host cells including plant cells are well known in the art of plant biotechnology. Any method may be used to transform the recombinant expression vector into plant cells to yield the transgenic plants of the invention. The wildtype plants cells may be e.g. from Fontane, Agria, Bientje, Sarpo Mira, Cara, Valor, Innovator, Diamant.

**[0084]** Transformation can also be carried out by bacterial infection by means of *Agrobacterium* (for example EP 0 116 718), viral infection by means of viral vectors (EP 0 067 553; US 4,407,956; WO 95/34668; WO 93/03161) or by means of pollen (EP 0 270 356; WO 85/01856; US 4,684,611). *Agrobacterium* based transformation techniques are well known in the art. The *Agrobacterium* strain (e.g., *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*) comprises a plasmid (Ti or Ri plasmid) and a T-DNA element which is transferred to the plant following infection with *Agrobacterium*. The T-DNA (transferred DNA) is integrated into the genome of the plant cell. The T-DNA may be localized on the Ri- or Ti-plasmid or is separately comprised in a so-called binary vector. Methods for the *Agrobacterium*-mediated transformation are described, for example, in Horsch RB et al. (1985) *Science* 225:1229. The transformation of potatoe by *Agrobacteria* is described in, for example WO 2008/034876). Transformation may result in transient or stable transformation and expression. Although a nucleotide sequence of the present invention can be inserted into any plant and plant cell falling within these broad classes, it is particularly useful in potato plant cells.

**[0085]** The genetically modified plant cells can be regenerated via all methods with which the skilled worker is familiar. Suitable methods can be found in the abovementioned publications by S.D. Kung and R. Wu (White FF, Vectors for Gene Transfer in Higher Plants, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15 - 38; Jenes B et al. Techniques for Gene Transfer, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 128-143) Potrykus (Potrykus (1991) *Annu Rev Plant Physiol Plant Molec Biol* 42:205- 225.) or Höfgen and Willmitzer (Höfgen R, Willmitzer L (1988) Storage of competent cells for *Agrobacterium* transformation. *Nucleic Acids Res* 16:9877).

**[0086]** The recombinant construct may comprise a mutated *ahs*-gene as a selection marker. Plants carrying the construct are resistant to imidazolines. For selection of transgenic potato plants chemical compounds inhibiting the AHAS enzyme can be used. Useful compounds are the imidazoline type herbicides. Especially useful compounds are selected from the group consisting of imazethapyr (Pursuit®), imazamox (Raptor®), imazamethabenz (Assert®), imazapyr (Arsenal®), imazapic (Cadre®) and imazaquinon (Scepter®). For selection of transgenic plants chemical compounds as described in the review article by Duggleby, R.G. and Pang, S.S. in *Journal of Biochemistry and Molecular Biology* 33(1), 1-36 (2000) can be used.

**[0087]** The transformed plant tissue may be exposed to 0,5 µM imazamox such selecting the plant material carrying the construct.

**[0088]** Following DNA transfer and regeneration, putatively transformed plants may also be evaluated, for instance using Southern analysis, for the presence of the whole recombinant construct, copy number and/or genomic organisation.

**[0089]** Gene targeting in plants is possible, but it is a quite rare event (Hanin & Paszkowski 2003 *Current Opinion Plant Biol.* 6(2):157-62). However, the person skilled in the art will know how to improve gene targeting frequency. For example, one could increase gene targeting frequency by expressing proteins, which facilitate the process of homologous recombination such as yeast RAD54 (Shaked et al. 2005 *Proc Natl Acad Sci USA* 102(34):12265-9). Another approach is to facilitate detection of gene targeting lines by a strong positive-negative selection system (Iida & Terada 2005 *Plant Mol. Biol* 59: 205-219). In such approach a negative selectable marker is located outside of the homologous sequences on the transformation construct. In consequence, only those transgenic plants with random insertion of the transgenic sequences contain the negative selectable marker, while transgenic lines obtained through gene targeting do not comprise the negative selectable marker.

**[0090]** Furthermore, gene targeting frequency can be drastically increased by introducing a DNA double strand break at or near the desired insertion site. The person skilled in the art will know how to achieve this. For example, natural occurring homing endonucleases (also referred to as meganucleases, e.g. I-CreI) can be modified such that they recognize and cut a novel DNA sequence, i.e. the sequence at or near the desired insertion site in the genome (WO 07/047859, WO 07/049156). Alternatively, one could design so called zinc finger nucleases, which are comprised of a

unspecific nuclease domain (usually obtained from FokI nuclease) linked to a zinc finger, which specifically recognizes the desired DNA sequence (compare for example Trends Biotechnol. 2005 23(12):567-9; Cell Mol Life Sci. 2007 64(22): 2933-44; WO 08/021207). Another method is the usage of TAL (transcription activator like) effectors linked to a DNA specific nuclease (e.g. FokI) as described in Mahfouz et al. 2011. De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks; PNAS 108(6):2623-2628. By using this method, a TAL effector variant binding to the desired target sequence can be easily generated by changing the well defined aminoacids binding to the DNA (the code can be found in WO/2010/079430).

**[0091]** Gene targeting may be used to obtain a line similar to the elite-event D by inserting a transgenic construct comprising the recombinant construct at essentially the same insertion site as found in elite-event D. The person skilled in the art will know that the insertion site may differ in a few base pairs or up to a few kilo base pairs, but still obtaining a similar line with similar beneficial characteristics as compared to deposited elite-event. Gene targeting may in particular be used to establish a line similar to deposited elite-event D in a potato variety other than Fontane. It may be of interest to establish such a corresponding line based on other varieties more particularly suited for environmental conditions found in different potato growing regions.

## Figures

### [0092]

Figure 1: Vector card VCPMA 16

Figure 2: Sequences of the present application

Figure 3: Overview of primers

Figure 4: Chart comparing the relative yield of events A to D, Bintje (standard variety) with Fontane (mother variety of events A to D). The average yield/ha of Fontane, events A - D and Bintje was measured over 3 years at 15 locations in the field. The average yield/ha of the Fontane variety was set to 100% and relative yields of events A - D and Bintje were calculated accordingly.

Figure 5: Chart shows the result of Phytophthora screening of Fontane compared with events A to D and Bintje. Diseased leaf area was scored in the field after natural infection. The mother variety Fontane was set to 100%. All events show full resistance against Phytophthora infestans.

## Examples

**[0093]** The following examples are not intended to limit the scope of the claims to the invention, but are rather intended to be exemplary of certain embodiments. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the present invention.

### Example 1: General methods

**[0094]** The cloning steps carried out for the purposes of the present invention such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking DNA fragments, transformation of E. coli cells, bacterial cultures, phage multiplication and sequence analysis of recombinant DNA, are carried out as described by Sambrook et al. Cold Spring Harbor Laboratory Press (1989), ISBN 0-87969-309-6.

**[0095]** The chemical synthesis of oligonucleotides can be affected, for example, in the known fashion using the phosphoramidite method (Voet, Voet, 2nd Edition, Wiley Press New York, pages 896-897). The sequencing of recombinant DNA molecules is carried out with an MWG-Licor laser fluorescence DNA sequencer following the manual of the manufacturer based on the method by Sanger (Sanger et al., Proc. Natl. Acad. Sci. USA 74, 5463 (1977)).

### Example 2 Cloning of transformation vector VC-PMA16

**[0096]** pSUNAHASmod was used as backbone for the construction of VCPMA16. pSUNAHASmod is based on the plasmid pSUN1 (WO 02/00900). The T-DNA of pSUNAHASmod contains a mutated AHAS (Acetohydroxyacid-Synthase)-gene (S653N), which enhances the resistance of the transformed plant against imidazolinone herbicides (e.g. Imazamox: (R/S)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid). The use of the mutated AHAS gene as selection marker is described in Andersson et al. (2003) Plant Cell Rep 22:261-267 and WO 2004/005516. The AHAS selection cassette was constructed by fusing the nos promoter fragment from pGPTVKan (Becker et al., Molecular plant Biology 20, 1195 - 1197), the mutated AHAS gene from Arabidopsis and the nos-terminator from pGPTVKan (Becker et al., 1992 Molecular plant Biology 20, 1195 - 1197).

[0097] The blb1-gene fragment, including the 1173 bp blb1-promotor-region and the 406 bp bib1 terminator region was ligated into pSUNAHASmod by using the XbaI restriction site.

[0098] The blb2-gene expression cassette comprises the Rpi-blb2 gene (3890 bp), the blb2-promoter sequence (1530 bp) and 2530 bp blb2 termination sequence. To insert the blb2-expression cassette into the bib1 containing pSUNAHASmod, the vector was cut with PstI. The resulting sticky restriction sites were blunted and the blb2 expression cassette was inserted in a blunt-blunt ligation. The resulting vector was named VCPMA16 (Figure 1).

### Example 3 Transformation of *Agrobacterium tumefaciens* (*A. tumefaciens*) with VCPMA16

[0099] The construct VCPMA16 was transformed into the *A. tumefaciens* strains LBA4404, AGL0 or AGL1 by using direct transformation as described by Walkerpeach & Velten (Agrobacterium-mediated gene transfer to plant cells: co-integrate and binary vector systems, Gelvin SB, Schilperoot RA (Hrsg.), Plant Molecular Biology Manual, 2nd edn, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. B1/1-B1/19, 1994). Transformed bacteria were grown on YEB agar plates containing 1 µg/ml spectinomycin.

### Example 4 Cultivation and transformation of potato cultivar Fontane using *A. tumefaciens*

[0100] The potato variety Fontane was transformed with VCPMA16 by using Agrobacterium mediated transformation as described by Visser (Visser RGF, 1991, "Regeneration and transformation of potato by Agrobacterium tumefaciens." In Lindsey K (ed), "Plant Culture Manual", Kluwer Academic Publishers, Dordrecht, Netherlands. Seiten B5/1 - B5/9) but using Imazamox as selection marker (WO 2004/005516; Andersson et al., 2003, plant Cell Rep 22: 2261-267).

[0101] Potato leaf or shoot segments were incubated for 1-3 days on MC-plates (M300-Plates (4,4 g/l MS-Medium, 2 mg/l NAA, 1 mg/l BAP, 30 g/l Sucrose, pH 5,2) covered with 1,5 - 2 ml liquid M100-Medium (4,4g/l MS-Medium, 30 g/l Sucrose, 0,5 mg/ml Thiamin-Hydrochloride, 0,5 mg/ml Pyridoxin-Hydrochloride, 1 mg/l Nikotinic acid, 0,5 mg/l Kinetin, 29,8 mg/l FeSO<sub>4</sub>\*7H<sub>2</sub>O, 1 mg/l 2,4-D, 2 g/l Casein-Hydrolysate, pH 6,5) and covered with a sterile filter paper.

[0102] After 1-3 days the tissue segments were incubated with *A. tumefaciens* (containing VCPMA16) in MS10-Medium (4,4 g/l MS-Medium, 10 g/l Sucrose, pH 5,8). After 8 - 10 min. the tissue segments were transferred to M300 plates (see above). After 1 - 3 days the tissue segments were transferred to MS400-plates (4,4 g/l MS-Medium, 2 mg/l zeatine, 0,01 mg/l NAA, 0,1 mg/l GA3, 10 g/l Sucrose, 400 mg/l Claforane or carbenicilline, pH 5,8) and incubated for another 3 - 5 days.

### Example 5 Selection of the transformed potato plantlets

[0103] After 3 - 5 days the tissue segments were transferred to MS400 plates (see above) containing 0,5 µmol Imazamox as selection agent. Every 2 weeks the tissue segments were conveyed to new MS400 plates containing 0,5 µmol Imazamox. Growing (regenerated) shoots were harvested and transferred to MS30 plates (4,4 g/l MS-Medium, 30 g/l Sucrose, 200 mg/l claforane, pH 5,8) for further cultivation.

### Example 6 DNA extraction from transformed potato shoots

[0104] DNA was extracted from putative transgenic shoots by using the Wizard Magnetic 96 DNA Plant System (Promega, Mannheim) Kit according to the instructions of the manufacturer.

[0105] Example 7 Detection of Rpi-blb1 und Rpi-blb2 in transformed potato plants using real-time PCR To detect the presence of blb1 and blb2 a real time PCR was performed using the DNA from putative transgenic potato shoots (see above) as template. Following primers were used:

blb1:

5'-TGT TGA ACA CTG TAA CAT GCT AAA ATG-3' (forward Primer; SEQ ID No. 49)

5'-AGT TGT GGA CAT CCC CGAATT-3' (backward Primer; SEQ ID No. 50)

5'-AGA GGG ATT GCA GCA CCT AAC AAC CCT C-3' (Probe; SEQ ID No. 51)

blb2:

5'-TTC AAA ACC CCA AAT AAG TTT CAA C-3' (forward Primer; SEQ ID No. 52)

5'-CCA TGC TTG CTG TAC TTT GCA-3' (backward Primer; SEQ ID No. 53)



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5'-CGT TAC CCA GTC CTT CGG CG-3' (Probe; SEQ ID No. 54)

[0106] The samples were analyzed using a Roche Lightcycler480 by using 20-50 ng genomic DNA, 900 mM PCR primer (see above), 200 nM probe (see above) in 1 x LightCycler 480 Probe Master (for detailed protocol see manual of the manufacturer).

[0107] The amplification cycles of the PCR were:

1 cycle of 15 min at 95°C for denaturation, followed by 40 cycles of in each case 10 seconds at 95°C (Ramp Rate 4,8°C/sec) and 30 sec at 60°C (Ramp Rate 2,5°C /sec).

[0108] If the PCR of both fragments resulted in a positive signal, the shoots were transferred into the greenhouse to conduct Phytophthora resistance tests.

### Example 8 Determination of the resistance level of blb1 and blb2 containing transgenic potato shoots against Phytophthora infestans

[0109] The blb1 and blb2 containing transgenic potato shoots (as determined above) were transferred into soil and adapted to soil for 2 days at 22°C with 12 h day-length and 100% humidity in a growth cabinet (Binder KBW 400). Afterwards plants were grown in the greenhouse or phytochambers under similar conditions but 70% humidity.

[0110] After 4 weeks plants were inoculated with Phytophthora infestans spores. To prove the broad spectrum resistance mediated by blb1 and blb2, a multitude of different Phytophthora isolates, e.g. Blue13, Us-22 and many locally collected strains were tested collected from all over the world, either in mixtures or as single isolates.

[0111] All Phytophthora isolates were cultivated on pea-agar

Table 2

Pea-agar:	- 150 g peas
	- 1000 ml Millipore-ater
	- cook for 75 min. in a steamer
	- cool down for- 1 hour, incubate for 24h at RT
	- strain media and refill with 1l Millipore water
	- add 5g Glucose and 20g agar-agar
	- adjust pH to 6.5
	- autoclave for 15 min
	- pour plates under sterile conditions

[0112] Plant were inoculated with a spore density of 2,5xE05 spores/ml. The spore density was evaluated by using a Thoma counting chamber. For inoculation the complete plant was sprayed with spore suspension and transferred into a dark mist chamber. After 12-18 hours the plants were moved to the greenhouse (21°C, 12h light, >90% humidity) for one week.

[0113] First disease symptoms occurred after approx. 1 week. The rating of disease symptoms was done by trained personal evaluating the diseased leaf area, necrotic lesions, clorotic lesions and potential sporulation of P. infestans.

[0114] These values were integrated into a disease rating ranging from 0 to 100%. In the scoring system 0% disease means no macroscopically visible symptoms, whereas 100% means that all inoculated leaves are completely brownish and covered with mycelia, so the plant is essentially dead. Inoculation of the susceptible mother variety Fontane potato variety generally leads to a strong infection of all leaves. All leaves are heavily infected and green tissue is rare. In contrast the inoculation of the transformed Fontane Event A to D always leads completely healthy plants with a disease rating of 0% for all used Phytophthora isolates. As susceptible control the standard variety Bintje was used, which is known to be fully susceptible to Phytophthora infection

### Example 9 DNA isolation and quantitation methods for FST identification

[0115] Young leaf tissue of the fungal resistant potato events were collected for DNA isolation and characterization. Upon collection, the leaf tissues were frozen with liquid nitrogen and lyophilized.

**[0116]** DNA was isolated from potato leaf tissue using a modified cetyl trimethyl ammonium bromide (CTAB) method (Carlson et al., 1991). Dry leaf tissue was ground with a pestle and a mortar. The ground tissue was incubated with preheated extraction buffer consisting of 2% (w/v) CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 1% (w/v) polyvinylpyrrolidone (PVP), 20 mM ethylenediamine tetraacetic acid (EDTA), pH 9.5 (5 ml/1 g fresh leaf tissue) and beta-mercaptoethanol (2.5  $\mu$ l/ml buffer) at 74°C for 20 min. After centrifugation at 2440 x g for 10 min, the supernatant was extracted twice with an equal volume of chloroform/isoamyl alcohol (24:1). DNA was precipitated with 0.7 volume of isopropanol and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) with 0.5 mg/ml RNase A (Invitrogen; Carlsbad, CA 92008 USA) added to a final concentration of about 500 ng/ $\mu$ l. The isolated DNA was quantified with Hoechst 33258 dye (Invitrogen) using calf thymus DNA (Invitrogen) as the DNA standard on an FLx800™ Microplate Reader (BioTek Instruments, Winooski, VT 05404, USA) according to the fluorometer user manual.

#### Example 10: Tail PCR amplification of flanking sequences

**[0117]** Oligonucleotide primers. T-DNA specific primers which are complementary to the AHAS coding sequence and the Blb2 promoter region in VC-PMA16, respectively, were synthesized (Table 3).

Table 3 T-DNA specific primers for cloning of flanking sequences using tail PCR

Name	Sequence	Position in VC-PMA16	Comment
07-038_P25	AACGATGTCATAACGGAAGG (SEQ-ID-NO. 55)	16136	Specific primer for tail PCR (LB1)
07-038_P26	AGAGCATTTGAAGCAGATCTAGGGT (SEQ-ID-NO.56)	464	Specific primer for tail PCR (RB1)
07-038_P27	CGGATTAAATACTGAGAGCTCGAAT (SEQ-ID-NO.57)	16163	Specific primer for tail PCR (LB2)
07-038_P28	CAGATCTAGGGTTTTATCTCGG (SEQ-ID-NO.58)	454	Specific primer for tail PCR (RB2)
07-038_P29	TGCCGGTCTTGCGATGATTA (SEQ-ID-NO.59)	16241	Specific primer for tail PCR (LB3)
07-038_P30	AGATCTAGGGTTTTATCTCGGGATT (SEQ-ID-NO. 60)	450	Specific primer for tail PCR (RB3)
LB = left flanking primer, RB = right flanking primer			

**[0118]** In addition, four arbitrary degenerate (AD) primers were synthesized according to Liu et al 1995):

TG(A/T)GNAG(A/T)ANCA(G/C)AGA-3' (AD1) (SEQ-ID-NO. 61), AG(A/T)GNAG(A/T)ANCA (A/T)AGG-3' (AD2) (SEQ-ID-NO. 62), CA(A/T)CGICNGAIA(G/C)GAA-3' (AD3, I indicates inosine) (SEQ-ID-NO. 63), and TC(G/C)TICGNACIT(A/T)GGA-3' (AD4) (SEQ-ID-NO. 64). These AD primers have average T<sub>m</sub>'s of 47-48°C as calculated with the formula  $69.3 + 0.41 (\%GC) - 650/L$ , where L is primer length (cf. Fig. 2I)

**[0119]** Tail PCR was performed basically following Liu et al procedure (Liu et al 1995). Primary TAIL-PCR reactions (20  $\mu$ l) contained 1x PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 or 2.0 mM MgCl<sub>2</sub>, 0.001 % gelatin), 200  $\mu$ M each of dNTPs, 25 ng of genomic DNA, 1 unit of Taq polymerase (Invitrogen), 0.2  $\mu$ M T-DNA specific primers (07-038\_P25 and 07-038\_P26) and a given AD primer (2  $\mu$ M for AD1, 3  $\mu$ M for AD2 or 4  $\mu$ M for AD3 and AD4). Primary TAIL-PCR was executed according to the PCR program in Liu et al (1995) in Perkin-Elmer thermal cyclers 9700. Aliquots (1  $\mu$ l) from 50-fold dilutions of the primary PCR products were applied directly to secondary TAIL-PCR reactions (20  $\mu$ l) containing 1x PCR buffer, 1 unit of Taq DNA polymerase, 200  $\mu$ M each of dNTPs, 0.2  $\mu$ M T-DNA specific primers (07-038\_P27 and 07-038\_P28) and the same AD primer used in the primary reaction (1.5  $\mu$ M for AD1, 2.0  $\mu$ M for AD2 and AD3 and AD4). After amplification with 12 super cycles, the secondary TAIL-PCR products (1  $\mu$ l aliquots of 10 fold dilutions) were re-amplified in 50  $\mu$ l tertiary reactions with 20 reduced-stringency cycles. Components and their concentrations were the same as in the secondary reaction except that another nested PCR primer was used (07-038\_P29 for LB and 07-038\_P30 for RB). Amplified products from the reactions were analyzed by agarose gel electrophoresis. Strongly amplified products were recovered and purified with Zymoclean Gel DNA recovery kit (Zymo Research, CA 92614, USA). The purified DNA was quantified with Hoechst 33258 dye (Invitrogen) using calf thymus DNA (Invitrogen) as the DNA standard on an FLx800™ Microplate Reader (BioTek Instruments, Winooski, VT 05404, USA) according to

the fluorometer user manual.

#### Example 11 DNA sequencing

**[0120]** The purified tertiary PCR products were sequenced with Sanger sequencing using BigDye terminator v3.0 kit according to the manufacturer's protocol (Applied Biosystems, California 92008, USA). The same specific primer used in the tertiary PCR (unlabeled) was used for sequencing.

#### Example 12: Identification of specific events by PCR

**[0121]** Unless otherwise specified, standard methods as described in Sambrook et al., Molecular Cloning: A laboratory manual, Cold Spring Harbor 1989, Cold Spring Harbor Laboratory Press are used.

**[0122]** Genomic DNA was prepared from the particular potato events by using the DNeasy Plant Mini Kit (Quiagen) for processing of single samples and the DNeasy 96 Plant Kit (Quiagen) for processing of samples in 96 well format. Both kits were used according to the instructions described in the manual. The junction between flanking region and T-DNA insert was amplified from the cDNA by PCR as described in the protocol of the Phusion hot-start, Pfu Ultra, Pfu Turbo or Herculase DNA polymerase (Stratagene, Santa Clara, CA, US).

**[0123]** The composition for the protocol of the Pfu Ultra, Pfu Turbo or Herculase DNA polymerase was as follows: 1x PCR buffer, 0.2 mM of each dNTP, 100 ng cDNA of Arabidopsis thaliana (var Columbia-0), 50 pmol forward primer, 50 pmol reverse primer, 1 u Pfu Ultra, Pfu Turbo or Herculase DNA polymerase.

**[0124]** The amplification cycles were as follows:

1 cycle of 60 seconds at 98°C, followed by 35 cycles of in each case 10 seconds at 98°C, 30 seconds at specific annealing temperature (see table) and 60 seconds at 72°C, followed by 1 cycle of 10 minutes at 72°C, then 4°C.

**[0125]** The following primer sequences and annealing temperatures were used to specifically amplify event-specific FST-T-DNA junctions:

Table 4

Event	Flanking site	Sense primer	SEQ ID No.	Antisense primer	SEQ ID No.	Annealing temperature [°C]	Product length (bp)
A	LB	TAATTCAGTACAT TAAAGACGTCCG	65	GTCCCATAGTCA TTTCTTGATCA	66	50	63
	RB	TGTCTCTGATAG GCTAATAAACTAT G	67	TAGATCTGATTG TCGTTTCCC	68	48	91
B	LB	ATGACGTTATTTA TGAGATGGGT	69	ATTTAAAAGGCA AAACGTGC	70	49	100
	RB	TTCATGTCAAGTT CAATTTCAGG	71	ACTCACATTAAT TGC GTTGCG	72	51	94
C	LB	GCTTGGAATAAT TGTCATTAGATTG	73	GCCTTGACCTTT GAATTATTTAC	74	49	118
	RB	TCTGATGCAGAA TTTTCTAACTCAA	75	TTCCTACTAGAT CTGATTGTCGTT TC	76	52	317
D	LB	TCAAACGGATGT TAATTCAGTACAT T	4	CCAGTTCCCAAT TGACTACTAGAA A	5	52	131

(continued)

Event	Flanking site	Sense primer	SEQ ID No.	Antisense primer	SEQ ID No.	Annealing temperature [°C]	Product length (bp)
	RB	CCAAGATAGTGT TTCAGGAAAGTTA TT	12	AAATTCATGGTA GAACTGGAGGA G	13	52	287

**[0126]** The resulting PCR products were analyzed on a 1.5% Agarose-gel. PCR products occur specifically to identify the event. Detailed conditions are given in Table 4.

### Example 13 Determination of yield by field trials

**[0127]** The various potato events were tested in the field to determine their yield potential. The yield of the events A, B, C and the elite event D, all showing full Phytophthora resistance, was compared to the non transgenic mother line Fontane and other standard potato varieties, like e.g. Bintje under disease free conditions (full plant protection scheme of transgenic events and control varieties according to good agricultural practice).

**[0128]** Yield trials were performed on more than 15 locations across 3 years (2008-2010). For every experiment a randomized block design with 3-5 block-repetitions was used. Each block was about 10-15 m<sup>2</sup> in size and planted with 4-6 potato plants per m<sup>2</sup>.

**[0129]** Potatoes were planted in April or May according to local conditions. All plant cultivation management, including plant protection, was performed based on good agricultural practice (GAP). Potato tubers were harvested two weeks after haulm killing in September/October. Harvest was performed either by hand or mechanically. The tuber yield/ha was determined by weighing of the freshly harvested potato tubers at the place of harvest. As control the potato variety Bintje was used. The yield/ha of the standard potato variety Fontane was set to 100% and the relative yield of the non-transgenic line Bintje and the transformed Fontane events A to D was calculated.

**[0130]** Only the elite event D showed the same yield/ha compared to the non-transgenic mother line, whereas the other events (e.g. A, B, C) showed a ~10% yield decrease (cf. Figure 4).

### Example 14 Determination of Phytophthora resistance by field trials

**[0131]** The various potato events were tested in the field to determine their resistance phenotype against Phytophthora infestans. All events and the nontransgenic controls (Fontane, as non-transgenic mother line and Bintje as susceptible standard variety) were grown in the field without any fungicide treatments targeting Phytophthora infestans. Resistance trials on more than 20 locations across 5 years (2006-2010) were performed. For every experiment a randomized block design with 3-5 block-repetitions was used. Each block was about 1 -15 m<sup>2</sup> in size and planted with 4-6 potato plants per m<sup>2</sup>.

**[0132]** Potatoes were planted in April or May according to local conditions. All plant cultivation management, excluding plant protection, was performed based on good agricultural practice (GAP). Phytophthora infection occurs naturally.

**[0133]** The rating of disease symptoms was done by trained personnel evaluating the diseased leaf area, necrotic lesions, chlorotic lesions and potential sporulation of *P. infestans*.

**[0134]** These values were integrated into a disease rating ranging from 0 to 100%. In the scoring system 0% disease means no macroscopically visible symptoms, whereas 100% means that all inoculated leaves are completely brownish and covered with mycelia, so the plant is essentially dead. The mother variety Fontane generally showed a strong infection of all leaves. All leaves are heavily infected and green tissue is rare. In contrast the inoculation of all events A to D led to completely healthy plants with a disease rating of 0%. As susceptible control the standard variety Bintje was used, which is known to be fully susceptible to Phytophthora infection and which showed strong infection (Figure 5).

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## SEQUENCE LISTING

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<130> PF71652

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15 <210> 76  
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<212> DNA  
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20 <220>  
<223> Primer

25 <400> 76  
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## Claims

- 30 1. Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue thereof comprising a nucleotide sequence having at least 80 % identity with SEQ-ID-No. 1.
2. Phytophthora-resistant transgenic potato plant, seed, tuber, plant cell or tissue thereof comprising
- 35 a) a recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and  
b) further comprising a junction sequence selected from the group consisting of
- 40 i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 126 and 136 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
- ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 505 and 515 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
- 45 iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 625 and 635 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9,  
and/or
- 50 iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 4752 and 4762 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,  
and/or
- further comprising a junction sequence selected from the group consisting of
- 55 v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 282 and 292 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 877 and 887 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,

- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 827 and 837 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or
- viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 9905 and 9915 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

3. Phytophthora-resistant transgenic potato, seed, tuber, plant cell or tissue according to claim 1 comprising

- a) a recombinant construct having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and further comprising a junction sequence selected from the group consisting of
- b)

- i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 131 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
- ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment 510 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
- iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 630 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or
- iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 4757 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11

and/or

further comprising a junction sequence selected from the group consisting of

- v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 287 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 882 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 832 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or
- viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9910 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

4. Method for providing a Phytophthora-resistant transgenic potato plant comprising the following steps:

- a) introducing a recombinant nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 into the genome of potato plant cells,
- b) integrating said recombinant nucleic acid into the genome,
- c) regenerating plant from said plant cells,
- d) selecting plant comprising a nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and a junction sequence selected from the group consisting of

- i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 126 and 136 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
- ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 505 and 515 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
- iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 625 and 635

basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9,

and/or

iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 4752 and 4762 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,

and/or

further comprising a junction sequence selected from the group consisting of

v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 282 and 292 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,

vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 877 and 887 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,

vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 827 and 837 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17,

and/or

viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of between 9905 and 9915 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

5. Method for providing a Phytophthora-resistant transgenic potato plant according to claim 4, wherein in step d) a plant is selected comprising a nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and a junction sequence selected from the group consisting of

i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 131 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,

ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment 510 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,

iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 630 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or

iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 4757 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11,

and/or further comprising a junction sequence selected from the group consisting of

v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 287 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,

vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 882 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,

vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 832 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17,

and/or

viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9910 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19.

6. Kit comprising the primer pairs for the detection of the specific integration place, selected from the group consisting of

SEQ-ID-No. 4 and 5,

SEQ-ID-No. 6 and 7,

SEQ-ID-No. 8 and 9,



SEQ-1 D-No. 10 and 11,  
SEQ-ID-No. 12 and 13,  
SEQ-ID-No. 14 and 15,  
SEQ-ID-No. 16 and 17, and/or  
SEQ-ID-No. 18 and 19.

7. Detection method for the detection of the specific integration place comprising

a) isolating a nucleic acid sequence from a potato plant, seed, tuber, plant cell or tissue thereof as a test sample,  
b) exposing said test sample, a positive and a negative sample with nucleotide sequence selected from at least one set of primer pairs defined in claim 6 under PCR-conditions, and

- i) evaluating the amplification of a nucleotide fragment selected from the group consisting of a nucleotide fragment of between 126 and 136 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
- ii) of between 505 and 515 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
- iii) of 625 and 635 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9,

and/or

- iv) of 4752 and 4762 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11

and/or

selected from the group consisting of a nucleotide fragment

- v) of between 282 and 292 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- vi) of 877 and 292 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- vii) of 827 and 837 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequence of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or
- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9905 and 9915 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19

compared with said positive and negative control.

8. Detection method according to claim 7 evaluating the amplification of a nucleotide fragment selected from the group consisting of a nucleotide fragment

- i) of 131 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,
- ii) of 510 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,
- iii) of 630 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or
- iv) of 4757 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11 and/or evaluating the amplification of a nucleotide fragment selected from the group consisting of a nucleotide fragment selected from the group consisting of a nucleotide fragment
- iv) of 287 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,
- v) of 882 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,
- vi) of 832 basepairs when using a polymerase chain reaction with two primers having the nucleotide sequences

of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or

vii) a nucleic acid sequences that can be used to amplify a nucleotide fragment of 9910 basepairs, using a polymerase chain reaction with two primers having the nucleotide sequence of SEQ-ID-No. 18 and SEQ-ID-No. 19

compared with said positive and negative control.

9. Plant, seed, tuber, plant cell or tissue thereof detectable by the kit of claim 6 or by the detection method of claims 7 or 8.

10. Polynucleotide comprising

- a) a recombinant nucleic acid having at least 80 % identity with SEQ-ID-No. 2 or SEQ-ID-No. 3 and
- b) further comprising a junction sequence selected from the group consisting of

- i) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 131 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 4 and SEQ-ID-No. 5,

- ii) a nucleic acid sequence that can be used to amplify a nucleotide fragment 510 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 6 and SEQ-ID-No. 7,

- iii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 630 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 8 and SEQ-ID-No. 9, and/or

- iv) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 4757 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 10 and SEQ-ID-No. 11

and/or

- v) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 287 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 12 and SEQ-ID-No. 13,

- vi) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 882 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 14 and SEQ-ID-No. 15,

- vii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 832 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 16 and SEQ-ID-No. 17, and/or

- viii) a nucleic acid sequence that can be used to amplify a nucleotide fragment of 9910 basepairs using a polymerase chain reaction with two primers having the nucleotide sequences of SEQ-ID-No. 18 and SEQ-ID-No. 19 stably integrated into a potato plant cell nucleus.

11. Polynucleotide comprising a nucleotide sequence having at least 80 % identity SEQ-No. 1 stably integrated into a potato plant cell nucleus.

Figure 1 of 5

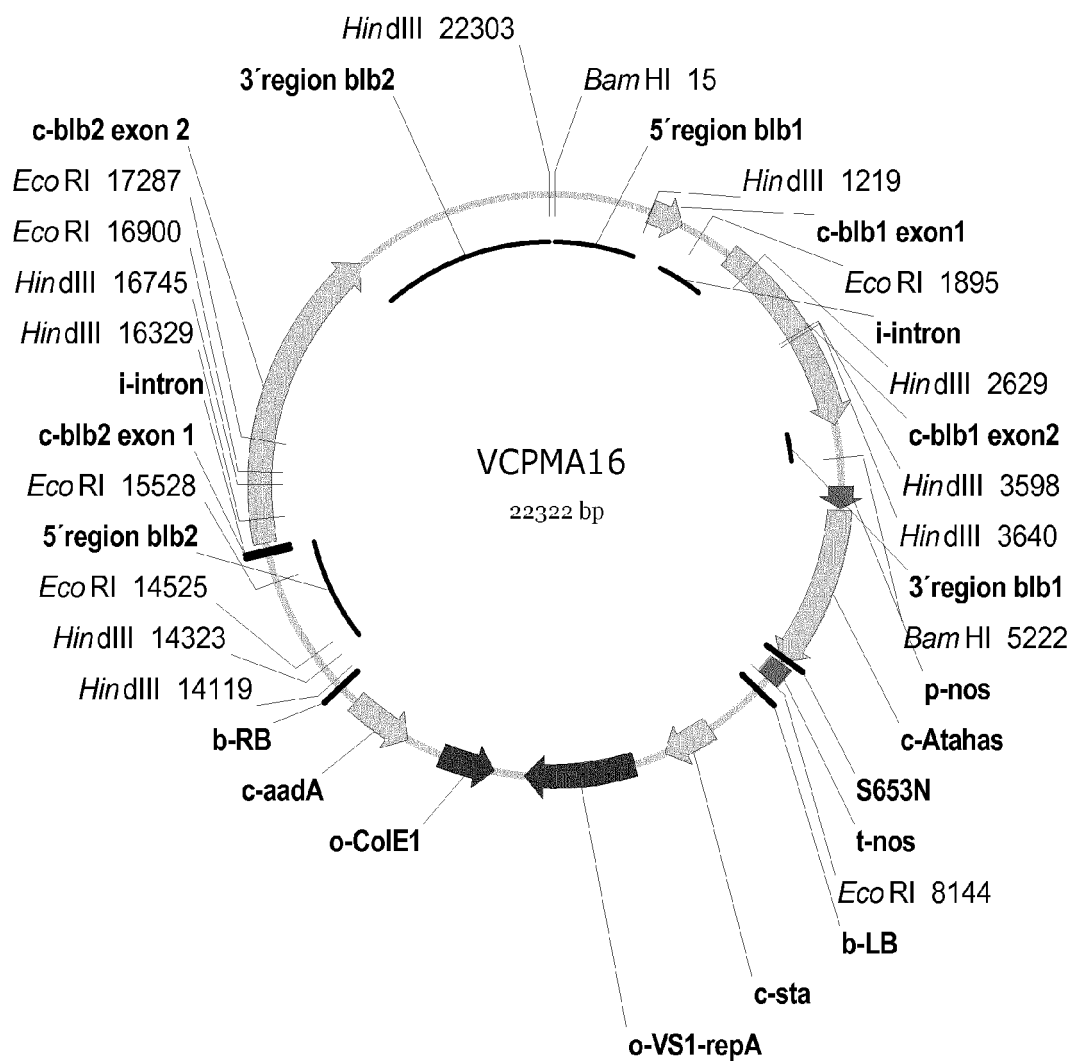


Figure 2 of 5: Sequences

Figure 2a of 5

Insert with flanking regions - SEQ-ID-No. 1

Lower case bold – flanking region at right border

Upper case *italic-underlined* – Blb2 expression cassette (promoter – gene –terminator)

Upper case bold – Blb1 expression cassette (promoter – gene –terminator)

Upper case bold *italic* – AHAS expression cassette (promoter – gene –terminator)Lower case *italic* – flanking region at left border

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Figure 2a - continued

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Figure 2a - continued

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 CAGTCTCTAATCTCGAATTTGAAAAAATTGTTGTTGTATGACTTTCTCTGACATCCGATGCAC  
 TATCAACAATAGCAAGACTGGAGGTTGGAGAGGAATCCTTTATTATACAATCATTTCAGGGAGAA  
 GAATGGAACATGGGGGAGGAAGACACTTTTGAGAATCTGAAATGTGTTAGAGCCACAAGCTACA  
 GAAGTATTGAATTTGTCATGAATATCAACATTCTTCATCCTAGTTAATTCTTTTTCAATTTTAT  
 ATAGACTCTCATTTTAACTACTAATATTCTTCTATTTGTGACTTCTTTTCTGCAGGTGGCAACT  
 TTAAATTCATAAAGTATAGGATTGATGACAACTCGAAAAATATCTTAATGAGGTGAAGTTTGA  
 GCAGTCAGCAGATGGTGGTTCCAACCTCTAAGTTGACAAGCACATACTATCCCGGAGGGCGATT  
 CAAGCCTGATGCATATGGTTAGTGTGGCTAGAGCAGACAGGATGTATTACCTGGATATCTACCA  
 AGACGAATCCACAATCAGTTTTATGTCAAGCAATACATGAAGTAACTCCCGATAGAACAGTAAA  
 AGCAAGATGTGTAGGTGTATCTCGACTCTAAGAGATTGTACATTCCTCTTTGAGATTTTTACTG  
 CTAATACAAATTTACACCTCAGAAGCGAATCTAGAATTTCTAGAGCATGAATGCACCACTAATG  
 AAAGGAGAAAAAAGGAAGTATGAAGTGGGAATTTGATCCTTGTTTCTAGGTATATAAAATTTAT  
 CATTCAACTATACTTCATTTAGCAAACAACCTCTCTTTGCCATTATTTCTCAAACAAGGGCTTCT  
 AATATTGCTAAACTAAAGACTGTCAAAAGGTAAGTTTCATCTTCAAACCTCTCTTGTTTACTTTAT  
 CTAAAGGGGAACTATGAAAAACAAGAAACATCAGGAATGTCCCGTAAACAAAGCAGCCTCATGC  
 ACAAACATCCAACGTTGGTAGGATTAATGGAGGGATCGCATCCAGGAGGATACTGTAGAAAA  
 ATTAGTGGCTTCTTTCACCGCTCAAACCCATGATCTATAGGTTACATGGAGACAACCTTTATGGT  
 TGCTCGTAGGCTCCCGTCAATTCTCATAAACCACAACACCAAAGTTGCATCAGACATCATCTTC  
 ATTCACAAGCTGACAATCTCCACAAGTCTTAGTCAACTTGTAATATGAATATTAGCCAGGTAGA  
 CGTACATATTTACAAAATTGAGTTTCCCTATATAATATGGTTTGAAGGAATGAAACATGATGGGG  
 AGGGTAGATAAAATAATATATGAGGCATAAAATAGGAAAGATATTTGTAGTGAGAGGTTTTGA  
 CTTTTTATGCTGCTTTTGATCTTCAGTTTCTTGATTCTTTTTCTACTGCTTTCCTCTTCTTC

Figure 2a - continued

TCCTGAGTAAAGTTTTATGTAGGTACTTTTTATACGTCCGATCGTGAGAAGTTGAAAGAAAGCT  
 CTCTATAGCTATGTTAGGTGCCACATAAAAAAATGAAATATTACAAAAACCTGATAATAAAA  
 TACACTAATCTAAGATATTCACCTGCAACATACATGCAAAATATATATATATAAAATTTTCATGAA  
 AATTATAACAAATAATAGATGTGAACATATAACTTTAAAAATAATATTACATCCATAAAGCTTA  
 AATTCTAGATCCCCGGTCGACTCTAGAGGATCCCCACTCCATCCGTTCACTTTTGATTTGTCATG  
 TTGCACTTTTTCGAAAGTCAATTTGACTAATTTTTTAAAGCTAAATTAGATTACACTAATTCATA  
 TTTTAAACAGAAAAATTAGATATTCAAAAACATACAAAAAATATTATACATTGCAATTTTTTTG  
 CATATCAATATGATAAAAAAATATATCGTAAAATATTAGTCAAATTTTTTATAATTTGACTCAA  
 ATCATGAAAAGTATAATAATTAATAGTGGACGGAGGAAGTATTGTCTTTCCAGATTTGTGGCCA  
 TTTTTGGTCCAAGGGCCATTAGCAGTTCTCTTCATTTTCTACTTCTGTCTCATATTAGATTGGGC  
 ATCTTACTAAAAATATTGTCTCATATTACTTGATTATTTATTAATCAAAAAGAATTAATTAA  
 TTTTTCTCATTTTACCCCTACAATTAATATAGTTTTTAAAGTTTTTAAACAAATTTTGAAGAAT  
 CAAAATTTCTTTTTGCAAGAGACTTATTAATATAAACAAAGGATAAAATAATAAAATTTGTCAA  
 TTTATTGACGATCACTTAATAATCATATAAAATAGAAAATGTTTATCTAATATGAGACGGAGAA  
 AATATATCCTAAAAATTTTTTGGACAGATATGTGATATTCTAACCATTCACTAGACTATATTAT  
 GCATTTTAGCCGCCAATGACTTATTTAGCTTTAATTAATTAGGAAAGAGGAAACTGCCAATGA  
 GGAAGAGTAGGGGCGTAGTTGCTGTGCGACGAAAAAAGATAATACTCACTCTTTTCGATTTTTTA  
 TTTTTATTATCACTTTTAACCTATCATGTAAAAAGATAATTATTTTTTTCATGCTTTATCCTT  
 AGTATTAAACAATTTAATAGGGATTATTTTGTAAAATATTATATGAATAATTGTTTTCGTAAT  
 GAATTTGTCCGGTCAAACAATGATAAATAAAAATGAATGAAGAGAGTAGAAAACAAAACAAAAG  
 AACAAAGTTGACAACCTTGAGAGATTAAGAGGGTCCAAAACGCCTTGGATTTTGAGATTCCATATG  
 TGAAATTTCCATGAAATAATTGAATTTGTATTATTACAAGTCAAACCTTTCCATTTTCATTCCAAC  
 TAGCCATCTTGGTTTCAAATTACACATTCATTCAATTCACAGATCTAATATCTTAATAGTGAT  
 TTCCACATATGGCTGAAGCTTTCAATCAAGTTCTGCTAGACAATCTCACTTCTTTCCTCAAAGG  
 GGAAGTTGTATTGCTTTTCGGTTTTCAAGATGAGTTCCAAAGGCTTTCAAGCATGTTTTCTACA  
 ATTCAAGCCGTCCTTGAAGATGCTCAGGAGAAGCAACTCAACAACAAGCCTCTAGAAAATTGGT  
 TGCAAAAACCTCAATGCTGCTACATATGAAGTCGATGACATCTTGGATGAATATAAAACCAAGGC  
 CACAAGATTCTCCAGTCTGAATATGGCCGTTATCATCCAAAGGTTATCCCTTTCCGTCACAAG  
 GTCGGGAAAAGGATGGACCAAGTGATGAAAAAATAAAGGCAATTGCTGAGGAAAGAAAGAATT  
 TTCATTTGCACGAAAAAATTGTAGAGAGACAAGCTGTTAGACGGGAAACAGGTACTCATCTTAA  
 ATTAGTATTACAACAACCTAAGTTTATATTCAATTTTTTTGGCAATTATCAAATTCAGAAAAGGGT  
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 TCTTTAGAAATCCACCTGTCTAACTCATCCACTACCCATTCCCTTTGCTTTGAATTTCTTTCTT  
 TACCTATAAACTTTGGAACACTCGATCCGTTTTGCTTTTCTTAACAAAGCAGCTCAGAGAAAAGA  
 GGTTTTCTTCTATTCTGTTTCTCTGTGTGCTGCACTTGGGTCTTAATCCCATTAATAACAGGG  
 CATGTTAATCCCAACGACGGTAGCCTTTCTTGACAGCTGACTGTAAATTTTGTCTAACAAAGAA  
 AAAAAAAGATTAGACATGTTTTTCTTGTCTATTGATTAGGCTGGATTTCTTTCAGAGTGGAACA  
 TAGGGGATATATTGGACCAAAGTAGAATGGGTATATATTAAAGTATTTCTGATAGAACAGGA  
 GTATATTGTGCGAAAATATCCTCTATTTTCTGTTGTCTCCTAATGAGTTTGAATGTAATAATAT  
 TCTCATGTGGACATTGCTTGCACCAGGTTCTGTATTAACCGAACCAGGTTTATGGAAGAGAC  
 AAAGAGAAAGATGAGATAGTAAAATCCTAATAAACAATGTTAGTGATGCCCAACACCTTTTCAG  
 TCCTCCCAATACTTGGTATGGGGGGATTAGGAAAAACGACTCTTGCCCAAATGGTCTTCAATGA  
 CCAGAGAGTTACTGAGCATTTCCATTCCAAAATATGGATTGTGTCTCGGAAGATTTTGATGAG  
 AAGAGGTTAATAAAGGCAATTGTAGAATCTATTGAAGGAAGGCCACTACTTGGTGAGATGGACT  
 TGGCTCCACTTCAAAGAAGCTTCAGGAGTTGCTGAATGGAAAAAGATACTTGCTTGTCTTAGA  
 TGATGTTTGAATGAAGATCAACAGAAGTGGGCTAATTTAAGAGCAGTCTTGAAGGTTGGAGCA  
 AGTGGTGCTTCTGTTCTAACCCTACTCGTCTTGAAAAGGTTGGATCAATTATGGGAACATTGC  
 AACCATATGAAGTGTCAAATCTGTCTCAAGAAGATTGTTGGTTGTTGTTTCATGCAACGTGCATT

Figure 2a - continued

TGGACACCAAGAAGAAATAAATCCAAACCTTGTGGCAATCGGAAAGGAGATTGTGAAAAAAGT  
 GGTGGTGTGCCTCTAGCAGCCAAAACCTTGGAGGTATTTTGTGCTTCAAGAGAGAAGAAAGAG  
 CATGGGAACATGTGAGAGACAGTCCGATTTGGAATTTGCCTCAAGATGAAAGTTCTATTCTGCC  
 TGCCCTGAGGCTTAGTTACCATCAACTTCCACTTGATTTGAAACAATGCTTTGCGTATTGTGCG  
 GTGTTCCCAAAGGATGCCAAAATGGAAAAAGAAAAGCTAATCTCTCTCTGGATGGCGCATGGTT  
 TTCTTTTATCAAAAGGAAACATGGAGCTAGAGGATGTGGCGATGAAGTATGGAAAGAATTATA  
 CTTGAGGTCTTTTTTCCAAGAGATTGAAGTTAAAGATGGTAAACTTATTTCAAGATGCATGAT  
 CTCATCCATGATTTGGCAACATCTCTGTTTTTCAGCAAACACATCAAGCAGCAATATCCGTGAAA  
 TAAATAAACACAGTTACACACATATGATGTCCATTGGTTTCGCCGAAGTGGTGTTTTTTTACAC  
 TCTTCCCCCTTGGAAAAGTTTATCTCGTTAAGAGTGCCTAATCTAGGTGATTCGACATTTAAT  
 AAGTTACCATCTTCCATTGGAGATCTAGTACATTTAAGATACTTGAACCTGTATGGCAGTGGCA  
 TGCGTAGTCTTCCAAAGCAGTTATGCAAGCTTCAAATCTGCAAACCTTGTATCTACAATATTG  
 CACCAAGCTTTGTTGTTTGCCAAAAGAAACAAGTAAACTTGGTAGTCTCCGAAATCTTTTACTT  
 GATGGTAGCCAGTCATTGACTTGTATGCCACCAAGGATAGGATCATTGACATGCCTTAAGACTC  
 TAGGTCAATTTGTTGTTGGAAGGAAGAAAGGTTATCAACTTGGTGAAGTAGGAAACCTAAATCT  
 CTATGGCTCAATTAATAATCTCGCATCTTGAGAGAGTGAAGAATGATAAGGACGCAAAAGAAGCC  
 AATTTATCTGCAAAAGGGAATCTGCATTCTTTAAGCATGAGTTGGAATAACTTTGGACCACATA  
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 AAAAATCTATGGCTTCAGAGGAATCCATCTCCAGAGTGGATGAATCACTCAGTATTGAAAAAT  
 ATTGTCTCTATTCTAATTAGCAACTTCAGAACTGCTCATGCTTACCACCCTTTGGTGATCTGC  
 CTTGTCTAGAAAGTCTAGAGTTACACTGGGGGTCTGCGGATGTGGAGTATGTTGAAGAAGTGA  
 TATTGATGTTTCTTGGATTCCCCACAAGAATAAGGTTCCATCCTTGAGGAACTTGATATA  
 TGGGACTTTGGTAGTCTGAAAGGATTGCTGAAAAAGGAAGGAGAAGAGCAATTCCTGTGCTTG  
 AAGAGATGATAATTCACGAGTGCCCTTTCTGACCCTTTCTTCTAATCTTAGGGCTCTTACTTC  
 CCTCAGAATTTGCTATAATAAAGTAGCTACTTCATTCCCAGAAGAGATGTTCAAAAACCTTGCA  
 AATCTCAAATACTTGACAATCTCTCGGTGCAATAATCTCAAAGAGCTGCCTACCAGCTTGGCTA  
 GTCTGAATGCTTTGAAAAGTCTAAAAATCAATTGTGTTGCGCACTAGAGAGTCTCCCTGAGGA  
 AGGGCTGGAAGGTTTATCTTCACTCACAGAGTTATTTGTTGAACACTGTAACATGCTAAAATGT  
 TTACCAGAGGGATTGCAGCACCTAACAACCCTCACAAGTTTAAAAATTCGGGGATGTCCACAAC  
 TGATCAAGCGGTGTGAGAAGGGAATAGGAGAAGACTGGCACAAAATTTCTCACATTCCTAATGT  
 GAATATATATATTTAAGTTATTTGCTATTGTTTCTTTGTTTGTGAGTCTTTTTTGGTTCCTGCCA  
 TTGTGATTGCATGTAATTTTTTTCTAGGGTTGTTTCTTTATGAGTCTCTCTCTCATTGGATGTA  
 ATTTTCTTTTGGAAACAAATCTGTCAATTGATTTGTATTATACGCTTTTCAGAATCTATTACTTA  
 TTTGTAATTGTTTCTTTGTTTGTAAATTGTGAGTATCTTATTTTATGGAATTTTCTGATTTTAT  
 TTTGAAAACAAATCAATGATTTGTAAGATCCATCTGTATTATACTCCCTTCGTCTCATTTTATG  
 TGTACCTGTGCGATTTGAGATTCAAACAAATCTATCTTTGATCGTAAATTTTTTAATAGATCT  
 TTTAAACATTTTGAATTATCAATTATTGTGACTTTAGTGGCTAGACTAGTGGATCCGATATCGC  
 CCAGCTTCACGCTGCCGCAAGCACTCAGGGCGCAAGGGCTGCTAAAGGAAGCGGAACACGTAGA  
 AAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGGCTATCTGGACAAG  
 GGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATAGCTAGAC  
 TGGGCGGTTTTATGGACAGCAAGCGAACCAGGAAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTG  
 GGAAGCCCTGCAAAGTAACTGGATGGCTTTCTTGCCGCAAGGATCTGATGGCGCAGGGGATC  
 AAGATCATGAGCGGAGAATTAAGGGAGTCACGTTATGACCCCGCCGATGACGCGGGACAAGCC  
 GTTTTACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGA  
 GTTTAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAGTCGCCTAAGGTCA  
 CTATCAGCTAGCAAAATTTTCTGTCAAAAATGCTCCACTGACGTTCCATAAATTCCTCTCGGT  
 ATCCAATTAGAGTCTCATATTCCTCTCAATCCAGATCCCCGGGTACCATGGCGGCGGCAACAA  
 CAACAACAACATCTTCTTCGATCTCCTTCTCCACCAACCATCTCCTTCTCTCCTCAAATC  
 ACCATTACCAATCTCCAGATTCTCCCTCCCATCTCCCTAAACCCCAACAAATCATCCTCCTCC



Figure 2a - continued

TCCCGCCGCGCGGTATCAAATCCAGCTCTCCCTCCTCCATCTCCGCCGTGCTCAACACAACCA  
 CCAATGTCAACCACTCCCTCTCCAACCAACCTACCAAACCCGAAACATTTCATCTCCCGATT  
 CGCTCCAGATCAACCCCGCAAAGGCGCTGATATTCTCGTCGAGGCTTTAGAACGTCAAGGCGTA  
 GAAACCGTATTTCGCTTACCCTGGAGGTGCATCAATGGAGATTACCAAGCCTTAACCCGCTCTT  
 CCTCAATCCGTAACGTCTTCTCGTCACGAACAAGGAGGTGTATTTCGACGAGAAGGATACGC  
 TCGATCCTCAGGTAAACCAGGTATCTGTATAGCCACTTCAGGTCCCGGAGCTACAAATCTCGTT  
 AGCGGATTAGCCGATGCGTTGTTAGATAGTGTTCCTCTTGTAGCAATCACAGGACAAGTCCCTC  
 GTCGTATGATTGGTACAGATGCGTTTCAAGAGACTCCGATTGTTGAGGTAACGCGTTTCGATTAC  
 GAAGCATAACTATCTTGTGATGGATGTTGAAGATATTCTAGGATTATTGAGGAGGCTTTCTTT  
 TTAGCTACTTCTGGTAGACCTGGACCTGTTTTGGTTGATGTTCTTAAAGATATTCAACAACAGC  
 TTGCGATTCTTAATTGGGAACAGGCTATGAGATTACCTGTTTATATGTCTAGGATGCCTAAACC  
 TCCGGAAGATTCTCATTTGGAGCAGATTGTTAGGTTGATTTCTGAGTCTAAGAAGCCTGTGTTG  
 TATGTTGGTGGTGGTTGTTTGAACCTTAGCGATGAATTGGGTAGGTTTGTGAGCTTACGGGAA  
 TCCCTGTTGCGAGTACGTTGATGGGGCTGGGATCTTATCCTTGTGATGATGAGTTGTCTGTTACA  
 TATGCTTGGAATGCATGGGACTGTGTATGCAAATTACGCTGTGGAGCATAGTGATTTGTTGTTG  
 GCGTTTGGGGTAAGGTTTGATGATCGTGTACGGGTAAACTTGAGGCTTTTGCTAGTAGGGCTA  
 AGATTGTTTCATATTGATATTGACTCGGCTGAGATTGGGAAGAATAAGACTCCTCATGTGTCTGT  
 GTGTGGTGTGTTAAGCTGGCTTTGCAAGGGATGAATAAGGTTCTTGAGAACCAGCGGAGGAG  
 CTAAACTTGATTTTGGAGTTTGGAGGAATGAGTTGAACGTACAGAAACAGAAGTTTCCGTTGA  
 GCTTTAAGACGTTTGGGGAAGCTATTCTCCACAGTATGCGATTAAGGTCCTTGATGAGTTGAC  
 TGATGGAAAAGCCATAATAAGTACTGGTGTGCGGCAACATCAAATGTGGGCGGCGCAGTTCTAC  
 AATTACAAGAAACCAAGGCAGTGGCTATCATCAGGAGGCCTTGAGGCTATGGGATTTGGACTTC  
 CTGCTGCGATTGGAGCGTCTGTTGCTAACCCCTGATGCGATAGTTGTGGATATTGACGGAGATGG  
 AAGTTTTATAATGAATGTGCAAGAGCTAGCCACTATTCTGTGTAGAGAATCTTCCAGTGAAGGTA  
 CTTTTATTAAACAACCAGCATCTTGGCATGGTTATGCAATGGGAAGATCGGTTCTACAAAGCTA  
 ACCGAGCACACATTTCTCGGAGATCCGGCTCAGGAGGACGAGATATTCCCGAACATGTTGCT  
 GTTTGCAGCAGCTTGCGGGATTCCAGCGGCGAGGGTGACAAAGAAAGCAGATCTCCGAGAAGCT  
 ATTCAGACAATGCTGGATACACCAGGACCTTACCTGTTGGATGTGATTTGTCCGCACCAAGAAC  
 ATGTGTTGCCGATGATCCCGAATGGTGGCACTTTCAACGATGTCATAACGGAAGGAGATGGCCG  
 GATTAAATACTGAGAGCTCGAATTTCCCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAG  
 ATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCAT  
 GTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGC  
 AATTATACATTTAATACGCGATAGAAAACAAATATAGCGCGCAAACCTAGGATAAATTATCGCG  
 CGCGGTGTCTATCTATGTTACTAGATCGGGAATTCAGTGGCCGTGCTTTTACAACGACTCAGCTG  
 CTTGGTAATAATTGTCATTAGATTGTTTTTATGCATAGATGCACTCGAAATCAGCCAATTTTAG  
 ACAAGTATCAAACGGATGTTAATTCAGTACATTAAAGACGTCCGCAATGTGTTATTAAAGTTGTC  
 TAAGTtgtccttggtttttattagttggttattggttgttttgccttttcaatttctagtagtcaat  
 tgggaactggaagtacaaagtgaatttttagacttatgtatacaaatgaaaatgtatcaaaa  
 agtagtgacacttataaaaaagaacaattctttcttctgagaacatcttcaaccacctctatatt  
 tactctccattctctatatttagtgagtaaaatagagaatgggcactccaaccacctccatat  
 cactctccattcttcatatttagagagtcataattttttattattttttattactttctaa  
 ttaatatattttttacatatataatgtcattaattaaatatctaataattcataattcttttttaa  
 tgtcatattataatttttaaaaaatatatttttaatatataactactttcatccgaattaatagt  
 attttaatttttttctaatttttcgacaataataatattgatttttattattaattttcactataa  
 ataatacacaaaaattaaaatgataagatgaaaaaatttaaaaaatacaatacataatatga  
 taattttaataacaggataacaatacaatacatgacataataatttaatacaagataaaaataca  
 atacataacataataatcaattcgtgatgaacaagaatcatgcaaaaaagatattgaacgtgc  
 attcgaagtttttgcaatcacgtttttgcaattattgcaagaccgtcacgttttttgagaaaaggaa  
 gtgtgacatgatataatgactacatgtattataactgcacacccatgataattgaggatgaacatg

Figure 2a - continued

atcttaatgcaccaaatacaagatgccgtagaggctccaactccaacgacaaaaatgatggtaga  
tgaaaatcttcgggttgaacaatTTTTtagctagacataaaaaagttaaggacaaaaatgctcat  
tttgaactccgtaatgcattaatagagcatttatgacagcaacgtgataatttcgaaacttgag  
tgtttatgtaattatatttcactttttatttgaatttgtccattaatttgtatattatataatat  
ttatttacaatttatgttattttaaaaaatttagaataaaaaataaaattttgaaaaataaaatttt  
atatcacatgaaaattatataaaagtaattattggggaaaaaaaataaaaggatttatattatgaaa  
taagaaaaataagaatgaatagaaaataataataatataatattgaggagaaaataattatTTTTT  
tagagagtgaatatagagaattgggttgaaattgattgtctgaaaaataaaaaactctatattt  
ggagagtaaaaatagagtgtagggttgagacgtca

Figure 2b of 5

Insert without flanking region - SEQ-ID-No. 2

Upper case ***italic-underlined*** – Blb2 expression cassette (promoter – gene –terminator)Upper case **bold** – Blb1 expression cassette (promoter – gene –terminator)Upper case ***bold italic*** – AHAS expression cassette (promoter – gene –terminator)

CAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTGGCGCGCCAAGCTTGCAATGC  
 CTGCAGGTCGACTCTAGAGGATCTAGAATCACCGAACCTCCCCTCGGTACAGCTCCTCCAGTTC  
TACCATGAATTTCATCCACTGATTCTCTTCAATCGCCATTGCAGATTCTCTCGATCTATGCTC  
AAAAAATCCCGAGATAAAACCCTAGATCTGCTTCAAATGCTCTGATACCATGTAATTTCAGTGA  
ATTCTAACTAAACAATGGAGAGAATTAACTATTTTAGAAAAGACTGATTGAAGGAGAAGAAGAGA  
GAAAAATTCTATATTGAACTCATGAACCAAATGAATGAAAAAATAATGAGAAGAACTATACT  
ATTACAATCTATATATCTCTATTTATATTCTAATCTGAAGCAGTTAATTTAACTGACTCTAACA  
ACTAGACTGATAGGTGTACATTTTCTGTTAGTGCAGTGCAGTGCATTTAACTAACTGCTTAAACA  
TAAAGAATGTTGTTGCGAACTTCATTGCAATAGCTTCAATGAGAAGCAAACATGTGTACCTGTAA  
AGACACACAGTAAAAAGTGTAAATAATGAATAAATATGAATAAATCAAATAATAAATTAAAAAATA  
AAAACACATCCAATTAACATTGGAGGTCTTGAAAATCGATGGTAATTAACAAAGACCCCTGTGA  
AATTTAAGTCTGTAATTGAAAATTTGAGTATAGGTTAGGGGACATTTGACTATTTTCTCATTTT  
CTTTATCTTTTTCTTAATTTGTGGCAGACAAGTGAGGAGGCCCTGTAATTGATTCATGCTT  
TTGCTTTCTTGACTTTTTGGAACAATACTATGCATCATATTTGGTCTTAATTATTCCTCTGTTT  
ATTTCCAGAATTTGAGCTCTATACATCTAATAACAAAGCAAGCAGAGGATATATAGTTTCATC  
AACTAAAAGGTTAGTCAACTCATCTAATATTTGCTACTCTCATCTCTATTGAAGTACAGTTAT  
GGAAAAGTAGAAGTGATGTAAGAAAAATGAAAGAACTTTAGTAGGTTAGTTGGATCTAACAAAG  
AGAAAGGGAAATAAATTCAGGAGAAAAGAGAGAGGTTAAATACTTACTCACACCACCGATTTAC  
AACAAATCACTTAATTGTGGTTAGTTAATGTATACTTTCACCTCATTAAATTATTACTTACCCA  
TGATAAGTTGTATTAATTTGGTATTAATATCCGGTGCGGGTGAATTCTTACCGGGTGAGAGGGA  
TGGGGTTGGAGAGTGTGGAGTGAACAGAAGCAGATGTTTTAGATTTTTTCTAAGATGACGAAAG  
ATTCCTCCTCACTAATGAAAATATATTACTATACGCTATTAGAGATAGAAAGGTTCCGGTACCAGT  
TGGTCTCGTTTCTGGATGAACCCCATTTTTACAAGTCATTTTCTTCAATTCAAATCGCAAGTGT  
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TATTCTTATCATTTTCATCTTCTTTCTCCTGATAAAGTTTTATGTACTTTTTATGCATCAGGTCT  
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TCATTGGTATGTTATTTGATAGAGTGAAGTGTAAAGTATTGAATTGTAGATATCATGTGGCTTT  
AAAAATTTGATATGTGTTATTTTGGCAGGAGTCATTTTCTGCTCTTCGCAAGGATGCTGCCAAT  
GTTCTGGATTTCTAGAGAGATTAAAGAATGAAGAAGATCAAAAGGCTGTTGATGTGGATCTGA  
TTGAAAGCCTGAAATTGAAGCTGACATTTATTTGTACATATGTCCAGCTTTCTTATTCCGATTT  
GGAGAAGTTTGAAAGATATAATGACTAGAAAAAGACAAGAGGTTGAGAATCTGCTTCAACCAATT  
TTGGATGATGATGGCAAAGACGTCGGGTGTAAATATGTCCTTACTAGCCTCGCCGGTAATATGG  
ATGACTGTATAAGCTTGTATCATCGTTCTAAATCAGATGCCACCATGATGGATGAGCAATTGGG  
CTTCCTCCTCTTGAATCTCTCTCATCTATCCAAGCATCGTGCTGAAAAGATGTTTCTGGAGTG  
ACTCAATATGAGGTTCTTCAGAATGTATGTGGCAACATAAGAGATTTCCATGGATTGATAGTGA  
ATTGTTGCATTAAGCATGAGATGGTTGAGAATGTCTTATCTCTGTTTCAACTGATGGCTGAGAG  
AGTAGGACGCTTCCTTTGGGAGGATCAGGCTGATGAAGACTCTCAACTCTCCGAGCTAGATGAG  
GATGATCAGAATGATAAAGACCCCTCAACTCTTCAAGCTAGCACATCTACTCTTGAAGATTGTTT  
CAACTGAATTTGGAGGTTATGCACATATGTTATAAAACTTTGAAAGCTTCAACTTCAACAGAAAT  
TGGACGCTTCATTAAGAAGCTCCTGGAAACCTCTCCGGACATTCTCAGAGAATATCTGATTCAT  
CTACAAGAGCATATGATAACTGTTATTACCCCTAACACTTCAGGGGCTCGAAACATTCATGTCA  
TGATGGAATTCCTATTGATTATTCTTTCTGATATGCCGCCCCAAGGACTTTATTCATCATGACAA

Figure 2b - continued

ACTTTTTGATCTCTTGGCTCGTGGTGTAGCACTTACCAGGGAGGTATCAACTCTTGTACGCGAC  
 TTGGAAGAGAAATTAAGGATTAAAGAGAGTACTGACGAAACAAATTGTGCAACCCTAAAGTTTC  
 TGGAAAATATTGAACTCCTTAAGGAAGATCTCAAACATGTTTATCTGAAAGTCCCGGATTTCATC  
 TCAATATTGCTTCCCCATGAGTGATGGACCTCTCTTCATGCATCTGCTACAGAGACACTTAGAT  
 GATTTGCTGGATTCCAATGCTTATTCAATTGCTTTGATAAAGGAACAAATTGGGCTGGTGAAAG  
 AAGACTTGGAATTCATAAGATCTTTTTTCGCGAATATTGAGCAAGGATTGTATAAAGATCTCTG  
 GGAACGTGTTCTAGATGTGGCATATGAGGCAAAAGATGTCATAGATTCAATTATTGTTTCGAGAT  
 AATGGTCTCTTACATCTTATTTTCTCACTTCCCATTACCAGAAAGAAGATGATGCTTATCAAAG  
 AAGAGGTCTCTGATTTACATGAGAACATTTCCAAGAACAGAGGTCTCATCGTTGTGAACTCTCC  
 CAAGAAACCAGTTGAGAGCAAGTCATTGACAACCTGATAAAATAATTGTAGGTTTTGGTGAGGAG  
 ACAAACCTTGATACTTAGAAAGCTCACCAGTGGACCGGCAGATCTAGATGTCATTTTCGATCATTG  
 GTATGCCGGGTTTTAGGTAAAACCTACTTTGGCGTACAAAGTATACAATGATAAATCAGTTTCTAG  
 CCATTTTCGACCTTCGTGCATGGTGCACGGTCGACCAAGTATATGACGAGAAGAAGTTGTTGGAT  
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 AACTACGGAAACAATTGTTTGGAAAGAGGTATCTTATTGTCTTAGATGACGTGTGGGATACTAA  
 TACATGGGATGAGCTAACAAGACCTTTTCTGATGGTATGAAAGGAAGTAGAATTATTTTGACA  
 ACTCGAGAAAAGAAAAGTTGCTTTGCATGGAAAGCTCTACACTGATCCTCTTAACCTTCGATTGC  
 TAAGATCAGAAGAAAAGTTGGGAGTTATTAGAGAAAAGGGCATTGGAACGAGAGTTGCCCTGA  
 TGAACATTGGATGTTGGTAAAGAAAATAGCCGAAAATTGTAAAGGGCTTCCTTTGGTGGTGGAT  
 CTGATTGCTGGAATCATTGCTGGGAGGGAAAAGAAAAGAGTGTGTGGCTTGAAAGTTGTAAATA  
 ATTTGCATTCCCTTTATTTTGAAGAATGAAGTGGAAGTGATGAAAGTTATAGAAAATAAGTTATGA  
 CCACCTTACCTGATCACCTGAAGCCATGCTTGCTGTACTTTGCAAGTGCGCCGAAGGACTGGGTA  
 ACGACAATCCATGAGTTGAAACTTATTTGGGGTTTTGAAGGATTTGTGGAAGACAGATATGA  
 AGAGTCTGGAAGAAGTGGTGAAAATTTATTTGGATGATTTAATTTCCAGTAGCTTGGTAATTTG  
 TTTCAATGAGATAGGTGATTACCCTACTTGCCAACCTTCATGATCTTGTGCATGACTTTTGTGTTG  
 ATAAAAGCAAGAAAGGAAAAGTTGTGTGATCGGATAAGTTCAAGTGCTCCATCAGATTTGTTGC  
 CACGTCAAATTAGCATTGATTATGATGATGATGAAGAGCACCTTTGGGCTTAATTTTGTCTGTT  
 CGGTTCAAATAAGAAAAGGCATTCCGGTAAACACCTCTATTCTTTGACCATAAATGGAGATGAG  
 CTGGACGACCATCTTTCTGATACATTTTCATCTAAGACACTTGAGGCTTCTTAGAACCTTGCACC  
 TGGAATCCTCTTTTATCATGGTTAAAGATTCTTTGCTGAATGAAATATGCATGTTGAATCATT  
 GAGGTACTTAAGCATTGGGACAGAAGTTAAATCTCTGCCTTTGTCTTTCTCAAACCTCTGGAAT  
 CTAGAAATCTTGTGTTGTGGATAACAAAGAATCAACCTTGATACTATTACCAGAAATTTGGGATC  
 TTGTAAAGTTGCAAGTGCTGTTACGACTGCTTGTCTTTCTTTGATATGGATGCAGATGAATC  
 AATACTGATAGCAGAGGACACAAAGTTAGAGAACCTTGACAGCATTAGGGGAACCTCGTGCTTTCC  
 TATTGGAAAGATACAGAGGATATTTTCAAAGGCTTCCCAATCTTCAAGTGCTTCATTTCAAAC  
 TCAAGGAGTCATGGGATTATTCAACAGAGCAATATTGGTTCCCGAAATTGGATTTCCCTAACTGA  
 ACTAGAAAACTCACTGTAGATTTTGAAGATCAAACACAAATGACAGTGGGTCCTCTGCAGCC  
 ATAAATCGGCCATGGGATTTTCACTTTCCCTTCGAGTTTGAAGATTGCAATTGCATGAATTTTC  
 CTCTGACATCCGATTCACTATCAACAATAGCGAGACTGCTGAACCTTGAAGAGTTGTACCTTTA  
 TCGTACAATCATCCATGGGGAAGAATGGAACATGGGAGAAGAAGACACCTTTGAGAATCTCAAA  
 TGTGTTGATGTTGAGTCAAGTGATTCTTTCCAAGTGGGAGGTTGGAGAGGAATCTTTTCCACGC  
 TTGAGAAATTAGAAGTGTCCGACTGTCATAATCTTGAGGAGATTCCGTCTAGTTTTGGGGATAT  
 TTATTCCTTGAAAATTATCGAACTTGTAAGGAGCCCTCAACTTGAAAATTCCGCTCTCAAGATT  
 AAGGAATATGCTGAAGATATGAGGGGAGGGGACGAGCTTCAGATCCTTGGCCAGAAGGATATCC  
 CGTTATTTAAGTAGTTTTTTGAGCATTATGGTTGAAAAGTAGATTGCACTTTGCTGGGTAGATTG  
 TATATGGTTAAGAAAATTCTGTTACAGTTGTTATGAAACATTTTTATTTGACTTTTCTGAGTTT  
 CTTTGTAGAAAACCTCAGAAGTTTTTAACAAAAATTATAGTTTTTATAAATACAATGTGGATTGTC  
 CTTTGGCTGTCCAACCTGGTCTGAAGTCTCATATGCTCAGAGCACTATCGTTCAACCTCAATCA  
 AGGTACTGATTTAAAATGACATCTATACTACTTTATCACAAACCAACGAACCTTCATCTCAAA

Figure 2b - continued

AGCTAGGCCAGGAAGTGAAGAGGTTGTAGAGAGCTTATAAGCACTCATGACTTCCTTTTCTCGA  
 ACATTCAACCAACGTAGGCTGAAATCCCACCTCTGAACGAAAATAAGTGTTTGTATTATCAAATTA  
 ACTCTCGTAGTAGAACACTGAAATACCTTCTTCTAAACGTTCAACAAATGGGATTTCCAGCACT  
 CAAAGTGAATGAAAGGTTACATTAATCTTCAAAAAGAATTACGACAATTCATGACCACAAGTA  
 CATTGACAGCACCATTTCAACAGAAGAACAAGTCAATGCTGCATCTTCATCAATAATCCGAGTG  
 TCGAACCTCCTTCTGACACTGTCTGTATATGTAAAGTTTCTCAACAGGGCAACTTTCTGGTCT  
 TCGTATCTGGATGACCCCTCTCGTCTATAACTTCAACATTAAGCCCTGGCAACTTCTGGACCA  
 CAGCTTACATGCTTCAAACTTACTGAACAATTAGACATCCAAAGGGATCGCATTGTCTCCAGC  
 TTTGCAGCATTAGCCAACAGAGCCTCATCGCCAAAGGGGCAGTCTCTAATCTCGAATTTGAAAA  
 AATTGTTGTTGTATGACTTTCTCTGACATCCGATGCACTATCAACAATAGCAAGACTGGAGGT  
 TGGAGAGGAATCCTTTATTATACAATCATTGAGGAGAAGAATGGAACATGGGGGAGGAAGACA  
 CTTTTGAGAATCTGAAATGTGTTAGAGCCACAAGCTACAGAAGTATTGAATTTGTCATGAATAT  
 CAACATTCTTCATCCTAGTTAATTTCTTTTTCAATTTTAAATAGACTCTCATTTTAACTACTAAT  
 ATTCTTCTATTTGTGACTTCTTTTCTGCAGGTGGCAACTTTAAATTCATAAAGTATAGGATTGA  
 TGACAAACTCGAAAAATATCTTAATGAGGTGAAGTTTGAGCAGTCAGCAGATGGTGGTTCCAAC  
 TCTAAGTTGACAAGCACATACTATCCCGGAGGGCGATTTCAAGCCTGATGCATATGGTTAGTGT  
 GGCTAGAGCAGACAGGATGTATTACCTGGATATCTACCAAGACGAATCCACAATCAGTTTTATG  
 TCAAGCAATACATGAAGTAACTCCCGATAGAACAGTAAAAGCAAGATGTGTAGGTGTATCTCGA  
 CTCTAAGAGATTGTACATTCCTCTTTGAGATTTTTACTGCTAATACAAATTTACACCTCAGAAG  
 CGAATCTAGAATTTCTAGAGCATGAATGCACCACCTAATGAAAGGAGAAAAAAGGAAGTATGAAG  
 TGGGAATTTGATCCTTGTTTCTAGGTATATAAAATTTATCATTCAACTATACTTCATTTAGCAA  
 ACAACTCTCTTTGCCATTATTTCTCAACAAGGGCTTCTAATATTGCTAAACTAAAGACTGTCA  
 AAAGGTAAGTTTCATCTTCAAACTCTCTTGTTTACTTTATCTAAAGGGGAACCTATGAAAAACAAG  
 AAACATCAGGAATGTCCCGTAAACAAGGCAGCCTCATGCACAAAACATCCAACGTTGGTAGGAT  
 TAATGGAGGGATCGCATCCCAGGAGGATACTGTAGAAAAATTAGTGGCTTCTTTACCGCTCAA  
 ACCCATGATCTATAGGTTACATGGAGACAACCTTTATGGTTGCTCGTAGGCTCCCGTCAATTCTC  
 ATAAACCACAACACCAGGTTGCATCAGACATCATCTTCATTCAAGCTGACAATCTCCACAA  
 GTCTTAGTCAACTTGTAATATGAATATTAGCCAGGTAGACGTACATATTTACAAAATTGAGTTT  
 CCTATATAATATGGTTTGAAGGAATGAAACATGATGGGGAGGGTAGATAAAATAATATATGAGG  
 CATAAAAATAGGAAAGATATTTGTAGTGAGAGGTTTGTACTTTTTATGCTGCTTTTGATCTTCA  
 GTTTCTTGATTCTTTTTCTACTGCTTTCCTCTTCTTCTCCTGAGTAAAGTTTATGTAGGTA  
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 AACATACATGCAAAATATATATATATAAATTTTCATGAAAAATTATAACAAATAATAGATGTGAA  
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 AAAAATATAACAAAAATATTATACATTGCAATTTTTTGCATATCAATATGATAAAAAAATATA  
 TCGTAAAATATTAGTCAAAATTTTTTATAATTTGACTCAAATCATGAAAAGTATAATAATTAATA  
 GTGGACGGAGGAAGTATTGTCTTTCCAGATTTGTGGCCATTTTGGTCCAAGGGCCATTAGCAG  
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 ATTACTTGATTATTTATTAAATCAAAAAGAATTAATTAATTTTTTCTCATTTTACCCCTACAAT  
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 ATTAATATAAACAAGGATAAAATAAATAAATTTGTCAATTTATTGACGATCACTTAATAATCA  
 TATAAATAGAAAAATGTTTATCTAATATGAGACGGAGAAAAATATATCCTAAAAATTTTTTGGAC  
 AGATATGTGATATTCTAACCATTCACTAGACTATATTATGCATTTTAGCCGCAATGACTTATT  
 TCAGCTTTAATTAATTAGGAAAGAGGAACTGCCAATGAGGAAGAGTAGGGGCGTAGTTGCTGT  
 CGACGAAAAAAGATAAATACTCACTCTTTTCGATTTTTATTTTTATTTATCACTTTTAACCTAT  
 CATGTAAAAAGATAATTATTTTTTTTCATGCTTTATCCTTAGTATTAAACAATTTAATAGGGATT

Figure 2b - continued

ATTTTGTAAATATTTATATGAATAATTGTTTTGTAATGAATTTGTCCGGTCAAACAATGATA  
 AATAAAATGAATGAAGAGAGTAGAAAACAAAACAAAAGAACAAGTTGACAACCTTGAGAGATTA  
 AAAGGGTCCAAAACGCCCTTGATTTTGGATTTCATATGTGAAATTTCCATGAAATAATTGAAT  
 TTGTATTATTACAAGTCAAACCTTTCCATTTCAATCCAACTAGCCATCTTGGTTTCAAATTACA  
 CATTCATTCAATCACAGATCTAATATTCTTAATAGTGATTTCACATATGGCTGAAGCTTTCAT  
 TCAAGTTCTGCTAGACAATCTCACTTCTTTCCTCAAAGGGGAACCTTGATTGCTTTTCGGTTTT  
 CAAGATGAGTTCCAAAGGCTTTCAAGCATGTTTTCTACAATTCAGCCGTCCTTGAAGATGCTC  
 AGGAGAAGCAACTCAACAACAAGCCTCTAGAAAATTGGTTGCAAAAACCTCAATGCTGCTACATA  
 TGAAGTCGATGACATCTTGGATGAATATAAAACCAAGGCCACAAGATTCTCCAGTCTGAATAT  
 GGCCGTTATCATCCAAAGGTTATCCCTTTCCGTCACAAGGTCGGGAAAAGGATGGACCAAGTGA  
 TGAAAAACCTAAAGGCAATTGCTGAGGAAAAGAAAGATTTTCATTTGCACGAAAAAATTGTAGA  
 GAGACAAGCTGTTAGACGGGAAACAGGTACTCATCTTAAATTAGTATTACAACAACCTAAGTTTA  
 TATTCATTTTTTTGGCAATTATCAAATTCAGAAAAGGGTTAAATATACTCATGTCCTATCGTAA  
 ATAGTGTATATATACCTCTCGTTGTACTTTGATCTGAATATACTTGTCAAATCTGGCAAGCTC  
 AGAATCAAATTATCCACCCCAACTTTTAAATACTCGATATCTTTAGAAATCCACCTGTCTAACT  
 CATCCACTACCCATTCCCTTTGCTTTGAATTCCTTTCTTTACCTATAAACTTGGAACTCTGAT  
 CCGTTTTGCTTTTCTTAACAAAGCAGCTCAGAGAAAAGAGGTTTTCTTCTATTCTGTTTCTCTG  
 TGTGCTGCACCTGGGTCCTTAATCCCATTAAAAACAGGGCATGTTAATCCCAACGACGGTAGCC  
 TTTCTGACAGCTGACTGTAAATTTTGTCTAACAAAGAAAAAAGATTAGACATGTTTTTCC  
 TTGTCAATTGATTAGGCTGGATTTCTTTCAGAGTGGAACATAGGGGATATATTGGACCAAAAGTA  
 GAATGGGTATATATTTAAAGTATTTCTGATAGAACAGGAGTATATTGTGCGAAAAATATCCTCTA  
 TTTTCTGTTGTCTCCTAATGAGTTTGAATGTAATAATATTCTCATGTGGACATTGCTTGACCA  
 GGTCTGTATTAAACCGAACCGCAGGTTTATGGAAGAGACAAAGAGAAAGATGAGATAGTGAAAA  
 TCCTAATAAAACAATGTTAGTGATGCCCAACACCTTTTCACTCCTCCCAATACTTGGTATGGGGGG  
 ATTAGGAAAAACGACTCTTGCCCAAATGGTCTTCAATGACCAGAGAGTTACTGAGCATTTCAT  
 TCCAAAATATGGATTTGTGTCTCGGAAGATTTTGTATGAGAAGAGGTTAATAAAGGCAATTGTAG  
 AATCTATTGAAGGAAGGCCACTACTTGGTGAGATGGACTTGGCTCCACTTCAAAGAAGCTTCA  
 GGAGTTGCTGAATGGAAAAAGATACTTGGTTGTCTTAGATGATGTTTGAATGAAGATCAACAG  
 AAGTGGGCTAATTTAAGAGCAGTCTTGAAGGTTGGAGCAAGTGGTGCTTCTGTTCTAACCCTA  
 CTCGTCTTGAAAAGGTTGGATCAATTATGGGAACATTGCAACCATATGAACTGTCAAATCTGTC  
 TCAAGAAGATTGTTGGTTGTTGTTTCATGCAACGTGCATTTGGACACCAAGAAGAAATAAATCCA  
 AACCTTGTGGCAATCGGAAAGGAGATTGTGAAAAAAGTGGTGGTGTGCCTCTAGCAGCCAAAA  
 CTCTTGGAGGTATTTTGTGCTTCAAGAGAGAAGAAAGAGCATGGGAACATGTGAGAGACAGTCC  
 GATTTGGAATTTGCCCTCAAGATGAAAGTTCTATTCTGCCTGCCCTGAGGCTTAGTTACCATCAA  
 CTTCCACTTGATTTGAAACAATGCTTTGCGTATTGTGCGGTGTTCCCAAAGGATGCCAAAATGG  
 AAAAAGAAAAGCTAATCTCTCTCTGGATGGCGCATGGTTTTCTTTTATCAAAGGAAACATGGA  
 GCTAGAGGATGTGGGCGATGAAGTATGGAAGAATTATACTTGAGGTCTTTTTTCCAAGAGATT  
 GAAGTTAAAGATGGTAAAACCTTATTTCAAGATGCATGATCTCATCCATGATTTGGCAACATCTC  
 TGTTTTTCAGCAAACACATCAAGCAGCAATATCCGTGAAATAAATAAACACAGTTACACACATAT  
 GATGTCCATTGGTTTTCGCCGAAGTGGTGTTTTTTTTACACTCTTCCCCCTTGGAAGGTTTTATC  
 TCGTTAAGAGTGCTTAATCTAGGTGATTCGACATTTAATAAGTTACCATCTTCCATTGGAGATC  
 TAGTACATTTAAGATACCTTGAACCTGTATGGCAGTGGCATGCGTAGTCTTCAAAGCAGTTATG  
 CAAGCTTCAAATCTGCAAACCTTTGATCTACAATATTGCACCAAGCTTTGTTGTTGGAAGGAA  
 GAAAGGTTATCAACTTGGTGAACCTAGGAAACCTAAATCTCTATGGCTCAATTAATAATCTCGCAT  
 CTTGAGAGAGTGAAGAATGATAAGGACGCAAAAGAAGCCAATTTATCTGCAAAAGGGAATCTGC  
 ATTTCTTTAAGCATGAGTTGGAATAACTTTGGACCACATATATATGAATCAGAAGAAGTTAAAGT  
 GCTTGAAGCCCTCAAACCACACTCCAATCTGACTTCTTTAAAAATCTATGGCTTCAGAGGAATC

Figure 2b - continued

CATCTCCAGAGTGGATGAATCACTCAGTATTGAAAAATATTGTCTCTATTCTAATTAGCAACT  
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 CTGGGGGTCTGCGGATGTGGAGTATGTTGAAGAAGTGATATTGATGTTTCTGGATTCCCC  
 ACAAGAATAAGGTTTCCATCCTTGAGGAACTTGATATATGGGACTTTGGTAGTCTGAAAGGAT  
 TGCTGAAAAAGGAAGGAGAAGAGCAATTCCCTGTGCTTGAAGAGATGATAATTCACGAGTGCCC  
 TTTTCTGACCCTTTCTTCTAATCTTAGGGCTCTTACTTCCCTCAGAATTTGCTATAATAAGTA  
 GCTACTTCATTCCAGAGAGATGTTCAAAAACCTTGCAAATCTCAAATACTTGACAATCTCTC  
 GGTGCAATAATCTCAAAGAGCTGCCTACCAGCTTGGCTAGTCTGAATGCTTTGAAAAGTCTAAA  
 AATTCAATTGTGTTGCGCACTAGAGAGTCTCCCTGAGGAAGGGCTGGAAGGTTTATCTTCACTC  
 ACAGAGTTATTTGTTGAACACTGTAACATGCTAAAATGTTTACCAGAGGGATTGCAGCACCTAA  
 CAACCCTCACAAGTTTAAAAATTCGGGGATGTCCACAACGATCAAGCGGTGTGAGAAGGGAAT  
 AGGAGAAGACTGGCACAAAATTTCTCACATTCCTAATGTGAATATATATATTTAAGTTATTTGC  
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 AGGTTTGTCTTTATGAGTCTCTCTCTCATTGGATGTAATTTTCTTTTGAAAACAAATCTGTC  
 AATTGATTTGTATTATACGCTTTCAGAACTATTACTTATTTGTAATTGTTTCTTTGTTTGTA  
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 AGATCCATCTGTATTATACCTTTCGTCTCATTATGTGTGCACCTGTGCGGATTTGAGATTC  
 AAACAAATCTATCTTTGATCGTAAATTTTAAATAGATCTTTTAAACATTTTGAAATTATCAATTA  
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 CAGGGCGCAAGGGCTGCTAAAGGAAGCGGAACACGTAGAAAAGCCAGTCCGCGAGAAACGGTGCTG  
 ACCCCGGATGAATGTCAGCTACTGGGCTATCTGGACAAGGGAAAACGCAAGCGCAAAGAGAAAAG  
 CAGGTAGCTTGCAGTGGGCTTACATGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGCG  
 AACCAGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTGGAT  
 GGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAA**GATCATGAGCGGAGAATTAAGGG**  
**AGTCACGTTATGACCCCCGCCGATGACGCGGGACAAGCCGTTTTACGTTTGGAAGTACAGAAC**  
**CGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTTCTGGAGTTTAATGAGCTAAGCACATACGTC**  
**AGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAGGTCATATCAGCTAGCAAATATTTCTTGT**  
**CAAAAATGCTCCACTGACGTTCCATAAAATCCCCTCGGTATCCAATTAGAGTCTCATATTCAT**  
**CTCAATCCAGATCCCCGGGTACCATGGCGCGGCAACAACAACAACAACATCTTCTTCGAT**  
**CTCCTTCTCCACCAAACCATCTCCTTCTCCTCCAAATCACCATTACCAATCTCCAGATTCTCC**  
**CTCCCATTCTCCCTAAACCCCAACAAATCATCCTCCTCCTCCCGCCGCCGCGGTATCAAATCCA**  
**GCTCTCCCTCCTCCATCTCCGCCGTGCTCAACACAACCACCAATGTCACAACCCTCCCTCTCC**  
**AACCAAACCTACCAAACCCGAAACATTCATCTCCCGATTGCTCCAGATCAACCCCGCAAAGGC**  
**GCTGATATTCTCGTCGAGGCTTTAGAACGTCAAGGCGTAGAAACCGTATTGCTTACCCTGGAG**  
**GTGCATCAATGGAGATTACCAAGCCTTAACCCGCTCTTCTCAATCCGTAACGTCTTCTCTCG**  
**TCACGAACAAGGAGGTGTATTGCGCAGCAGAAGGATACGCTCGATCCTCAGGTAAACCAGGTATC**  
**TGTATAGCCACTTCAGGTCCCGGAGCTACAAATCTCGTTAGCGGATTAGCCGATGCGTTGTTAG**  
**ATAGTGTTCCTCTTGTAGCAATCACAGGACAAGTCCCTCGTCGTATGATTGGTACAGATGCGTT**  
**TCAAGAGACTCCGATTGTTGAGGTAACGCGTTCGATTACGAAGCATAACTATCTTGTGATGGAT**  
**GTTGAAGATATTCTTAGGATTATTGAGGAGGCTTTCTTTTAGCTACTTCTGGTAGACCTGGAC**  
**CTGTTTTTGGTTGATGTTTCTTAAAGATATTCAACAACAGCTTGCGATTCTAATTGGGAACAGGC**  
**TATGAGATTACCTGGTTATATGTCTAGGATGCCTAAACCTCCGGAAGATTCTCATTGGAGCAG**  
**ATTGTTAGGTTGATTCTGAGTCTAAGAAGCCTGTGTTGTATGTTGGTGGTGGTTGTTTGAAGT**  
**CTAGCGATGAATTGGGTAGGTTTGTGAGCTTACGGGAATCCCTGTTGCGAGTACGTTGATGGG**  
**GCTGGGATCTTATCCTTGTGATGATGAGTTGTCGTTACATATGCTTGGAAATGCATGGGACTGTG**  
**TATGCAAATTACGCTGTGGAGCATAGTGATTTGTTGTTGCGTTTTGGGGTAAGGTTTGATGATC**  
**GTGTCACGGGTAACTTGAGGCTTTTGTAGTAGGGCTAAGATTGTTTCATATTGATATTGACTC**  
**GGCTGAGATTGGGAAGAATAAGACTCCTCATGTGTCTGTGTGTGGTGATGTTAAGCTGGCTTTG**  
**CAAGGGATGAATAAGGTTCTTGAGAACCAGCGGAGGAGCTTAAACTTGATTTTGAGTTTGA**

Figure 2b - continued

*GGAATGAGTTGAACGTACAGAAACAGAAGTTTCCGTTGAGCTTTAAGACGTTTGGGGAAGCTAT  
TCCTCCACAGTATGCGATTAAGGTCCCTTGATGAGTTGACTGATGGAAAAGCCATAATAAGTACT  
GGTGTCGGGCAACATCAAATGTGGGCGGCGCAGTTCTACAATTACAAGAAACCAAGGCAGTGGC  
TATCATCAGGAGGCCTTGGAGCTATGGGATTTGGACTTCCTGCTGCGATTGGAGCGTCTGTTGC  
TAACCCTGATGCGATAGTTGTGGATATTGACGGAGATGGAAGTTTTATAATGAATGTGCAAGAG  
CTAGCCACTATTTCGTGTAGAGAATCTTCCAGTGAAGGTACTTTTATTAAACAACCAGCATCTTG  
GCATGGTTATGCAATGGGAAGATCGGTTCTACAAAGCTAACCGAGCACACACATTTCTCGGAGA  
TCCGGCTCAGGAGGACGAGATATTTCCCGAACATGTTGCTGTTTGCAGCAGCTTGCGGGATTCCA  
GCGGCGAGGGTGACAAAGAAAGCAGATCTCCGAGAAGCTATTCAGACAATGCTGGATACACCAG  
GACCTTACCTGTTGGATGTGATTTGTCCGCACCAAGAACATGTGTTGCCGATGATCCCGAATGG  
TGGCACTTTCAACGATGTCATAACGGAAGGAGATGGCCGGATTAAATACTGAGAGCTCGAATTT  
CCCCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCG  
ATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGA  
CGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGA  
AAACAAAATATAGCGCGCAAAC TAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT  
CGGGAATTCAC TGCCGTCGTTTTACAACGACTCAGCTGCTTGGTAATAATTGTCATTAGATTG  
TTTTTATGCATAGATGCACTCGAAATCAGCCAATTTTAGACAAGTATCAAACGGATGTTAATTC  
AGTACATTAAAGACGTCCGCAATGTGTTATTAAGTTGTCTAAG*



Figure 2c of 5

Insert without flanking region without ahas marker- SEQ-ID-No. 3

Upper case *italic-underlined* – Blb2 expression cassette (promoter – gene –terminator)

Upper case **bold** – Blb1 expression cassette (promoter – gene –terminator)

CAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTGGCGCGCCAAGCTTGCATGC  
 CTGCAGGTCTGACTCTAGAGGATCTAGAATCACCGAACCTCCCCTCGGTACAGCTCCTCCAGTTC  
 TACCATGAATTTTCATCCACTGATTCTCTTCAATCGCCATTGCAGATTCTCTCGATCTATGCTC  
 AAAAAATCCCGAGATAAAACCCTAGATCTGCTTCAAATGCTCTGATACCATGTAATTTCACTGA  
 ATTCTAATACTAAACAATGGAGAGAATTAATACTATTTTAGAAAAGACTGATTGAAGGAGAAGAAGAGA  
 GAAAAATTCTATATTGAACCTCATGAACCAAAATGAATGAAAAAATAATGAGAAGAAGTATACT  
 ATTACAATCTATATATCTCTATTTATATTCTAATCTGAAGCAGTTAATTTAACTGACTCTAACA  
 ACTAGACTGATAGGTGTACATTTTCTGTTAGTGCAGTGCAGTTTAACTAACTGCTTAACA  
 TAAAGAATGTTGTTGGAACCTTCATTTCGAATAGCTTCAATGAGAAGCAAACATGTGTACCTGTAA  
 AGACACACAGTAAAGTGTTAATAATGAATAAATATGAATAAATCAAATAATAAATTAATAAATA  
 AAAACACATCCAATTAACATTGGAGGTCTTGAAAATCGATGGTAATTAACAAAGACCCTTGTGA  
 AATTTAAGTCTGTAATTGAAAATTTGAGTATAGGTTAGGGGACATTTGACTATTTTCTCATTTT  
 CTTTATCTTTTTCTTAATTTGTGGCAGACAAGTGAGGAGGCCCTGTAATTGATTTCATGCTT  
 TTGCTTTCTTGACTTTTTGGAACAATACTATGCATCATATTTGGTCTTAATTATTCCTCTGTTT  
 ATTTCCAGAATTTTGAGCTCTATACATCTAATAACAAAGCAAGCAGAGGATATATAGTTTCATC  
 AACTAAAAGGTTAGTCAACTCATCTAATATTTGCTACTCTCATCTCTATGAAGTACAGTTAT  
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 AGAAAGGGAAATAAATGTCAGGAGAAAGAGAGAGGTTAAATACTTACTCACACCACCGATTAC  
 AACAAATCACTTAATTGTGGTTAGTTAATGTATACTTTACCTCATTAAATTTACTTACCCA  
 TGATAAGTTGTATTAATTTGGTATTAATATCCGGTGCGGGTGAATTCCTACCGGGTGAGAGGGA  
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 ACCTTTATCATCTTCCACTAATTAAGTCCTCTTAAGTTTCGCGTGAAAATAGTGAAATTATTGAT  
 TATTCTTATCATTTTCATCTTCTTCTCTGATAAAGTTTTATGTACTTTTTATGCATCAGGTCT  
 TGAGAACTTGGAAAGGAAAAGTAGAATCATGGAAAACGAAAAGATAATGAAGAAGCAAACAAC  
 TCATTGGTATGTTATTTGATAGAGTGAAGTGTAAAGTATTGAATTGTAGATATCATGTGGCTTT  
 AAAAATTTGATATGTGTTATTTTGGCAGGAGTCATTTTCTGCTCTTCGCAAGGATGCTGCCAAT  
 GTTCTGGATTTCTAGAGAGATTAAAGAATGAAGAAGATCAAAGGCTGTTGATGTGGATCTGA  
 TTGAAAGCCTGAAATTGAAGCTGACATTTATTTGTACATATGTCCAGCTTTCTTATTCCGATTT  
 GGAGAAAGTTTGAAGATATAATGACTAGAAAAAGACAAGAGGTTGAGAATCTGCTTCAACCAATT  
 TTGGATGATGATGGCAAAGACGTCGGGTGTAAATATGTCCTTACTAGCCTCGCCGGTAATATGG  
 ATGACTGTATAAGCTTGTATCATCGTTCTAAATCAGATGCCACCATGATGGATGAGCAATTGGG  
 CTTCTCTCTTGAATCTCTCTCATCTATCCAAGCATCGTGCTGAAAAGATGTTTCCTGGAGTG  
 ACTCAATATGAGGTTCTTCAGAATGTATGTGGCAACATAAGAGATTTCCATGGATTGATAGTGA  
 ATTTGTTGCATTAAAGCATGAGATGGTTGAGAATGTCTTATCTCTGTTTCAACTGATGGCTGAGAG  
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 GATGATCAGAATGATAAAGACCCTCAACTCTTCAAGCTAGCACATCTACTCTTGAAGATTGTTT  
 CAACTGAATTTGGAGGTTATGCACATATGTTATAAAACTTTGAAAGCTTCAACTTCAACAGAAAT  
 TGGACGCTTCATTAAAGAAGCTCCTGGAAACCTCTCCGGACATTCTCAGAGAATATCTGATTTCAT  
 CTACAAGAGCATATGATAACTGTTATTACCCCTAACACTTCAGGGGCTCGAAACATTCATGTCA  
 TGATGGAATTCCTATTGATTATTCTTTCTGATATGCCGCCAAGGACTTTATTCATCATGACAA  
 ACTTTTTGATCTCTTGGCTCGTGTTGTAGCACTTACCAGGGAGGTATCAACTCTTGTACGCGAC

TTGGAAGAGAAATTAAGGATTAAAGAGAGTACTGACGAAACAAATTGTGCAACCCCTAAAGTTTC  
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 GGAACTGTTCTAGATGTGGCATATGAGGCAAAAGATGTCATAGATTCAATTATTGTTTCGAGAT  
 AATGGTCTCTTACATCTTATTTTCTCACTTCCCATTACCAGAAAGAAGATGATGCTTATCAAAG  
 AAGAGGTCTCTGATTACATGAGAACATTTCCAAGAACAGAGGTCTCATCGTTGTGAACTCTCC  
 CAAGAAACCAGTTGAGAGCAAGTCATTGACAACCTGATAAAATAATTGTAGGTTTTGGTGAGGAG  
 ACAAACCTTGATACTTAGAAAGCTCACCAGTGGACCGGCAGATCTAGATGTCATTTTCGATCATTG  
 GTATGCCGGGTTTAGGTAAAACCTACTTTGGCGTACAAAGTATACAATGATAAATCAGTTTCTAG  
 CCATTTTCGACCTTCGTGCATGGTGCACGGTGCACCAAGTATATGACGAGAAGAAGTTGTTGGAT  
 AAAATTTTCAATCAAGTTAGTGACTCAAATTCAAAATTGAGTGAGAATATTGATGTTGCTGATA  
 AACTACGGAAACAATTGTTTGGAAAGAGGTATCTTATTGTCTTAGATGACGTGTGGGATACTAA  
 TACATGGGATGAGCTAACAAGACCTTTTCCCTGATGGTATGAAAGGAAGTAGAATTATTTTGACA  
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 TAAGATCAGAAGAAAGTTGGGAGTTATTAGAGAAAAGGGCATTGGAACGAGAGTTGCCCTGA  
 TGAATATTGGATGTTGGTAAAGAAATAGCCGAAAATTGTAAAGGGCTTCCTTTGGTGGTGGAT  
 CTGATTGCTGGAATCATTGCTGGGAGGGAAAAGAAAAAGAGTGTGTGGCTTGAAGTTGTAAATA  
 ATTTGCATTCCCTTTATTTTGAAGAATGAAGTGGAAAGTGATGAAAGTTATAGAAATAAGTTATGA  
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 TTGTAAAGTTGCAAGTGCTGTTACGACTGCTTGTTCTTTCTTTGATATGGATGCAGATGAATC  
 AATACTGATAGCAGAGGACACAAAGTTAGAGAACTTGACAGCATTAGGGGAACTCGTGCTTTCC  
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 AAGGAATATGCTGAAGATATGAGGGGAGGGGACGAGCTTCAGATCCTTGGCCAGAAGGATATCC  
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 CCTATATAATATGGTTTGAAGGAATGAAACATGATGGGGAGGGTAGATAAAAAATATATGAGG  
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 TTTCTGACAGCTGACTGTAAATTTTGTCTAACAAAGAAAAAAGATTAGACATGTTTTTCC  
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ATCAGCCAATTTTAGACAAGTATCAAACGGATGTTAATTCAGTACATTAAAGACGTCCGCAATG  
TGTTATTAAGTTGTCTAAG

Figure 2d of 5

Event D	Border	Sense primer	SEQ- ID- No.	Antisense primer	SEQ- ID- No.	Annealing temperature [°C]	Product length (bp) (range)
	LB	TCAAACGGATGTTAATTCAGTACATT	4	CCAGTTCCTCAATTGACTACTAGAAA	5	52	131 (126 to 136)
	LB	TCTGTGAATTACGTTAAGC	6	CTCAGAAGAAAGAATTGTTC	7	48	510 (505 to 515)
	LB	GTTTCTTAAGATTGAATCCTGTTGC	8	GCCCATCTCTATTTTACTCACTAA	9	51	630 (625 to 635)
	RB	CCAAGATAGTGTTCAGGAAAGTTATT	12	AAATTCATGGTAGAACTGGAGGAG	13	52	287 (282 to 292)
	RB	AACTGAATTTTGGGATTGAG	14	GAGTCAGTTAAATTAACGTTCAG	15	49	882 (877 to 887)
	RB	ACAAGAATAGCAAGGATTATCC	16	GAAGTTCGAACAACATTCTT	17	49	832 (827 to 837)
Left-flanking region to b1b1	LB	GTGAACTAGGAAACCTAAATC	10	CAACTAATAAAACCAAGGAC	11	52	4757 (4752 to 4762)
Right flanking region to B1b1	RB	AACTGAATTTTGGGATTGAG	18	ATGTAGCAGCATTGAGTTT	19	50	9910 (9905 to 9915)

Figure 2 e of 5

## Flanking genomic sequence left border

GAAACTCAGTTGGAGATTACGTTTAAAGGGATGGCCTACATGGATAAGGTTATCTACAATGATGC  
 AATGGAGATAATAATAGTACTCCTATGTGATTCTTCGTTTAAATTTGTTTATTTTATTTTCAT  
 TTAATTTGATTTGATACGTAGTTTAAAAAATAAAAAAGATATTTAAATTTTGTGATGTTAGAT  
 GAAACATACGTTAAATGCATCAAAATGTCATTTAAATTTGTCTTGAACATATAGTATTAGAAAG  
 TTGAAATTAAAGAGCTATCAAAAAAGAAGAAGATATGACTAAAAAATAGCACAAAGCAAATTG  
 AAATAAAAGAGTAATTAACAATAACAATTTTTTTTTTAAATGATACATAATACTCACTCTTTC  
 TCAATATGTGGCACAAAAATATCAAGAATCAAATTTTAAATTTTGAATATAAAATTTAGAAATA  
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 GATTATCCATTTAGGAAATAAGATGAAAGAATCAAATCAACTAATAAAAACTTTAATCCTATAA  
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 CTTTAAAGACCAAGATAGTGTTTCAGGAAAGTTATTTCAATTTCCAAATTTAAATAAAAGAAAAT  
 AATTATTCAAATTCCTTATGTCTTTTTTATTGATTTATAGTCGAAGATCTTTCAAAAATAATTT  
 TTCTATAGGTAAAAAAGGATAATTT (SEQ-ID-No. 20)

## Flanking genomic sequence right border

TTGTCCTTGGTTTTATTAGTTGTTATTGTTGTTTTGTCTTTTCAATTTCTAGTAGTCAATTGGG  
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 GTGACACTTATAAAAAGAACAATTCTTCTCTGAGAACATCTTCAACCCACCTCTATTTTACT  
 CTCCATTCTCTATATTTAGTGAGTAAAATAGAGAATGGGCACTCCAACCCACCTCCATATCACT  
 CTCCATTCTTCATATTTAGAGAGTCATATTATTTTTATTATTATTTTTATTACTTTTCTAATTAA  
 TATATTATTTTACATATAATGTCATTAATTAATATCTAATATTCATAATTCCTTTTTAAATGTC  
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 TTAAATACAGGATAACAATACAATACATGACATAATAATTTAAATACAAGATAAAATACAATAC  
 ATAACATAATAATCAATTCGTGATGAAACAAGAATCATGCCAAAAAGATATTGAACGTGCATTC  
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 AAAATAAGAATGAATAGAAATAATAATAATATAATATTGAGGAGAAATAATTATTTTTTTTAGA  
 GAGTGAAATAGAGAATTGGGTTGAAATTGATTGTCTGAAAAATAAAAACTCTATATTTGGAG  
 AGTAAATAGAGTGTAGGGTTGGAGACGTCA (SEQ-ID-NO.21)

Figure 2 e - continued

**Part of flanking genomic sequence left border including part of insert**

Partial insertion sequence is bold (T-DNA, Vector), Primer-binding sites are underlined:

Gtttettaagattgaatectgttgcgggtcttgcgatgattatcatataatttctgttgaattacgttaagcatgtaataattaacatgta  
atgcatgacgttatttatgagatgggttttatgattagagtccecgcaattatacatttaatacgcgatagaaaacaaaatatagcgc  
gcaaactaggataaattatcgcgcggtgtcatctatgttactagatcggggaattcactggcgcgtgtttacaacgactcagctgc  
ttggtaataattgtcattagattgttttatgcatagatgcactcgaaatcagccaattttagacaagtatcaaacggatgttaattca  
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aattgggaactggagtaaaaagtgaatttttagacttatgtatacaaatgaaatgtatcaaaaagtagtgacactataaaaagaacaattc  
ttcttctgagaacatctcaaccacctctattttactctcattctctatatttagtgagtaaaatagagaatgggcactccaaccacctccata  
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ccgtagaggctccaactccaacgacaaaaatgatgglagatgaaaatctcggttgaacaatttttagctagacataaaaaagtttaaggaca  
aaaatgctcattttgaactccgtaatgcattaatagagcatttatgacagcaacgtgataattcgaaacttgagtgtttatgtaattatatttcacttt  
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ataatattgaggagaaataattatttttttagagagtgaaatagagaattgggtgaaattgattgtctgaaaaataaaaaactctatatttga  
gagtaaaatagagtgtaggggttgagagctca (SEQ-ID-No. 22)

**Part of flanking genomic sequence right border including part of insert**

Partial insertion sequence is bold (T-DNA, Vector), Primer-binding sites are underlined:

gaaactcagttggagattacgtttaagggtatgcctcatatggataagggtatctacaatgatgcaatggagataataatagtactcctatgtgat  
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ataaaagaaaataatttcaaaattctatgtctttttattgatttatagtcgaagatcttcaaaaataattttctataggtaaaaaaggataatttc  
aatttcacacaggaaacagctatgaccatgattacgccaagctggcgcccaagcttgcattgectgcaggctgactctagaggatc  
tagaatcaccgaacctcccctcggtacagctcctccagttctaccatgaatttcatecaetgattctcttcaatcgccattgcagattc  
tctcgatctatgtcaaaaaatcccgagataaaaacctagatctgcttcaaatgctctgataccatgtaatttcagtgaaatttcaacta  
aacaatggagagaattaacttttttagaaagactgattgaaggagaagaagagagaaaaattctatattgaactcatgaacaaa  
atgaatgaaaaaataatgagaagaactatactattacaatctatatactctatttatattetaatctgaagcagtttaatttaactga  
ctctaacaactagactgatagggtgtacattttctgttagtgactgcagtgcatftaaactaactgcttaacataaagaatgttgttcga  
acttc (SEQ-ID-No. 23)



Figure 2 f of 5

**Partial-sequences of the insert with flanking region**

Insertion sequence is bold (T-DNA, Vector), Primer-binding sites are underlined:

**Event: A**

Left border - PMA16

**Agcatgtaataa**taacatgtaatgcatgacgttattatgagatgggttttatgattagagtcccgaattatacatttaacgcgatagaa  
**aacaaa**atagcgcgcaaactaggataatatcgcgcggggtgcatctatgtactagatcgggaattcactggccgtcgtttacaa  
**cgactcagctgcttgg**taataattgccattagattgttttatgcatagatgcactcgaaatcagccaatttagacaagtatcaaacggatgtt  
**aattcag**tacattaagacgtccgcaatgtgaactcattgatcaagaatgactatgggacattaagtaaaaatcataagacattttttcata  
**ttccata**ataaaagaataaacagaatattagtggaaaaaatgaaaattctaaatgtagggggtggggtggggaaggagatacattaggttc  
**taaaa**ataataaaaaacgaactaaccaagatgatcaaatcgaaaaaatgcgtaattggtttgatttaataattttaaaatgaataaatgattg  
**atttag**tggcaaaagttgatctaaatgatctctgagtacctttagccccactaattcctttaaggataaacgtttcaattgtaaagacaaattag  
**actaa**atagctgaagaaggatagtttaacccaataatcaatgtaagacaatttaattatataaaagaaaaataaaactatttcaatag  
**ttag**ggacaagggaataaatcttaaaacaatcgcaatatcaaaattggcgctataaataaacttaaattaaactattctagtaaaagatgatg  
**tatt**ctaataatctctcagcttctcgataagaagtagtaattggatataggattgtaaggtcatgcgcagctct (SEQ-ID-No. 24)

## #1: Product of length 63 (rating: 171)

Contains region of the molecule from 281 to 343

Tm: 64.2 C TaOpt: 45.0 C GC: 36.5

Sense Primer:

TAATTCAGTACATTAAAGACGTCCG (SEQ-ID-No. 25)

Similarity: 100.0%

Length: 25 Tm: 51.8 C GC: 36.0

dH: -185.5 kcal/mol dS: -488.6 cal/mol dG: -38.0 kcal/mol

Antisense Primer:

GTCCCATAGTCATTCTTGATCA (SEQ-ID-No. 26)

Similarity: 100.0%

Length: 23 Tm: 50.1 C GC: 39.1

dH: -160.9 kcal/mol dS: -419.9 cal/mol dG: -33.9 kcal/mol

Tm Difference: 1.7

GC Difference: 3.1

Right border-PMA16

gattttatttataaaaaattgaaaaaattaatcaaactaaacaataaattattcatataattttataatatatatatacacacataatatataatt  
ttacaattatttataatcattataacttttcaccattatcatattgtctctgataggctataaactatgtattaatgttgctgtacatttctgatagtttaaa  
ctgaaggcgggaacgacaatcagatctagtaggaaacagctatgaccatgattacgccaagcttgcatgccctggcacgacaggtt  
cccgactggaagcgggcagtgagcgcaacgcaatatgtgagttagctcactcattaggcaccccaggttacactttatgcttccg  
gctcgatgttgttggaattgtgagcggatacaatttcacacaggaaacagctatgaccatgattacgccaagctggcgcgccaagct  
tgcatgcctgcaggtcgactctagaggatcagaatcaccgaacctcccctcggtacagctcctcagttctaccatgaatttcatccact  
gattctcttcaatcgccattgca (SEQ-ID-No. 27)

## #1: Product of length 91 (rating: 171)

Contains region of the molecule from 155 to 245

Tm: 66.5 C TaOpt: 46.1 C GC: 34.1

Sense Primer:

TGTCTCTGATAGGCTAATAAACTATG

Similarity: 100.0% (SEQ-ID-No. 28)

Length: 26 Tm: 48.7 C GC: 34.6

dH: -185.4 kcal/mol dS: -493.2 cal/mol dG: -36.6 kcal/mol

Antisense Primer:

TAGATCTGATTGTCGTTTCCC (SEQ-ID-No. 29)

Similarity: 100.0%

Length: 21 Tm: 48.4 C GC: 42.9

dH: -152.6 kcal/mol dS: -398.0 cal/mol dG: -32.2 kcal/mol

Tm Difference: 0.2

GC Difference: 8.2

Figure 2 g of 5

**partial-sequences of the insert with flanking region**

Insertion sequence is bold (T-DNA, Vector), Primer-binding sites are underlined:

**Event B**

Left border - PMA16

**Ggattaaactgagagctcgaatttccccgatcggtcaaacatttggcaataaagtttctaagattgaatcctgttgcgggtcttgcgatg  
attatcatataatttctgtgaattacgttaagcatgtaataaataacatgtaatgcatgacgttattatgagatgggttttatgattagagtc  
gcaattggattgtaccagcaacaagaagacataaaatgcacgttttgccttttaaatgagttagttttatttttatccttcaaattgggtgatgttaatt  
tgtcttttctaatacgaacttatacctagtgatacataagttctttaaataaaaatttagtccgagaataacttatgaaataattacgataaaggataaaact  
tcaatgaccaacacaaaactcg** (SEQ-ID-No.30)

**#1: Product of length 100 (rating: 171)**

Contains region of the molecule from 151 to 250

Tm: 67.1 C TaOpt: 46.8 C GC: 34.0

Sense Primer:

ATGACGTTATTTATGAGATGGGT(SEQ-ID-No. 31)

Similarity: 100.0%

Length: 23 Tm: 49.1 C GC: 34.8

dH: -169.1 kcal/mol dS: -445.1 cal/mol dG: -34.6 kcal/mol

Antisense Primer:

ATTTAAAAGGCAAAACGTGC(SEQ-ID-No. 32)

Similarity: 100.0%

Length: 20 Tm: 49.3 C GC: 35.0

dH: -164.9 kcal/mol dS: -432.6 cal/mol dG: -34.1 kcal/mol

Tm Difference: 0.2

GC Difference: 0.2

Right-border - PMA16

**Ttttttggatggagaaagcagagataatagttatagaattgttggtacccttagatttcaagagtatagaatgatgttagttcatttgagaatagcgct  
atagcagtatatgtcatataaaccagaaacatacaaccatcacctatctcgaatgatcaagccaaacttcccctgcatagtgcttggtgttctt  
agtttaaaacaataatataaacaacaataaataactaatggccgaataattatctgaatttaacaaattttattctcggaataatgatcaacttcc  
caacttagggctcctccacogtgaatcatgtcaacaccttctcccaagggcaataacttagtttctacggcttaagcaataatgcacaagggttga  
gagtcacactttgctttatcgataaattcaatgggaaaactaatgcaactgtccaaatcaccctcccaagtgaagaactcaatgtacagaaa  
ataggtgtttgggttatttctgcataaaattctgaltcagctcaaaatagtagtccaaacccgtgatagcgacaactagtctcactatagcgatctccaca  
ttctgclatagcgagacacttttccacctaactccagaaaaatgtcatttgtacaacacaaactctccttcatgtcgaagtcaatttcaggagttcat  
gccttggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccaccag  
gctttacactttatgcttccggctcgtatgtgtgtggaagttg** (SEQ-ID-No. 33)

**#1: Product of length 94 (rating: 171)**

Contains region of the molecule from 681 to 774

Tm: 73.2 C TaOpt: 51.7 C GC: 50.0

Sense Primer:

TTCATGTCAAGTTCAATTTCAAGG(SEQ-ID-No. 34)

Similarity: 100.0%

Length: 23 Tm: 51.2 C GC: 34.8

dH: -162.8 kcal/mol dS: -423.9 cal/mol dG: -34.6 kcal/mol

Antisense Primer:

ACTCACATTAATTGCGTTGCG(SEQ-ID-No. 35)

Similarity: 100.0%

Length: 21 Tm: 53.0 C GC: 42.9

dH: -161.7 kcal/mol dS: -418.3 cal/mol dG: -35.2 kcal/mol

Tm Difference: 1.8

GC Difference: 8.1

Figure 2 h of 5

**partial-sequences of the insert with flanking region**

Insertion sequence is bold (T-DNA, Vector), Primer-binding sites are underlined:

**Event C**

Left-border - PMA16

**Tgttgccggctctgcatgattatcatataatttctgttgaaatcgttaagcatgtaataattaacatgtaatgcatgacgttatttatgagatg  
ggttttatgattagagtcgccgaattatacatttaatacgcgatagaaaacaaaatagcgcgcaactaggataaattatcgcgcgcg  
gtgtcatctatgttactagatcgggaattcactggccgtcgttttacaacgactcagctgcttggttaataattgtcattagattgttttatgcat  
agatgcactc**caagaatgtaataattaatgtaataaaitcaagaatgtaataattaatgtaataaaitcaagggtcaaggcggttgcttgagaaat  
gagagattgtttagtgactaatgataatgtaataaagaagaccattttagcattcattttacattttcttactatgaatagcagaacaaacgacgcaaat  
agaacctttttctcttccattttctttaaatttgactagcaaatgatgaggtaccacaaatcacaataattaatacttttcaaagatatcaataaaitaat  
gatgatccttaaaatttaggtaaaagtgttgaaactgtcatgattcagaccgtcggtgattgacaccacactagccctccggtgggagaaccattacta  
caaccctaaactaacaatttatgaaaactaaagcatttaagatatagaagcagggtataatgacaataaaaatggagtcccataaactccaa  
ggtttttaacaacaactaatcaactaatgcggaagaacaaccccaaaaactaaacaacaccttaagcaagtctgagtcgaaaagagtggtgac  
ttaaacaagaaagatccatggcagcctgaaagaactgactcacccttgaatccggtcacgctcaatatccgcctaaagatgccctgtactcaacaa  
aaaaacaagcaagtacagtatcagtacacaaccacagagtactggttaggatcacgcgactatcctaataagtgaacacatgcaagtaaccac  
aacattataaaacaagcatataccgaacatatacagtattagtcattttcaagatcaatgtagcatcacacgttctcaataatacacattggttatcatt  
ttagagcagcatacagctctccaatatcaatcatcattag (SEQ-ID-No. 36)

#1: Product of length 118 (rating: 171)

Contains region of the molecule from 247 to 364

Tm: 64.6 C TaOpt: 45.0 C GC: 25.4

Sense Primer:

GCTTGGTAATAATTGTCATTAGATTG (SEQ-ID-No. 37)

Similarity: 100.0%

Length: 26 Tm: 50.8 C GC: 30.8

dH: -192.1 kcal/mol dS: -509.2 cal/mol dG: -38.5 kcal/mol

Antisense Primer:

GCCTTGACCTTTGAATTATTTAC (SEQ-ID-No. 38)

Similarity: 100.0%

Length: 23 Tm: 49.1 C GC: 34.8

dH: -177.4 kcal/mol dS: -469.3 cal/mol dG: -35.7 kcal/mol

Tm Difference: 1.8

GC Difference: 4.0

Figure 2 h - continued

## Right-border - PMA16

Atacaccataccttaggagaaaaacacaactaacgttattttatcctgcaaatggaaaggtctcaaccatagcaacggataggatcattgcagcctt  
taccagcagactgcttgagccaaacgaccccccttgcaagggtactgctggtgatctcctcagctcgataaaatcacatcaaatgactcccaa  
gtgttagatataaattgcgtcataattgaggggtctttaaagttgaattaacaataatattagttgatattgttgacacccattccactggccttttaa  
agtttagcaaggtaattttctttacttgaacaattggaataaaaaaataattattccttgcataatctcaattatatgatagtttaactatattagaaa  
agttaatcgtaataactcatataattgtcaaatattatacgtttgacctcaatctctccgaattacctaagcgtttgggcccataatttgaactctcg  
cttttaacctaaaagggtcgttataattgaattatgaactcactttatgtgacctaaactttcctcgttctgatgcagaatttttaactcaaattcataat  
attcgattctgttattgagtcacaaatcataatattgttcttctattaaatctgattatgaaatctctagtcgctcgtaatttataggctacaatctcatttcc  
ctcaatgaatattcaattctgttattgagttcaaatcataalattcgtttcttctattaaatctgattatgaaatctctagtcgctcgtaatttataggctaca  
atctcattttccaaacactgatagtttaaactgaaggcgggaaacgacaatcagatctagtaggaaacagctatgaccatgattacgcca  
**gcttgcacgcccgtggcagcagaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattagggc**  
**accccggtttacactttatgcttccggctcgatgtgtgtggaattgtgagcggataacaatttcacacaggaaacagctatgaccatg**  
**attacgccaagctggcgcgccaagcttgcacgctgcaggctgactctagaggatctagaatcaccgaacctcccctcggtacagctc**  
**ctc** (SEQ-ID-No. 39)

Contains region of the molecule from 589 to 905

Tm: 70.4 C TaOpt: 50.1 C GC: 30.9

Sense Primer:

TCTGATGCAGAATTTTCTAACTCAA (SEQ-ID-No. 40)

Similarity: 100.0%

Length: 25 Tm: 52.2 C GC: 32.0

dH: -177.8 kcal/mol dS: -465.8 cal/mol dG: -37.1 kcal/mol

Antisense Primer:

TTCCTACTAGATCTGATTGTCGTTTC (SEQ-ID-No. 41)

Similarity: 100.0%

Length: 26 Tm: 52.3 C GC: 38.5

dH: -184.4 kcal/mol dS: -484.7 cal/mol dG: -38.1 kcal/mol

Tm Difference: 0.1

GC Difference: 6.5

Figure 2 i of 5

**partial-sequences of the insert with partial flanking region**

Insertion sequence is bold (T-DNA, Vector), Primer-binding sites are underlined:

**Elite-Event D**

Left-border - PMA16

Attaaatactgagagctegaatttccccgatcggtcacaacatttggcaataaagtttcttaagattgaatcctgttgcgggtcttgcga  
 tgattatcatataatttctgttgaattacgttaagcatgtaataattaacatgtaatgcacgttatttatgagatgggttttatgatt  
 agagtccecgcaattatacatttaatacgcgatagaaaaacaaaatatagcgcgcgaactaggataaattatcgcgcgcgggtgcac  
 tatgttactagatcggaattcactggcgcgtgtttacaacgaactcagctgcttggtaataattgtcattagattgttttatgcatag  
 atgcactcgaaatcagccaattttagacaagtatcaaacggatgttaattcagtacattaaagacgtccgcaatgtgttattaagttg  
 tctaagttgtccttggtttattagttgttattgtgtttgtctttcaatttctagtagtcaattgggaactggaagtacaaagtgaatttttagactta  
 tgtatacaaatgaaaatgtatcaaaaagtagtgacactataaaaagaacaattctttcttgagaacatcttcaaccacctctattttactctc  
 cattctctatattttagtgagtataaatagagaatgggcactccaaccaccttcate (SEQ-ID-No. 42)

**Primer pair1**

Sense Primer:

TCAAACGGATGTTAATTCAGTACATT (SEQ-ID-No. 4)

Similarity: 100.0%

Length: 26 Tm: 53.2 C GC: 30.8

dH: -189.1 kcal/mol dS: -496.8 cal/mol dG: -39.2 kcal/mol

Antisense Primer:

CCAGTTCCCAATTGACTACTAGAAA (SEQ-ID-No.5)

Similarity: 100.0%

Length: 25 Tm: 52.9 C GC: 40.0

dH: -184.0 kcal/mol dS: -482.6 cal/mol dG: -38.3 kcal/mol

Tm Difference: 0.3

GC Difference: 9.2

**Primer pair2**

Tm: 72.1 C TaOpt: 47.7 C GC: 32.9

Sense Primer:

TCTGTTGAATTACGTTAAGC (SEQ-ID-No. 6)

Similarity: 100.0%

Length: 20 Tm: 42.4 C GC: 35.0

dH: -147.0 kcal/mol dS: -389.3 cal/mol dG: -29.2 kcal/mol

Antisense Primer:

CTCAGAAGAAAGAATTGTTC (SEQ-ID-No. 7)

Similarity: 100.0%

Length: 20 Tm: 40.4 C GC: 35.0

dH: -140.5 kcal/mol dS: -372.5 cal/mol dG: -27.7 kcal/mol

Tm Difference: 2.0

GC Difference: 0.0



Figure 2i – continued

Primer pair 2

Contains region of the molecule from 17 to 898  
 Tm: 71.5 C TaOpt: 48.9 C GC: 30.0  
 Sense Primer:  
 AACTGAATTTTGGGATTGAG (SEQ-ID-No. 14)  
 Similarity: 100.0%  
 Length: 20 Tm: 45.9 C GC: 35.0  
 dH: -150.1 kcal/mol dS: -393.9 cal/mol dG: -30.9 kcal/mol  
 Antisense Primer:  
 GAGTCAGTTAAATTAAGTCTTCAG (SEQ-ID-No. 15)  
 Similarity: 100.0%  
 Length: 25 Tm: 48.8 C GC: 36.0  
 dH: -179.0 kcal/mol dS: -474.3 cal/mol dG: -35.8 kcal/mol  
 Tm Difference: 2.9  
 GC Difference: 1.0

Primer pair 3

Contains region of the molecule from 118 to 949  
 Tm: 72.3 C TaOpt: 48.8 C GC: 32.3  
 Sense Primer:  
 ACAAGAATAGCAAGGATTATCC (SEQ-ID-No. 16)  
 Similarity: 100.0%  
 Length: 22 Tm: 46.8 C GC: 36.4  
 dH: -165.6 kcal/mol dS: -438.2 cal/mol dG: -33.2 kcal/mol  
 Antisense Primer:  
 GAAGTTCGAACAACATTCTT (SEQ-ID-No. 17)  
 Similarity: 100.0%  
 Length: 20 Tm: 43.4 C GC: 35.0  
 dH: -144.2 kcal/mol dS: -379.8 cal/mol dG: -29.2 kcal/mol  
 Tm Difference: 3.4  
 GC Difference: 1.4

Figure 2 j of 5

**Elite Event D****partial-sequences of the insert with partial flanking region**

Insertion sequence is bold (T-DNA, Vector), Primer-binding sites are underlined:

**Upper case bold – Blb1 expression cassette (promoter – gene –terminator)****Upper case bold italic – AHAS expression cassette (promoter – gene –terminator)****Lower case italic – flanking regions at left border**

Extra-primer for flanking region (left border) to BLB1 (over AHAS cassette):

Part of blb1 - blb1 terminator - ahas-cassette - flanking region left border

**GTGAACTAGGAAACCTAAATCTCTATGGCTCAATTAAATCTCGCATCTTGAGAGAGTGAAGAA**  
**TGATAAGGACGCAAAAGAAGCCAATTTATCTGCAAAAGGGAATCTGCATTCTTTAAGCATGAGT**  
**TGGAATAACTTTGGACCACATATATATGAATCAGAAGAAGTTAAAGTGCTTGAAGCCCTCAAAC**  
**CACATCCAATCTGACTTCTTTAAATCTATGGCTTCAGAGGAATCCATCTCCACAGTGGAT**  
**GAACTCACTCAGTATTGAAAAATATTGTCTCTATTCTAATTAGCAACTTCAGAAAGTGCATGC**  
**TTACCACCCCTTTGGTGATCTGCCTTGTCTAGAAAGTCTAGAGTTACACTGGGGTCTGCGGATG**  
**TGGAGTATGTTGAAGAAGTGGATATTGATGTTTCATTCTGGATTCCCACAAGAATAAGGTTTCC**  
**ATCCTTGAGGAAACTTGATATATGGGACTTTGGTAGTCTGAAAGGATTGCTGAAAAAGGAAGGA**  
**GAAGAGCAATTCCCTGTGCTTGAAGAGATGATAATTCACGAGTGCCCTTTTCTGACCCCTTTCTT**  
**CTAATCTTAGGGCTCTTACTTCCCTCAGAATTTGCTATAATAAAGTAGCTACTTCATTCCCGA**  
**AGAGATGTTCAAAACCTTGCAAATCTCAAATACTTGACAATCTCTCGGTGCAATAATCTCAAA**  
**GAGCTGCCTACCAGCTTGGCTAGTCTGAATGCTTTGAAAAGTCTAAAAATTCAATTGTGTTGCG**  
**CACTAGAGAGTCTCCCTGAGGAAGGGCTGGAAGGTTTATCTTCACTCACAGAGTTATTTGTTGA**  
**ACACTGTAACATGCTAAAAATGTTTACCAGAGGGATTGCAGCACCTAACAACCCCTACAAGTTTA**  
**AAAATTTCGGGGATGTCCACAACCTGATCAAGCGGTGTGAGAAGGGAATAGGAGAAGACTGGCACA**  
**AAATTTCTCACATTCCCTAATGTGAATATATATATTTAAGTTATTTGCTATTGTTTCTTTGTTTG**  
**TGAGTCTTTTTTGGTTCCCTGCCATTGTGATTGCATGTAATTTTTTTCTAGGGTTGTTTCTTTATG**  
**AGTCTCTCTCTCATTGGATGTAATTTTCTTTTGAAACAAATCTGTCAATTGATTGTATTATA**  
**CGCTTTCAGAATCTATTACTTATTTGTAATTGTTTCTTTGTTTGTAATTTGTGAGTATCTTATT**  
**TTATGGAATTTTCTGATTTTATTTTGAAACAAATCAATGATTGTGTAAGATCCATCTGTATTAT**  
**ACTCCCTTCGTCTCATTTTATGTGTCACCTGTGCGGATTTGAGATTCAAACAAATCTATCTTTG**  
**ATCGTAAATTTTAAATAGATCTTTTAAACATTTTGAATTATCAATTATTGTGACTTTAGTGGCT**  
**AGACTAGTGGATCCGATATCGCCCAGCTTCACGCTGCCGCAAGCACTCAGGGCGCAAGGGCTGC**  
**TAAAGGAAGCGGAACACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCA**  
**GCTACTGGGCTATCTGGACAAGGGAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGG**  
**GCTTACATGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCAGGAATTGCCAGCT**  
**GGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAACTGGATGGCTTTCTTGCCGCCAA**  
**GGATCTGATGGCGCAGGGGATCAAGATCATGAGCGGAGAATTAAGGGAGTCACGTTATGACCCC**  
**CGCCGATGACGCGGGACAAGCCGTTTTACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAGC**  
**CACTCAGCCGCGGGTTTCTGGAGTTTAAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGC**  
**GTTCAAAAGTCGCCTAAGGTCATATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCACTGA**  
**CGTTCCATAAATCCCTCGGTATCCAATTAGAGTCTCATATTCACTCTCAATCCAGATCCCCG**  
**GGTACCATGGCGGCGGCAACAACAACAACAACATCTTCTTCGATCTCCTTCTCCACCAAAC**  
**CATCTCCTTCTCCTCCAAATCACCATTACCAATCTCCAGATTCTCCCTCCCATTTCTCCCTAAA**  
**CCCCAACAAATCATCCTCCTCCTCCCGCCGCGCGGTATCAAATCCAGCTCTCCCTCCTCCATC**  
**TCCGCCGTGCTCAACACAACCACCAATGTCACAACCACTCCCTCTCAACCAAACCTACCAAAC**  
**CCGAAACATTATCTCCCGATTGCTCCAGATCAACCCCGCAAAGGCGCTGATATTCTCGTCTGA**



*GGCTTTAGAACGTCAAGGCGTAGAAACCGTATTCGCTTACCCTGGAGGTGCATCAATGGAGATT  
CACCAAGCCTTAACCCGCTCTTCCTCAATCCGTAAAGTCCTTCCTCGTCACGAACAAGGAGGTG*

Figure 2j - continued

TATTTCGCAGCAGAAGGATACGCTCGATCCTCAGGTAAACCAGGTATCTGTATAGCCACTTCAGG  
 TCCCGGAGCTACAAATCTCGTTAGCGGATTAGCCGATGCGTTGTTAGATAGTGTTCTCTTGTA  
 GCAATCACAGGACAAGTCCCTCGTCGTATGATTGGTACAGATGCGTTTCAAGAGACTCCGATTG  
 TTGAGGTAACGCGTTCGATTACGAAGCATAACTATCTTGTGATGGATGTTGAAGATATTCTTAG  
 GATTATTGAGGAGGCTTTCTTTTCTAGCTACTTCTGGTAGACCTGGACCTGTTTTGGTTGATGTT  
 CCTAAAGATATTCAACAACAGCTTGCGATTCTTAATTGGGAACAGGCTATGAGATTACCTGGTT  
 ATATGTCTAGGATGCCTAAACCTCCGGAAGATTCTCATTGAGCAGATTGTTAGGTTGATTTT  
 TGAGTCTAAGAAGCCTGTGTTGTATGTTGGTGGTGGTTGTTTGAAGTCTAGCGATGAATTGGGT  
 AGGTTTGTGAGCTTACGGGAATCCCTGTTGCGAGTACGTTGATGGGGCTGGGATCTTATCCTT  
 GTGATGATGAGTTGTGTTACATATGCTTGGAAATGCATGGGACTGTGTATGCAAATTACGCTGT  
 GGAGCATAGTGATTTGTTGTTGGCGTTTGGGGTAAGGTTTGTATGATCGTGTACGGGTAAACTT  
 GAGGCTTTTGTAGTAGGGCTAAGATTGTTTCATATTGATATTGACTCGGCTGAGATTGGGAAGA  
 ATAAGACTCCTCATGTGTCTGTGTGTGGTGTATGTTAAGCTGGCTTTGCAAGGGATGAATAAGGT  
 TCTTGAGAACCAGCGGAGGAGCTTAACTTGATTTTGGAGTTTGGAGGAATGAGTTGAACGTA  
 CAGAAACAGAAAGTTTCCGTTGAGCTTTAAGACGTTTGGGGAAGCTATTCCTCCACAGTATGCCA  
 TTAAGGTCTTGATGAGTTGACTGATGGAAAAGCCATAATAAGTACTGGTGTCTGGGCAACATCA  
 AATGTGGGCGGCGCAGTTCTACAATTACAAGAAACCAAGGCAGTGGCTATCATCAGGAGGCCCTT  
 GGAGCTATGGGATTTGGAATTCCTGCTGCGATTGGAGCGTCTGTTGCTAACCCTGATGCGATAG  
 TTGTGGATATTGACGGAGATGGAAGTTTATAATGAATGTGCAAGAGCTAGCCACTATTCGTGT  
 AGAGAATCTTCCAGTGAAGGTACTTTTATTAAACAACCAGCATCTTGGCATGGTTATGCAATGG  
 GAAGATCGGTTCTACAAAGCTAACCAGCACACACATTTCTCGGAGATCCGGCTCAGGAGGACG  
 AGATATTCCCGAACATGTTGCTGTTTGCAGCAGCTTGCGGGATTCCAGCGGCGAGGGTGACAAA  
 GAAAGCAGATCTCCGAGAAGCTATTCAGACAATGCTGGATACACCAGGACCTTACCTGTTGGAT  
 GTGATTTGTCCGCACCAAGAACATGTGTTGCCGATGATCCCGAATGGTGGCACTTTCAACGATG  
 TCATAACGGAAGGAGATGGCCGGATTAAATACTGAGAGCTCGAATTTCCCGATCGTTCAAACA  
 TTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATT  
 TCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGG  
 GTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCG  
 CAAACTAGGATAAATTATCGCGCGCGGTGTCTATGTTACTAGATCGGGAATTCAGTGGCCG  
 TCGTTTTACAACGACTCAGCTGCTTGGTAATAATTGTCATTAGATTGTTTTATGCATAGATGC  
 ACTCGAAATCAGCCAATTTTAGACAAGTATCAAACGGATGTTAATTCAGTACATTAAAGACGTC  
 CGCAATGTGTTATTAAGTTGTCTAAGTtgtccttggtttttattagttgttattgtgtgtttgtc  
 ttttcaatttctagtagtcaattgggaactggaagtacaaagttgaatttttagacttatgtat  
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 catcttcaaccacctctattttactctccattctctatatatttagtgagtataatagagaatgg  
 gcaactccaaccacctccatatcactctccattcttcatatttagagagtcataattttttat  
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 aatattcataaattcttttttaaatgtcatattataatttttaaaaaatatattattttaatatatact  
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 tttaaatacaagataaaaatacaatacataataataatcaattcgtgatgaaacaagaatcat  
 gccaaaaagatatattgaacgtgcattcgaagttttgcaatcacgttttgcaattattgcaagacc  
 gtcacgtttttgagagaaaggaagtgtagatgatataatgactacatgtattatactgcacacc  
 atgataattgaggatgaacatgatcttaatgcaccaaatacaagatgccgtagaggctccaaactc  
 caacgacaaaaatgatggttagatgaaaatcttcggtttgaacaatttttagctagacataaaaa  
 agttaaggacaaaaatgctcattttgaactccgtaatgcattaatagagcattttatgacagcaa

*cgtgataatttcgaaacttgagtgtttatgtaattatatttcacttttatttgaatttgtccat  
taatttgtatattatataatatatttatttacaatttatgttattttaaaaatttagaataaaaataa*

Figure2j - continued

aattttgaaaaataaaaattttatatcacatgaaaattatataaagtaattattggggaaaaaa  
 ataaaggatttatattatgaaataagaaaaataagaatgaatagaaataataataatataatatt  
 gaggagaaataattatTTTTTTtagagagtgaatatagagaattgggttgaaattgattgtctga  
 aaaaaataaaaaactctatatTTTggagagtaaaatagagtgtagggttgagacgtca (SEQ-  
 ID-No. 44)

Tm: 77.5 C TaOpt: 51.6 C GC: 43.2  
 Sense Primer:  
 GTGAAGTAGGAAACCTAAATC (SEQ-ID-No. 10)  
 Similarity: 100.0%  
 Length: 21 Tm: 42.0 C GC: 38.1  
 dH: -153.6 kcal/mol dS: -409.4 cal/mol dG: -29.8 kcal/mol  
 Antisense Primer:  
 CAACTAATAAAACCAAGGAC (SEQ-ID-No. 11)  
 Similarity: 100.0%  
 Length: 20 Tm: 41.1 C GC: 35.0  
 dH: -149.5 kcal/mol dS: -398.5 cal/mol dG: -28.9 kcal/mol  
 Tm Difference: 1.0  
 GC Difference: 3.1

Extra-primer for flanking region (right border) to BLB1 (over blb2.cassette):  
 Part of blb1 - blb1 terminator - ahas-cassette - flanking region left border (SEQ-ID-No.45)

Lower case bold – flanking regions at right border  
 Upper case *italic*— Blb2 expression cassette (promoter – gene –terminator)  
 Upper case **bold** – Blb1 expression cassette (promoter – gene –terminator)

gaaactcagttggagattacgtttaagggatggcctacatggataaggttatctacaatgatgc  
 aatggagataataatagttactcctatgtgatttcttcgttttaatttggtttattttattttcat  
 ttaatttgatttgatacgtagtttaaaaaaataaaaaagatatTTTaaattttgtgatgttagat  
 gaaacatacgttaaattgcatcaaaatgtcatttaaatttgtccttgaacatatagtttagaaag  
 ttgaaattaaagagctatcaaaaaagaagaagatatgactaaaaaaatagcacaagcaaattg  
 aaactaaaagagtaatttaacaataacaatttttttttaaaatgatacataatactcactctttc  
 tcaatatgtggcacaaaaatatcaagaatcaaactatttaatttttgactataaaatttagaaata  
 aatttttttaaatttcttaaaaaacaaaattttacaaagcaaatactatatataaaaaatattataaa  
 ttataataattaataattcaaaatagtttaaccatacaaaactgaattttgggattgagtttctat  
 tttaaattttcgtgagtaaagattaaagttcaaattaactttttataaaaatgagttttcaatttt  
 aaaaagtcaaacaaaatatatatagaatataaagtgaagaaacaaataaaaacaagaatagcaag  
 gattatccatttaggaataaagatgaagaatcaaatcaactaataaaaactttaatcctataa  
 gataagcttgccaataattttaaaatagcatgatgctgcttaagagggtatcaatatattgttatc  
 ctttaaagaccaagatagtggtttcaggaaagttattttcatttccaaatttaaataaaaagaaaat  
 aattattcaaaattcttatgtcttttttatttgatttatagtcgaagatctttcaaaaataattt  
 ttctataggtaaaaaaggataatttCAATTTACACAGGAAACAGCTATGACCATGATTACGCC  
 AAGCTGGCGCGCCAAGCTTGCATGCCTGCAGGTGCACTCTAGAGGATCTAGAATCACCGAACCT  
 CCCCTCGGTACAGCTCCTCCAGTTCTACCATGAATTTTCATCCACTGATTCCTCTTCAATCGCCA  
 TTGCAGATTCTCTCGATCTATGCTCAAAAAATCCCAGATAAAACCCTAGATCTGCTTCAAATG

Figure2j - continued

CTCTGATACCATGTAATTTTCAGTGAATTTCTAACTAAACAATGGAGAGAATTAAGTATTTTAGAA  
 AGACTGATTGAAGGAGAAGAAGAGAGAAAAATTCTATATTGAACTCATGAACCAAATGAATGA  
 AAAAAATAATGAGAAGAACTATACTATTACAATCTATATATCTCTATTTATATTCTAATCTGAA  
 GCAGTTAATTTAACTGACTCTAACTAGACTGATAGGTGTACATTTTCTGTTAGTGCAGTGC  
 AGTGCATTTAACTAACTGCTTAACATAAAGAATGTTGTTTCTGAACCTTCATTCGAATAGCTTCAAT  
 GAGAAGCAAACATGTGTACCTGTAAAGACACACAGTAAAAGTGTTAATAATGAATAAATATGAA  
 TAAATCAAATAATAAATTAATAAATAAACAACATCCAATTAACATTGGAGGTCTTGAAAATCGA  
 TGGTAATTAACAAAGACCCTTGTGAAATTTAAGTCTGTAATTGAAAATTTGAGTATAGGTTAGG  
 GGACATTTGACTATTTTCTCATTTTCTTTATCTTTTCTTAATTTGTGGCAGACAAGTGAGGAG  
 GCCCCACTGTAATTGATTCATGCTTTTGCTTTCTTGACTTTTGGAAACAATACTATGCATCATA  
 TTTGGTCTTAATTATTCTCTGTTTATTTCCAGAATTTTGAGCTCTATACATCTAATAACAAAG  
 CAAGCAGAGGATATATAGTTTCATCACTAAAGGTTAGTCAACTCATCTAATATTTGCTACT  
 CTCATCTCTATTGAAGTACAGTTATGGAAAAGTAGAAGTGATGTAAGAAAAATGAAAGAACTTT  
 AGTAGGTTAGTTGGATCTAACAAAGAGAAAGGGAAATAAATTGCAGGAGAAAGAGAGAGGTTAA  
 ATACTTACTCACACCACCGATTTACAACAAATCACTTAATTGTGGTTAGTTAATGTATACTTTT  
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 GTGAATTTCTACCGGGTGAGAGGGATGGGGTTGGAGAGTGTGGAGTGAACAGAAGCAGATGTTT  
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 CAAAAGGCTGTTGATGTGGATCTGATTGAAAGCCTGAAATTGAAGCTGACATTTATTTGTACAT  
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 GGTTGAGAATCTGCTTCAACCAATTTTGGATGATGATGGCAAAGACGTCGGGTGTAAATATGTC  
 CTTACTAGCCTCGCCGGTAATATGGATGACTGTATAAGCTTGTATCATCGTTCTAAATCAGATG  
 CCACCATGATGGATGAGCAATTGGGCTTCTCTCTTGAATCTCTCTCATCTATCCAAGCATCG  
 TGCTGAAAAGATGTTTCTGAGTGACTCAATATGAGGTTCTTCAGAATGTATGTGGCAACATA  
 AGAGATTTCCATGGATTGATAGTGAATTGTTGCATTAAGCATGAGATGGTTGAGAATGTCTTAT  
 CTCTGTTTCAACTGATGGCTGAGAGAGTAGGACGCTTCTTTGGGAGGATCAGGCTGATGAAGA  
 CTCTCAACTCTCCGAGCTAGATGAGGATGATCAGAATGATAAAGACCTCAACTCTTCAAGCTA  
 GCACATCTACTCTTGAAGATTGTTCCAACCTGAATTGGAGGTTATGCACATATGTTATAAACTT  
 TGAAAGCTTCAACTTCAACAGAAATTGGACGCTTCATTAAGAAGCTCCTGGAAACCTCTCCGGA  
 CATTCTCAGAGAATATCTGATTCATCTACAAGAGCATATGATAACTGTTATTACCCCTAACACT  
 TCAGGGGCTCGAAACATTCATGTCATGATGGAATTCCTATTGATTATTCTTTCTGATATGCCGC  
 CCAAGGACTTTATTCATCATGACAACTTTTTGATCTCTTGGCTCGTGTGTAGCACTTACCAG  
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 CATAGATTCAATTATTGTTTCGAGATAATGGTCTCTTACATCTTATTTTCTCACTTCCCATACC  
 AGAAAGAAGATGATGCTTATCAAAGAAGAGGTCTCTGATTTACATGAGAACATTTCCAAGAACA  
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GATCTAGATGTCATTTGATCATTGGTATGCCGGGTTTAGGTAAAACTACTTTGGCGTACAAAG  
TATACAATGATAAATCAGTTTCTAGCCATTTGACCTTCGTGCATGGTGCACGGTCGACCAAGT

Figure2j - continued

ATATGACGAGAAGAAGTTGTTGGATAAAAATTTTCAATCAAGTTAGTGACTCAAATTCAAAATTG  
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 GAAAGGAAGTAGAATTATTTTGACAACTCGAGAAAAGAAAGTTGCTTTGCATGGAAAGCTCTAC  
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 CATTTGGAAACGAGAGTTGCCCTGATGAACTATTGGATGTTGGTAAAGAAATAGCCGAAAATTG  
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 AGTGTGTGGCTTGAAGTTGTAAATAATTTGCATTCCTTTATTTTGAAGAATGAAGTGGAAAGTGA  
 TGAAAGTTATAGAAATAAGTTATGACCACCTTACCTGATCACCTGAAGCCATGCTTGCTGTACTT  
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GAAGTATTGAATTTGTCATGAATATCAACATTCTTCATCCTAGTTAATTCTTTTTCAATTTTA  
ATAGACTCTCATTTTAATCACTAATATTCTTCTATTTGTGACTTCTTTCTGCAGGTGGCAACT  
TTAAATTCATAAAGTATAGGATTGATGACAACTCGAAAAATATCTTAATGAGGTGAAGTTGA



Figure2j - continued

GCAGTCAGCAGATGGTGGTTCCAACCTCTAAGTTGACAAGCACATACTATCCCGGAGGGCGATTT  
 CAAGCCTGATGCATATGGTTAGTGTGGCTAGAGCAGACAGGATGTATTACCTGGATATCTACCA  
 AGACGAATCCACAATCAGTTTTATGTCAAGCAATACATGAAGTAACTCCCGATAGAACAGTAAA  
 AGCAAGATGTGTAGGTGTATCTCGACTCTAAGAGATTGTACATTCTCTTTGAGATTTTTACTG  
 CTAATACAAATTTACACCTCAGAAGCGAATCTAGAATTTCTAGAGCATGAATGCACCCTAATG  
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 ACAAACATCCAACGTTGGTAGGATTAATGGAGGGATCGCATCCAGGAGGATACTGTAGAAAA  
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TCTTTAGAAATCCACCTGTCTAACTCATCCACTACCCATTCCCTTTGCTTTGAATTCTTTTCTT  
TACCTATAAACTTGGAACACTCGATCCGTTTTGCTTTTCTTAACAAAGCAGCTCAGAGAAAAGA

Figure 2j continued

GGTTCCTTCTATTCTGTTTCTCTGTGTGCTGCACTTGGGTCCTTAATCCCATTAAAAACAGGG  
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 TAGGGGATATATTGGACCAAAAGTAGAATGGGTATATATTTAAAGTATTTCTGATAGAACAGGA  
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 TATATGAATCAGAAGAAGTTAAAGTGCTTGAAGCCCTCAAACCACACTCCAATCTGACTTCTTT  
 AAAATCTATGGCTTCAGAGGAATCCATCTCCCAGAGTGGATGAATCACTCAGTATTGAAAAAT  
 ATTGTCTCTATTCTAATTAGCAACTTCAGAACTGCTCATGCTTACCACCCTTTGGTGATCTGC  
 CTTGTCTAGAAAGTCTAGAGTTACACTGGGGGTCTGCGGATGTGGAGTATGTTGAAGAAGTGGA  
 TATTGATGTTTCATTCTGGATTCCCCACAAGAATAAGGTTTCCATCCTTGAGGAACTTGATATA  
 TGGGACTTTGGTAGTCTGAAAGGATTGCTGAAAAAGGAAGGAGAAGAGCAATTCCTGTGCTTG  
 AAGAGATGATAATTCACGAGTGCCCTTTTCTGACCCTTTCTTCTAATCTTAGGGCTCTTACTTC  
 CCTCAGAATTTGCTATAATAAAGTAGCTACTTCATTCCCAGAAGAGATGTTCAAAAACCTTGCA  
 AATCTCAAATACTTGACAATCTCTCGGTGCAATAATCTCAAAGAGCTGCCTACCAGCTTGGCTA  
 GTCTGAATGCTTTGAAAAGTCTAAAAATTCAATTGTGTTGCGCACTAGAGAGTCTCCCTGAGGA  
 AGGGCTGGAAGGTTTATCTTCACTCACAGAGTTATTTGTTGAACACTGTAACATGCTAAATGT  
 TTACCAGAGGGATTGCAGCACCTAACAACCCTCACAAGTTTAAAAATTCGGGGATGTCCACAAC  
 TGATCAAGCGGTGTGAGAAGGGAATAGGAGAAGACTGGCACAAAATTTCTCACATTCCTAATGT  
 GAATATATATATTTAAGTTATTTGCTATTGTTTCTTTGTTTGTGAGTCTTTTGGTTCCTGCCA  
 TTGTGATTGCATGTAATTTTTTTCTAGGGTTGTTTCTTTATGAGTCTCTCTCTCATTGGATGTA  
 ATTTTCTTTTGGAAACAAATCTGTCAATTGATTTGTATTATACGCTTTCAGAATCTATTACTTA  
 TTTGTAATTGTTTCTTTGTTTGTAAATTGTGAGTATCTTATTTTATGGAATTTTCTGATTTTAT

TTTGAAAACAAATCAATGATTTGTAAGATCCATCTGTATTATACTCCCTTCGTCTCATTTTATG  
TGTCACCTGTCGGATTTTCGAGATTCAAACAAATCTATCTTTGATCGTAAATTTTAAATAGATCT  
TTTAAACATTTTGAATTATCAATTATTGTGACTTTAGT

Figure2j - continued

Tm: 74.2 C TaOpt: 50.3 C GC: 35.0  
Sense Primer:  
AACTGAATTTTGGGATTGAG(SEQ-ID-No. 18)  
Similarity: 100.0%  
Length: 20 Tm: 45.9 C GC: 35.0  
dH: -150.1 kcal/mol dS: -393.9 cal/mol dG: -30.9 kcal/mol  
Antisense Primer:  
ATGTAGCAGCATTGAGTTTT(SEQ-ID-No. 19)  
Similarity: 100.0%  
Length: 20 Tm: 44.2 C GC: 35.0  
dH: -147.0 kcal/mol dS: -387.0 cal/mol dG: -29.8 kcal/mol  
Tm Difference: 1.7  
GC Difference: 0.0

Figure 2k of 5

Bib1-expression cassette (SEQ-ID-No. 46)

Upper case italic— promoter

Upper case bold – Bib1

Upper case italic underlined – terminator

TTGATTTGTCATGTTGCACTTTTCGAAAGTCAATTTGACTAATTTTTTAAAGCTAAATTAGATTA  
 CACTAATTCATATTTTTAAACAGAAAAATTAGATATTCAAAACTATACAAAAAATTATATACA  
 TTGCAATTTTTTGCATATCAATATGATAAAAAAATATATCGTAAAAATATTAGTCAAAATTTTTTA  
 TAATTTGACTCAAATCATGAAAAGTATAATAATTAATAGTGGACGGAGGAAGTATTGTCTTTCC  
 AGATTTGTGGCCATTTTTGGTCCAAGGGCCATTAGCAGTTCCTTCATTTTCTACTTCTGTCTC  
 ATATTAGATGGGCATCTTACTAAAAATATTTGTCTCATATTACTTGATTATTTATTAAATCAAA  
 AAGAATTAATTAATTTTTTCTCATTTTACCCCTACAATTAATATAGTTTTTAAAGTTTTAAACA  
 AATTTTGAAGAATCAAAATTTCTTTTGGCAAGAGACTTATTAATATAAAACAAAGGATAAAATAA  
 TAAAATTTGTCAATTTATTGACGATCACTTAATAATCATATAAAATAGAAAATGTTTATCTAAT  
 ATGAGACGGAGAAAATATATCCTAAAATATTTTTGGACAGATATGTGATATTCTAACCATTAC  
 TAGACTATATTATGCATTTTAGCCGCCAATGACTTATTCAGCTTTAATTAATTAGGAAAGAGG  
 AAAC TGCCAATGAGGAAGAGTAGGGGCGTAGTTGCTGTCGACGAAAAAAGATAATACTCACTC  
 TTTTCGATTTTTATTTTTATTTATCACTTTTAACTATCATGTAAAAAGATAATTATTTTTTTC  
 ATGCTTTATCCTTAGTATTAAACAATTTAATAGGGATTATTTTGTAAAATATTTATATGAATAA  
 TTGTTTTCGTAATGAATTTGTCCGGTCAAACAATGATAAATAAAATGAATGAAGAGAGTAGAA  
 AACAAAACAAAAGAACAAGTTGACAACCTTGAGAGATTAAAAGGGTCCAAAACGCCTTGGATTTT  
 GAGATTCCATATGTGAAATTTCCATGAAATAATTGAATTTGTATTATTACAAGTCAAACCTTCC  
 ATTTCAATCCAAC TAGCCATCTTGGTTTCAAATTACACATTCATTCAATTCACAGATCTAATAT  
 TCTTAATAGTGATTTCCACATATGGCTGAAGCTTTCAATCAAGTTCTGCTAGACAATCTCACTT  
 CTTTCCTCAAAGGGGAACCTTGATTGCTTTTCGGTTTTCAAGATGAGTTCCAAAGGCTTTCAAG  
 CATGTTTTCTACAATTCAGCCGTCCTTGAAGATGCTCAGGAGAAGCAACTCAACAACAAGCCT  
 CTAGAAAATTGGTTGCAAAAACCTCAATGCTGCTACATATGAAGTCGATGACATCTTGGATGAAT  
 ATAAAACCAAGGCCACAAGATTCTCCAGTCTGAATATGGCCGTTATCATCCAAAGGTTATCCC  
 TTTCCGTCACAAGGTCGGGAAAAGGATGGACCAAGTGATGAAAAACTAAAGGCAATTGCTGAG  
 GAAAGAAAGAATTTTCATTTGCACGAAAAAATTGTAGAGAGACAAGCTGTTAGACGGGAAACAG  
 GTACTCATCTTAAATTAGTATTACAACAACCTAAGTTTATATTCATTTTTTTGGCAATTATCAAA  
 TTCAGAAAAGGGTTAAATATACTCATGTCCTATCGTAAATAGTGTATATATACCTCTCGTTGTA  
 CTTTCGATCTGAATATACTTGTCAAATCTGGCAAGCTCAGAATCAAATTATCCACCCCACTTT  
 TAAATACTCGATATCTTTAGAAATCCACCTGTCTAACTCATCCACTACCCATTCCCTTTGCTTT  
 GAATTCCTTTCTTTACCTATAAACTTGGAACACTCGATCCGTTTTGCTTTTCTTAACAAAGCAG  
 CTCAGAGAAAAGAGGTTTTCTTCTATTCTGTTTCTCTGTGTGCTGCACTTGGGTCCCTTAATCCC  
 ATTAACAAACAGGGCATGTTAATCCCAACGACGGTAGCCTTTCCTGACAGCTGACTGTAAATTTT  
 GTCTAACAAAGAAAAAAGATTAGACATGTTTTTCTTGTGCTGATTAGGCTGGATTTCTT  
 TCAGAGTGGAACATAGGGGATATATTGGACCAAAAGTAGAATGGGTATATATTAAAGTATTTT  
 TGATAGAACAGGAGTATATTGTGCGAAAATATCCTCTATTTTCTGTTGTCTCCTAATGAGTTTG  
 AATGTAATAATATTTCTCATGTGGACATTGCTTGACCAGGTTCTGTATTAAACCGAACCGCAGGT  
 TTATGGAAGAGACAAAGAGAAAGATGAGATAGTGAAAATCCTAATAAACAATGTTAGTGATGCC  
 CAACACCTTTCAGTCCTCCCAATACTTGGTATGGGGGGATTAGGAAAAACGACTCTTGCCCCAA  
 TGGTCTTCAATGACCAGAGAGTTACTGAGCATTTCCATTCCAAAATATGGATTGTGTCTCGGA  
 AGATTTTGATGAGAAGAGGTTAATAAAGGCAATTGTAGAATCTATTGAAGGAAGGCCACTACTT  
 GGTGAGATGGAATGGCTCCACTTCAAAGAAGCTTCAGGAGTTGCTGAATGGAAAAAGATACT  
 TGCTTGTCTTAGATGATGTTTGAATGAAGATCAACAGAAGTGGGCTAATTTAAGAGCAGTCTT  
 GAAGGTTGGAGCAAGTGGTGCTTCTGTTCTAACCCTACTCGTCTTGAAAAGGTTGGATCAATT

Figure 2k - continued

ATGGGAACATTGCAACCATATGAACTGTCAAATCTGTCTCAAGAAGATTGTTGGTTGTTGTTCA  
 TGCAACGTGCATTTGGACACCAAGAAGAAATAAATCCAAACCTTGTGGCAATCGGAAAGGAGAT  
 TGTGAAAAAAGTGGTGGTGTGCCTCTAGCAGCCAAAACCTTGGAGGTATTTTGTGCTTCAAG  
 AGAGAAGAAAGAGCATGGGAACATGTGAGAGACAGTCCGATTTGGAATTTGCCTCAAGATGAAA  
 GTTCTATTCTGCCTGCCCTGAGGCTTAGTTACCATCAACTTCCACTTGATTTGAAACAATGCTT  
 TGCCTATTGTGCGGTGTTCCCAAAGGATGCCAAAATGGAAAAAGAAAAGCTAATCTCTCTCTGG  
 ATGGCGCATGGTTTTCTTTTATCAAAGGAAACATGGAGCTAGAGGATGTGGGCGATGAAGTAT  
 GGAAAGAATTATACTTGAGGTCTTTTTTCCAAGAGATTGAAGTTAAAGATGGTAAAACCTATTTT  
 CAAGATGCATGATCTCATCCATGATTTGGCAACATCTCTGTTTTTCAGCAAACACATCAAGCAGC  
 AATATCCGTGAAATAAATAAACACAGTTACACACATATGATGTCCATTGGTTTTCGCCGAAGTGG  
 TGTTTTTTTTTACACTCTTCCCCCCTTGGAAAAGTTTATCTCGTTAAGAGTGCTTAATCTAGGTGA  
 TTCGACATTTAATAAGTTACCATCTTCCATTGGAGATCTAGTACATTTAAGATACTTGAACCTG  
 TATGGCAGTGGCATGCGTAGTCTTCCAAAGCAGTTATGCAAGCTTCAAAATCTGCAAACTCTTG  
 ATCTACAATATTGCACCAAGCTTTGTTGTTTGCCAAAAGAAACAAGTAAACTTGGTAGTCTCCG  
 AAATCTTTTACTTGATGGTAGCCAGTCATTGACTTGTATGCCACCAAGGATAGGATCATTGACA  
 TGCCTTAAGACTCTAGGTCAATTTGTTGTTGGAAGGAAGAAAGGTTATCAACTTGGTGAAGTAG  
 GAAACCTAAATCTCTATGGCTCAATTAATAATCTCGCATCTTGAGAGAGTGAAGAATGATAAGGA  
 CGCAAAAGAAGCCAATTTATCTGCAAAAGGGAATCTGCATTCTTTAAGCATGAGTTGGAATAAC  
 TTTGGACCACATATATATGAATCAGAAGAAGTTAAAGTGCTTGAAGCCCTCAAACCACACTCCA  
 ATCTGACTTCTTTAAAAATCTATGGCTTCAGAGGAATCCATCTCCCAGAGTGGATGAATCACTC  
 AGTATTGAAAAATATTGTCTCTATTCTAATTAGCAACTTCAGAAACTGCTCATGCTTACCACCC  
 TTTGGTGATCTGCCTTGTCTAGAAAGTCTAGAGTTACACTGGGGGTCTGCGGATGTGGAGTATG  
 TTGAAGAAGTGGATATTGATGTTCAATTCTGGATTCCCCACAAGAATAAGGTTTCCATCCTTGAG  
 GAACTTGATATATGGGACTTTGGTAGTCTGAAAGGATTGCTGAAAAAGGAAGGAGAAGAGCAA  
 TTCCCTGTGCTTGAAGAGATGATAATTCACGAGTGCCCTTTTCTGACCCCTTCTTCTAATCTTA  
 GGGCTCTTACTTCCCTCAGAAATTTGCTATAATAAAGTAGCTACTTCATTCCCAGAAGAGATGTT  
 CAAAACCTTGCAAACTCAAATACTTGACAACTCTCTCGGTGCAATAATCTCAAAGAGCTGCCT  
 ACCAGCTTGGCTAGTCTGAATGCTTTGAAAAGTCTAAAAATTCAATTGTGTTGCGCACTAGAGA  
 GTCTCCCTGAGGAAGGGCTGGAAGGTTTATCTTCACTCACAGAGTTATTTGTTGAACACTGTAA  
 CATGCTAAAATGTTTACCAGAGGGATTGCAGCACCTAACCAACCCTCACAAAGTTTAAAAATTTCGG  
 GGATGTCCACAACTGATCAAGCGGTGTGAGAAGGGAATAGGAGAAGACTGGCACAAAATTTCTC  
 ACATTCCTAATGTGAATATATATATTTAAGTTATTTGCTATTGTTTCTTTGTTTGTGAGTCTTT  
TTGGTTTCCTGCCATTGTGATTGCATGTAATTTTTTTCTAGGGTTGTTTCTTTATGAGTCTCTCT  
CTCATTGGATGTAATTTTCTTTTGGAAACAAATCTGTCAATTGATTTGTATTATACGCTTTCAG  
AATCTATTACTTATTTGTAATTGTTTCTTTGTTTGTAAATTGTGAGTATCTTATTTTATGGAAT  
TTTCTGATTTTATTTTGAACAAATCAATGATTTGTAAGATCCATCTGTATTATACTCCCTTC  
GTCTCATTTTATGTGTCACCTGTGCGATTTCGAGATTCAAACAAATCTATCTTTGATCGTAAAT  
TTTTAATAGATCTTTTAAACATTTTGAATTATCAATTATTGTGACTTTAGT

Figure 2k - continued

B1b2-expression cassette (SEQ-ID-No. 47)

Upper case *italic*— promoterUpper case **bold** – B1b2Upper case *italic underlined* – terminator

AACCTCCCCCTCGGTACAGCTCCTCCAGTTCTACCATGAATTTTCATCCACTGATTCTCTTCAAT  
CGCCATTGCAGATTCTCTCGATCTATGCTCAAAAAATCCCGAGATAAAACCCTAGATCTGCTTC  
AAATGCTCTGATACCATGTAATTTTCAGTGAATTTCTAACTAAACAATGGAGAGAATTAACATTTT  
TAGAAAGACTGATTGAAGGAGAAGAAGAGAGAAAAATTCTATATTGAACTCATGAACCAAAATG  
AATGAAAAAATAATGAGAAGAACTATACTATTACAATCTATATATCTCTATTTATATTCTAAT  
CTGAAGCAGTTAATTTAACTGACTCTAACAACCTAGACTGATAGGTGTACATTTTCTGTTAGTGC  
ACTGCAGTGCATTTAACTAACTGCTTAACATAAAGAATGTTGTTTCGAACTTCATTCTGAATAGCT  
TCAATGAGAAGCAACATGTGTACCTGTAAAGACACACAGTAAAGTGTTAATAATGAATAAAT  
ATGAATAAATCAAATAATAAATTAATAAATAAACAACATCCAATTAACATTGGAGGTCTTGAAA  
ATCGATGGTAATTAACAAAGACCCTTGTGAAATTTAAGTCTGTAATTGAAAATTTGAGTATAGG  
TTAGGGGACATTTGACTATTTTCTCATTTTCTTTATCTTTTTCTTAATTTGTGGCAGACAAGTG  
AGGAGGCCCCACTGTAATTGATTCATGCTTTTGTCTTCTTGACTTTTTGGAACAATACTATGCA  
TCATATTTGGTCTTAATTATTCCTCTGTTTATTTCCAGAATTTTGAGCTCTATACATCTAATAA  
CAAAGCAAGCAGAGGATATATAGTTTCATCAACTAAAAAGGTTAGTCAACTCATCTAATATTTG  
CTACTCTCATCTCTATTGAAGTACAGTTATGGAAAAGTAGAAGTGATGTAAGAAAAATGAAAGA  
ACTTTAGTAGGTTAGTTGGATCTAACAAAGAGAAAGGGAAATAAATTGCAGGAGAAAGAGAGAG  
GTTAAATACTTACTCACACCACCGATTTACAACAAATCACTTAATTGTGGTTAGTTAATGTATA  
CTTTCACCTCATTAAATTATTACTTACCCATGATAAGTTGTATTAATTTGGTATTAATATCCGG  
TGCGGGTGAATTTCTTACCGGGTGAGAGGGATGGGGTTGGAGAGTGTGGAGTGAACAGAAGCAGA  
TGTTTTTAGATTTTTTCTAAGATGACGAAAGATTTCCCTCACTAATGAAAATATATTACTATACG  
CTATTAGAGATAGAAAGGTTCCGTACCAGTTGGTCTCGTTTCTGGATGAACCCCATTTTTACAA  
GTCATTTTCTTCAATTCAAATCGCAAGTGACCTTTATCATCTTCCACTAATTAAGTCCTCTTA  
AGTTCGCGTGAAAAATAGTGAAATTATTGATTATTCTTATCATTTTCATCTTCTTCTCTGATAA  
AGTTTTATGTACTTTTTATGCATCAGGTCTTGAGAACTTGGAAGGAAAAGTAGAATC**ATGGAA**  
**AAACGAAAAGATAATGAAGAAGCAAACAACCTATTGGTATGTTATTTGATAGAGTGAAGTGTAA**  
**AGTATTGAATTGTAGATATCATGTGGCTTTAAAAATTTGATATGTGTTATTTTGGCAGGAGTCA**  
**TTTTCTGCTCTTCGCAAGGATGCTGCCAATGTTCTGGATTTCTAGAGAGATTAAAGAATGAAG**  
**AAGATCAAAAGGCTGTTGATGTGGATCTGATTGAAAGCCTGAAATTGAAGCTGACATTTATTTG**  
**TACATATGTCCAGCTTCTTATTCCGATTTGGAGAAGTTTGAAGATATAATGACTAGAAAAAGA**  
**CAAGAGGTTGAGAATCTGCTTCAACCAATTTTGGATGATGATGGCAAAGACGTCGGGTGTAAAT**  
**ATGTCCTTACTAGCCTCGCCGGTAATATGGATGACTGTATAAGCTTGTATCATCGTTCTAAATC**  
**AGATGCCACCATGATGGATGAGCAATTGGGCTTCCTCCTCTTGAATCTCTCTCATCTATCCAAG**  
**CATCGTGCTGAAAAGATGTTTCTGGAGTGACTCAATATGAGGTTCTTCAGAATGTATGTGGCA**  
**ACATAAGAGATTTCCATGGATTGATAGTGAATTGTTGCATTAAAGCATGAGATGGTTGAGAATGT**  
**CTTATCTCTGTTTCAACTGATGGCTGAGAGAGTAGGACGCTTCCTTTGGGAGGATCAGGCTGAT**  
**GAAGACTCTCAACTCTCCGAGCTAGATGAGGATGATCAGAATGATAAAGACCCTCAACTCTTCA**  
**AGCTAGCACATCTACTCTTGAAGATTGTTCCAACCTGAATTGGAGGTTATGCACATATGTTATAA**  
**AACCTTTGAAAGCTTCAACTTCAACAGAAATTGGACGCTTCATTAAGAAGCTCCTGGAAACCTCT**  
**CCGGACATTCTCAGAGAATATCTGATTCATCTACAAGAGCATATGATAACTGTTATTACCCCTA**  
**ACACTTCAGGGGCTCGAAACATTTCATGTCATGATGGAATTCCTATTGATTATTCTTTCTGATAT**  
**GCCGCCCAAGGACTTTATTCATCATGACAAACTTTTTGATCTCTTGGCTCGTGTGTAGCACTT**  
**ACCAGGGAGGTATCAACTCTTGTACGCGACTTGGAAAGAGAAATTAAGGATTAAAGAGAGTACTG**  
**ACGAAACAAATTGTGCAACCCTAAAGTTTCTGGAAAATATTGAACCTCTTAAGGAAGATCTCAA**



Figure 2k - continued

ACATGTTTATCTGAAAGTCCCGGATTCATCTCAATATTGCTTCCCCATGAGTGATGGACCTCTC  
 TTCATGCATCTGCTACAGAGACACTTAGATGATTTGCTGGATTCCAATGCTTATTCAATTGCTT  
 TGATAAAGGAACAAATTGGGCTGGTGAAAGAAGACTTGGAATTCATAAGATCTTTTTTCGCGAA  
 TATTGAGCAAGGATTGTATAAAGATCTCTGGGAACGTGTTCTAGATGTGGCATATGAGGCAAAA  
 GATGTCATAGATTCAATTATTGTTGAGATAATGGTCTCTTACATCTTATTTTCTCACTTCCCA  
 TTACCAGAAAGAAGATGATGCTTATCAAAGAAGAGGTCTCTGATTTACATGAGAACATTTCCAA  
 GAACAGAGGTCTCATCGTTGTGAACTCTCCCAAGAAACCAGTTGAGAGCAAGTCATTGACAACCT  
 GATAAAATAAATTGTAGGTTTTGGTGAGGAGACAACTTGATACTTAGAAAGCTCACCAGTGGAC  
 CGGCAGATCTAGATGTCATTTTCGATCATTGGTATGCCGGGTTTAGGTAAAACCTACTTTGGCGTA  
 CAAAGTATACAATGATAAATCAGTTTCTAGCCATTTTCGACCTTCGTGCATGGTGCACGGTTCGAC  
 CAAGTATATGACGAGAAGAAGTTGTTGGATAAAATTTTCAATCAAGTTAGTGACTCAAATTCAA  
 AATTGAGTGAGAATATTGATGTTGCTGATAAACTACGGAAACAATTGTTTGGAAAGAGGTATCT  
 TATTGTCTTAGATGACGTGTGGGATACTAATACATGGGATGAGCTAACAAGACCTTTTCTGAT  
 GGTATGAAAGGAAGTAGAATTATTTTGACAACCTCGAGAAAAGAAAGTTGCTTTGCATGGAAAGC  
 TCTACACTGATCCTCTTAACCTTCGATTGCTAAGATCAGAAGAAAGTTGGGAGTTATTAGAGAA  
 AAGGGCATTGGAACGAGAGTTGCCCTGATGAACATTGGATGTTGGTAAAGAAATAGCCGAA  
 AATTGTAAAGGGCTTCCTTTGGTGGTGGATCTGATTGCTGGAATCATTGCTGGGAGGGAAAAGA  
 AAAAGAGTGTGTGGCTTGAAGTTGTAAATAATTTGCATTCTTTTATTTTGAAGAATGAAGTGGA  
 AGTGATGAAAGTTATAGAAATAAGTTATGACCACCTTACCTGATCACCTGAAGCCATGCTTGCTG  
 TACTTTGCAAGTGCGCCGAAGGACTGGGTAAACGACAATCCATGAGTTGAAACTTATTTGGGGTT  
 TTGAAGGATTTGTGGAAGACAGATATGAAGAGTCTGGAAGAAAGTGGTGAAGATTTATTTGGA  
 TGATTTAATTTCCAGTAGCTTGGTAATTTGTTTCAATGAGATAGGTGATTACCCTACTTGCCAA  
 CTTTCATGATCTTGTGCATGACTTTTGTGTTGATAAAAGCAAGAAAGGAAAAGTTGTGTGATCGGA  
 TAAGTTCAAGTGCTCCATCAGATTTGTTGCCACGTCAAATTAGCATTGATTATGATGATGATGA  
 AGAGCACTTTGGGCTTAATTTTGTCTCTGTTCTGTTCAAATAAGAAAAGGCATTCCGGTAAACAC  
 CTCTATTCTTTGACCATAAATGGAGATGAGCTGGACGACCATCTTTCTGATACATTTTCATCTAA  
 GACACTTGAGGCTTCTTAGAACCTTGACCTGGAATCCTCTTTTATCATGGTTAAAGATTCTTT  
 GCTGAATGAAATATGCATGTTGAATCATTGAGGTACTTAAGCATTGGGACAGAAGTTAAATCT  
 CTGCCTTTGTCTTTCTCAAACCTCTGGAATCTAGAAATCTTGTGTTGTGGATAACAAAGAATCAA  
 CTTGATACTATTACCGAGAATTTGGGATCTTGTAAGTTGCAAGTGCTGTTCCGACTGCTTG  
 TTCTTTCTTTGATATGGATGCAGATGAATCAATACTGATAGCAGAGGACACAAAGTTAGAGAAC  
 TTGACAGCATTAGGGGAACCTCGTGCTTTCTTATTGGAAAGATACAGAGGATATTTTCAAAAGGC  
 TTCCCAATCTTCAAGTGCTTCATTTCAAACCTCAAGGAGTCATGGGATTATTCAACAGAGCAATA  
 TTGGTTCCCGAAATTTGATTTTCTAACTGAAC TAGAAAACTCACTGTAGATTTTGAAAGATCA  
 AACACAAATGACAGTGGGTCTCTGCAGCCATAAATCGGCCATGGGATTTTCACTTTCTTTCGA  
 GTTTGAAAAGATTGCAATTGCATGAATTTCTCTGACATCCGATTCACTATCAACAATAGCGAG  
 ACTGCTGAACCTTGAAGAGTTGTACCTTTATCGTACAATCATCCATGGGGAAGAATGGAACATG  
 GGAGAAGAAGACACCTTTGAGAATCTCAAAATGTTTGATGTTGAGTCAAGTGATTCTTTCCAAGT  
 GGGAGGTTGGAGAGGAATCTTTTCCACGCTTGAGAAATTAGAACTGTCGGACTGTCATAATCT  
 TGAGGAGATTCCGTCTAGTTTTGGGGATATTTATTCCTTGAAAATTATCGAAGTTGTAAGGAGC  
 CCTCAACTTGAAAAATCCGCTCTCAAGATTAAGGAATATGCTGAAGATATGAGGGGAGGGGACG  
 AGCTTCAGATCCTTGGCCAGAAGGATATCCCGTTATTTAAGTAGTTTTTGAGCATTATGGTTGA  
 AAAGTAGATTGCACCTTTGCTGGGTAGATTGTATATGGTTAAGAAAATTCTGTTACAGTTGTTAT  
 GAAACATTTTTATTTGACTTTTCTGAGTTTCTTTTAGAAAACTCAGAAGTTTTTAACAAAAATT  
 ATAGTTTTTATAAATACAATGTGGATTTGCCCTTTGGCTGTCCAACTTGGTCTGAAGTCTCATAT  
 GCTCAGAGCACTATCGTTCAACCTCAATCAAGGTACTGATTTAAAATGACATCTATACTACTTT  
 ATCACAACCCCAACGAACCTTCATCTCAAAAGCTAGGCCAGGAAGTGAAGAGTTGTAGAGAGC

TTATAAGCACTCATGACTTCCTTTTCTCGAACATTCAACCAACGTAGGCTGAAATCCCACTCTG  
AACGAAAATAAGTGTTTGTTTATCAAATTAACCTCTCGTAGTAGAACACTGAAATACCTTCTTCT

Figure 2k - continued

AAACGTTCAACAAATGGGATTTCCAGCACTCAAAGTGAATGAAAGGTTACATTAATCTTCAAA  
 AAGAATTACGACAATTCATGACCACAAGTACATTGACAGCACCATTTCAACAGAAGAACAAGTC  
 AATGCTGCATCTTCATCAATAATCCGAGTGTGCAACCTCCTTCCTGACACTGTCCTGTATATGT  
 AAAGTTTCTCAACAGGGCAACTTTCTGGTCTCGTATCTGGATGACCCCTCTCGTCTATAACTTC  
 AACATTAAGCCCTGGCAACTTCTGGACCAACAGCTTACATGCTTCAAACTTACTGAACAATTA  
 GACATCCAAAGGGATCGCATTGTCTCCAGCTTTGCAGCATTAGCCAAACAGAGCCTCATCGCCAA  
 AGGGGCAGTCTCTAATCTCGAATTTGAAAAAATTGTTGTTGTATGACTTTCCTCTGACATCCGA  
 TGCATATCAACAATAGCAAGACTGGAGGTTGGAGAGGAATCCTTTATTATACAATCATTACAGG  
 GAGAAGAATGGAACATGGGGGAGGAAGACACTTTTGAGAATCTGAAATGTGTTAGAGCCACAAG  
 CTACAGAAGTATTGAATTTGTCTATGAATATCAACATTCTTCATCCTAGTTAATTCTTTTTCAAT  
 TTTTAATAGACTCTCATTTTAAATCACTAATATTCTTCTATTTGTGACTTCTTTTCTGCAGGTGG  
 CAACTTTAAATTCATAAAGTATAGGATTGATGACAACTCGAAAAATATCTTAATGAGGTGAAG  
 TTTGAGCAGTCAGCAGATGGTGGTTCCAACTCTAAGTTGACAAGCACATACTATCCCGGAGGGC  
 GATTTCAAGCCTGATGCATATGGTTAGTGTGGCTAGAGCAGACAGGATGTATTACCTGGATATC  
 TACCAAGACGAATCCACAATCAGTTTTATGTCAAGCAATACATGAAGTAACTCCCGATAGAACA  
 GTAAAAGCAAGATGTGTAGGTGTATCTCGACTCTAAGAGATTGTACATTCTCTTTGAGATTTT  
 TACTGCTAATACAAATTTACACCTCAGAAGCGAATCTAGAATTTCTAGAGCATGAATGCACCAC  
 TAATGAAAGGAGAAAAAAGGAAGTATGAAGTGGGAATTTGATCCTTGTTTTCTAGGTATATAAAA  
 TTTATCATTCAACTATACTTCATTTAGCAACAACCTCTCTTTGCCATTATTTCTCAAACAAGGG  
 CTTCTAATATTGCTAAACTAAAGACTGTCAAAGGTAAGTTCATCTTCAAACCTCTCTTGTTTAC  
 TTTATCTAAAGGGGAACTATGAAAAACAAGAAACATCAGGAATGTCCCGTAAACAAAGCAGCCT  
 CATGCACAAAACATCCAACGTTGGTAGGATTAATGGAGGGATCGCATCCCAGGAGGATACTGTA  
 GAAAAATTAGTGGCTTCTTTCACCGCTCAAACCCATGATCTATAGGTTACATGGAGACAACCTT  
 ATGGTTGCTCGTAGGCTCCCGTCAATTCTCATAAACCACAACACCAAAGTTGCATCAGACATCA  
 TCTTCATTCAACAAGCTGACAATCTCCACAAGTCTTAGTCAACTTGTAATATGAATATTAGCCAG  
 GTAGACGTACATATTTACAAAATTGAGTTTCCTATATAATATGGTTTGAAGGAATGAAACATGA  
 TGGGGAGGGTAGATAAAATAATATATGAGGCATAAAAAATAGGAAAGATATTTGTAGTGAGAGGT  
 TTTGACTTTTTATGCTGCTTTTGATCTTCAGTTTCTTGATTCTTTTTCTACTGCTTTCCTCTT  
 CTTTCTCCTGAGTAAAGTTTTATGTAGGTACTTTTTATACGTCCGATCGTGAGAACTTGAAAGA  
 AAGCTCTCTATAGCTATGTTAGGTGCCACATAAAAAAATGAAATATTACAAAAACCCTGATAA  
 TAAAAATACACTAATCTAAGATATTCAGTGCAACATACATGCAAAATATATATATATAAATTTTC  
 ATGAAAAATTATAACAAATAATAGATGTGAACATATAACTTTAAAAATAATATTACATCCATAAA  
 GCTTAAATTCTAGA

Figure 2k - continued

Ahas expression cassette (SEQ-ID-No. 48)

Upper case italic— promoter

Upper case bold – AHAS

Upper case italic underlined – terminator

*GATCATGAGCGGAGAATTAAGGGAGTCACGTTATGACCCCCGCCGATGACGCGGGACAAGCCGT*  
*TTTACGTTTGGAACTGACAGAACC****GCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGAGT***  
*TTAATGAGCTAAGCACATACGTCAGAAAACCATTATTGCGCGTTCAAAAGTCGCCTAAGGTCACT*  
*ATCAGCTAGCAAAATTTTCTTGTCAAAAAATGCTCCACTGACGTTCCATAAAATTCCCCTCGGTAT*  
*CCAATTAGAGTCTCATATTC****ACTCTCAATCCAGATCCCCGGGTACC******ATGGCGGCGGCAACAACA***  
***ACAACAACAACATCTTCTTCGATCTCCTTCTCCACCAAAACCATCTCCTTCCTCCTCCAAATCAC***  
***CATTACCAATCTCCAGATTCTCCCTCCCATTCTCCCTAAACCCCAACAAATCATCTCCTCCTC***  
***CCGCCGCCGCGGTATCAAATCCAGCTCTCCCTCCTCCATCTCCGCCGTGCTCAACACAACCACC***  
***AATGTCACAACCACTCCCTCTCCAACCAAACTACCAACCCGAAACATTCTCCTCCGATTGCG***  
***CTCCAGATCAACCCCGCAAAGGCGCTGATATTCTCGTCGAGGCTTTAGAACGTCAAGGCGTAGA***  
***AACCGTATTGCGTTACCCTGGAGGTGCATCAATGGAGATTCACCAAGCCTTAACCCGCTCTTCC***  
***TCAATCCGTAACGTCCTTCTCCTCGTCACGAACAAGGAGGTGTATTGCGCAGCAGAAGGATACGCTC***  
***GATCCTCAGGTAAACAGGTATCTGTATAGCCACTTCAGGTCCCGGAGCTACAAATCTCGTTAG***  
***CGGATTAGCCGATGCGTTGTTAGATAGTGTTCTTGTAGCAATCACAGGACAAGTCCCTCGT***  
***CGTATGATTGGTACAGATGCGTTTCAAGAGACTCCGATTGTTGAGGTAACGCGTTTCGATTACGA***  
***AGCATAACTATCTTGTGATGGATGTTGAAGATATTCTTAGGATTATTGAGGAGGCTTTCTTTTT***  
***AGCTACTTCTGGTAGACCTGGACCTGTTTTGGTTGATGTTTCTAAAGATATTCAACAACAGCTT***  
***GCGATTCTTAATTGGGAACAGGCTATGAGATTACCTGGTTATATGTCTAGGATGCCTAAACCTC***  
***CGGAAGATTCTCATTTGGAGCAGATTGTTAGGTTGATTTCTGAGTCTAAGAAGCCTGTGTTGTA***  
***TGTTGGTGGTGGTTGTTTGAACCTAGCGATGAATTGGGTAGGTTTGTGAGCTTACGGGAATC***  
***CCTGTTGCGAGTACGTTGATGGGGCTGGGATCTTATCCTTGTGATGATGAGTTGTCGTTACATA***  
***TGCTTGGAATGCATGGGACTGTGTATGCAAAATTACGCTGTGGAGCATAGTGATTTGTTGTTGGC***  
***GTTTGGGGTAAGGTTTGTGATCGTGTACGGGTAAACTTGAGGCTTTTGCTAGTAGGGCTAAG***  
***ATTGTTTCATATTGATATTGACTCGGCTGAGATTGGGAAGAATAAGACTCCTCATGTGTCTGTGT***  
***GTGGTGATGTTAAGCTGGCTTTGCAAGGGATGAATAAGGTTCTTGAGAACCGAGCGGAGGAGCT***  
***TAACTTGATTTTGGAGTTTGGAGGAATGAGTTGAACGTACAGAAACAGAAGTTTCCGTTGAGC***  
***TTTAAGACGTTTGGGGAAGCTATTCCTCCACAGTATGCGATTAAGGTCCTTGATGAGTTGACTG***  
***ATGGAAAAGCCATAATAAGTACTGGTGTGCGGCAACATCAAATGTGGGCGGCGCAGTTCTACAA***  
***TTACAAGAAACCAAGGCAGTGGCTATCATCAGGAGGCCTTGAGCTATGGGATTTGGA******CTTCT***  
***GCTGCGATTGGAGCGTCTGTTGCTAACCTGATGCGATAGTTGTGGATATTGACGGAGATGGAA***  
***GTTTTATAATGAATGTGCAAGAGCTAGCCACTATTCTGTAGAGAATCTTCCAGTGAAGGTACT***  
***TTTATTAAACAACCAGCATCTTGGCATGGTTATGCAATGGGAAGATCGGTTCTACAAAGCTAAC***  
***CGAGCACACACATTTCTCGGAGATCCGGCTCAGGAGGACGAGATATTCCCGAACATGTTGCTGT***  
***TTGCAGCAGCTTGCGGGATTCCAGCGGCGAGGGTGACAAAGAAAGCAGATCTCCGAGAAGCTAT***  
***TCAGACAATGCTGGATACACCAGGACCTTACCTGTTGGATGTGATTGTCCGCACCAAGAACAT***  
***GTGTTGCCGATGATCCCGAATGGTGGCACTTTCAACGATGTCATAACGGAAGGAGATGGCCGGA***  
***TTAAATACTGAGAGCTCGAATTTCCCCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGAT***  
***TGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGT***  
***AATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAA***  
***TTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCG***  
***CGGTGTCATCTATGTTACTAGATC***

Figure 2 I of 5

Primer for detection blb1 and blb-2 in transformed potato plants using real time-PCR

5'-TGT TGA ACA CTG TAA CAT GCT AAA ATG-3' (forward Primer; SEQ ID No. 49)

5'-AGT TGT GGA CAT CCC CGA ATT-3' (backward Primer; SEQ ID No. 50)

5'-AGA GGG ATT GCA GCA CCT AAC AAC CCT C-3' (Probe; SEQ ID No. 51)

Rpi-blb2:

5'-TTC AAA ACC CCA AAT AAG TTT CAA C-3' (forward Primer; SEQ ID No. 52)

5'-CCA TGC TTG CTG TAC TTT GCA-3' (backward Primer; SEQ ID No. 53)

5'-CGT TAC CCA GTC CTT CGG CG-3' (Probe; SEQ ID No. 54).

Figure 2 m of 5

Primers for Tail PCR-Amplification of flanking Sequences

Name	Sequence	Position in VC- PMA16	Comment
07-038_P25	AACGATGTCATAACGGAAGG (SEQ-ID-No. 55)	16136	Specific primer for tail PCR (LB1)
07-038_P26	AGAGCATTGAAGCAGATCTAGGGT (SEQ-ID-No. 56)	464	Specific primer for tail PCR (RB1)
07-038_P27	CGGATTAAATACTGAGAGCTCGAAT (SEQ-ID-No. 57)	16163	Specific primer for tail PCR (LB2)
07-038_P28	CAGATCTAGGGTTTTATCTCGG (SEQ-ID-No. 58)	454	Specific primer for tail PCR (RB2)
07-038_P29	TGCCGGTCTTGCGATGATTA (SEQ-ID-No. 59)	16241	Specific primer for tail PCR (LB3)
07-038_P30	AGATCTAGGGTTTTATCTCGGGATT (SEQ-ID-No. 60)	450	Specific primer for tail PCR (RB3)

In addition, four arbitrary degenerate (AD) primers were synthesized according to Liu et al 1995):

TGWGNAGWANCASAGA-3' (ADI) (SEQ-ID-No. 61),

W may be A or T

S may be G or C

N may be A, T, C, or G

AGWGNAGWANCAWAGG-3' (AD2) (SEQ-ID-No. 62),

W may be A or T

N may be A, T, C, or G

CAWCGICNGAIASGAA-3' (AD3,) (SEQ-ID-No. 63),

W may be A or T

S may be G or C

I is inosine

N may be A, T, C, or G

TCSTICGNACITWGGA-3' (AD4) (SEQ-ID-No. 64)

W may be A or T

S may be G or C

I is inosine

N may be A, T, C, or G

**Figure 2 n of 5**

Primers for detection of specific events A, B, and C

Event	Border	Sense primer	SEQ ID No.	Antisense primer	SEQ ID No.	Annealing temperature [°C]	Product length (bp)
<b>A</b>	LB	TAATTCAGTACATT AAAGACGTCCG	65	GTCCCATAGTCAT TTCTTGATCA	66	50	63
	RB	TGTCTCTGATAGGC TAATAAACTATG	67	TAGATCTGATTGT CGTTTCCC	68	48	91
<b>B</b>	LB	ATGACGTTATTTAT GAGATGGGT	69	ATTTAAAAGGCAA AACGTGC	70	49	100
	RB	TTCATGTCAAGTTC AATTCAGG	71	ACTCACATTAATT GCGTTGCG	72	51	94
<b>C</b>	LB	GCTTGGTAATAATT GTCATTAGATTG	73	GCCTTGACCTTTG AATTATTTAC	74	49	118
	RB	TCTGATGCAGAATT TTCTAACTCAA	75	TTCCTACTAGATC TGATTGTCGTTTC	76	52	317

Figure 3 of 5

Event D - flanking sequences and insert  
18723 bp

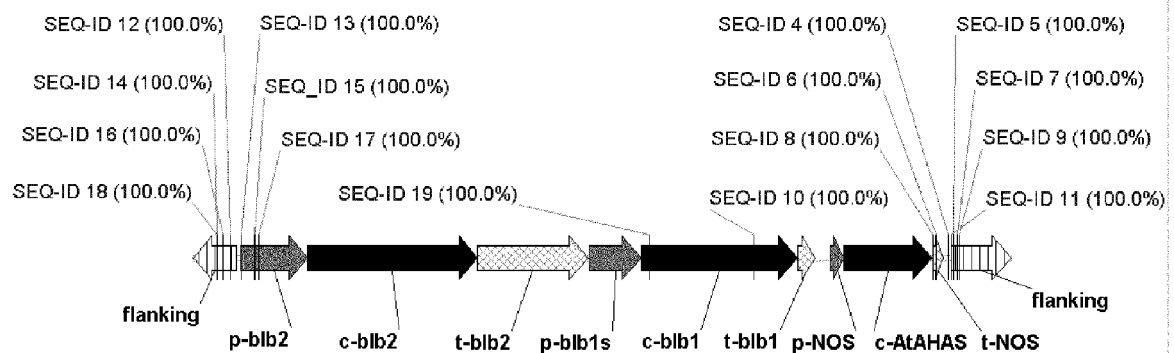




Figure 4 of 5

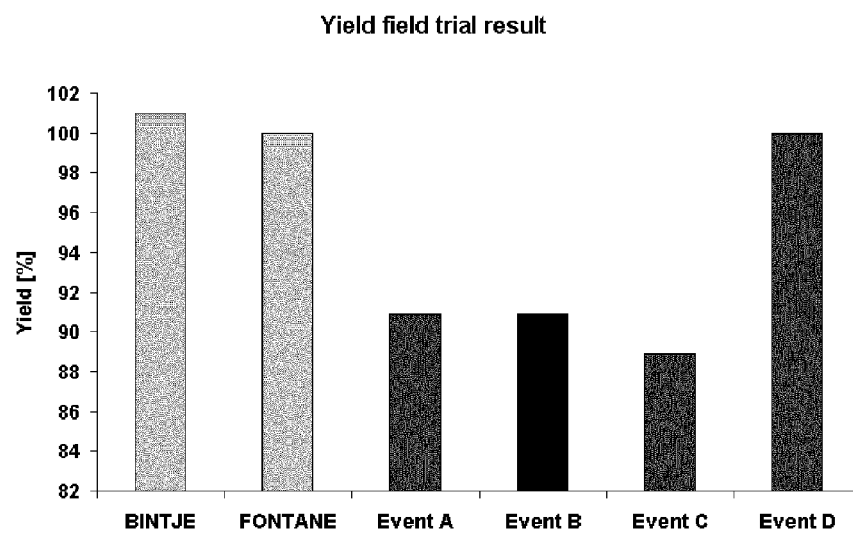
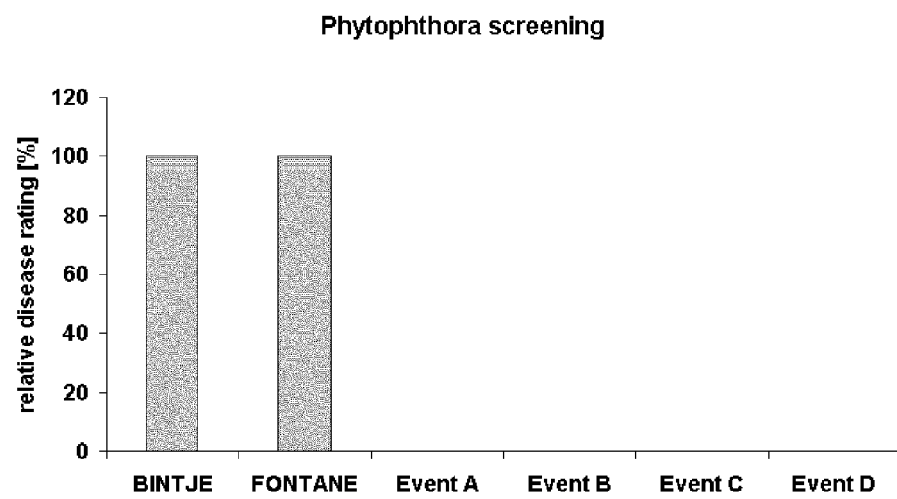


Figure 5 of 5





## EUROPEAN SEARCH REPORT

Application Number  
EP 12 16 8070

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X,D	W0 2008/034876 A1 (BASF PLANT SCIENCE GMBH [DE]; FRANK MARKUS [DE]; SPEAKMAN JOHN-BRYAN []) 27 March 2008 (2008-03-27) * abstract * * page 8, line 21 - page 9, line 7 * * page 22, line 11 - line 19 * * page 28, line 1 - line 2 * * page 39, line 9 - line 11 * * page 57, line 10 - line 17 *	1,9-11	INV. C12N15/82
X	MCCLOUGHLIN T: "INSPECTORS REPORT ON A LICENCE APPLICATION", INTERNET CITATION  , 13 April 2006 (2006-04-13), XP002466181, Retrieved from the Internet: URL:http://www.epa.ie/downloads/pubs/other/ /gmo/field/epa_gm_trial_inspection_report.pdf [retrieved on 2008-01-25] * page 10 *	1,9-11	TECHNICAL FIELDS SEARCHED (IPC)
X	ANONYMOUS: "Notification Number B/NL/05/03", INTERNET CITATION  , 30 August 2005 (2005-08-30), XP002466222, Retrieved from the Internet: URL:http://gmoinfo.jrc.ec.europa.eu/gmp_report.aspx?CurNot=B/NL/05/03 [retrieved on 2008-01-24] * the whole document *	1,9-11	C12N
The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 8 November 2012	Examiner Mundel, Christophe
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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EPO FORM 1503 03-82 (P04C01)



## EUROPEAN SEARCH REPORT

Application Number  
EP 12 16 8070

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	<p>Pavel Forint: "Decision - Reference Number: 4697/ENV/07", The Ministry of the Environment of the Czech Republic</p> <p>, 10 May 2007 (2007-05-10), XP002655466, Retrieved from the Internet: URL: <a href="http://www.mzp.cz/www/webdav_biosafety.nsf/biosafety/pdf/decision_4697_ENV_07.pdf">http://www.mzp.cz/www/webdav_biosafety.nsf/biosafety/pdf/decision_4697_ENV_07.pdf</a> [retrieved on 2011-08-01] * page 2 * * Purpose of the release; page 7 *</p> <p style="text-align: center;">-----</p>	1,9-11	
			TECHNICAL FIELDS SEARCHED (IPC)
The present search report has been drawn up for all claims			
Place of search <b>Munich</b>		Date of completion of the search <b>8 November 2012</b>	Examiner <b>Mundel, Christophe</b>
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	

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EPO FORM 1503 03/92 (P04C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 12 16 8070

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

08-11-2012

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		WO 2008034876 A1	27-03-2008
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