



(86) Date de dépôt PCT/PCT Filing Date: 1998/02/05
(87) Date publication PCT/PCT Publication Date: 1998/08/13
(45) Date de délivrance/Issue Date: 2007/01/09
(85) Entrée phase nationale/National Entry: 1999/06/29
(86) N° demande PCT/PCT Application No.: CA 1998/000055
(87) N° publication PCT/PCT Publication No.: 1998/035051
(30) Priorités/Priorities: 1997/02/10 (US60/036,946);
1997/06/04 (US08/868,786)

(51) Cl.Int./Int.Cl. *C12N 15/82* (2006.01),
C12N 9/10 (2006.01), *A01H 5/00* (2006.01)

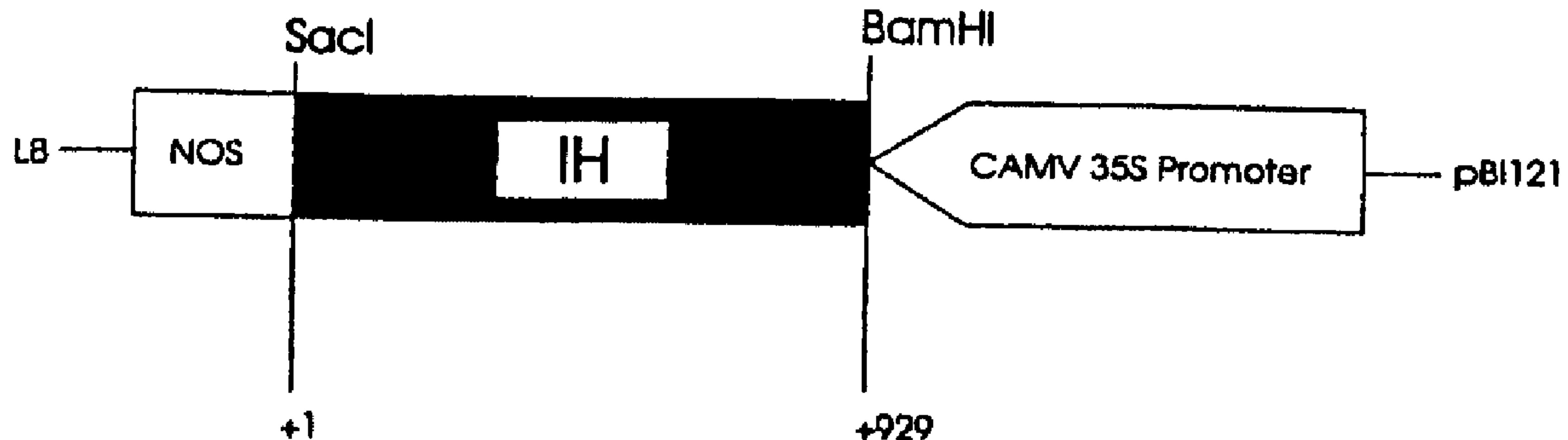
(72) Inventeurs/Inventors:
KAWCHUK, LAWRENCE MICHAEL, CA;
ARMSTRONG, JOHN DAVID, CA;
LYNCH, DERMOT ROBORG, CA;
KNOWLES, NORMAN RICHARD, CA

(73) Propriétaire/Owner:
HER MAJESTY THE QUEEN IN RIGHT OF CANADA AS
REPRESENTED BY THE MINISTER OF
AGRICULTURE AND AGRI-FOOD CANADA, CA

(74) Agent: MCKAY-CAREY & COMPANY

(54) Titre : POMMES DE TERRE TRANSGENIQUES AVEC REDUCTION DES NIVEAUX D'ACTIVITE DE LA
TUBERCULE-PHOSPHORYLASE DE TYPE L OU H ALPHA-GLUCAN ET AVEC REDUCTION DE
L'ADOUCCISSEMENT AU FROID

(54) Title: TRANSGENIC POTATOES HAVING REDUCED LEVELS OF ALPHA GLUCAN L- OR H-TYPE TUBER
PHOSPHORYLASE ACTIVITY WITH REDUCED COLD-SWEETENING



(57) **Abrégé/Abstract:**

Potato plants which exhibit reduced levels of α glucan L-type tuber phosphorylase (GLTP) or α glucan H-type tuber phosphorylase (GHTP) enzyme activity within the potato tuber are provided. The conversion of starches to sugars in potato tubers, particularly when stored at temperatures below 7 ° C, is reduced in tubers exhibiting reduced GLTP or GHTP enzyme activity. Reducing cold-sweetening in potatoes allows for potato storage at cooler temperatures, resulting in prolonged dormancy, reduced incidence of disease, and increased storage life. Methods for producing potato plants which produce tubers exhibiting reduced GLTP or GHTP enzyme activity are also provided. Reduction of GLTP or GHTP activity within the potato tuber may be accomplished by such techniques as suppression of gene expression using homologous antisense RNA, the use of co-suppression, regulatory silencing sequences, chemical and protein inhibitors, or the use of site-directed mutagenesis or the isolation of alternative alleles to obtain GLTP or GHTP variants with reduced starch affinity or activity.



**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

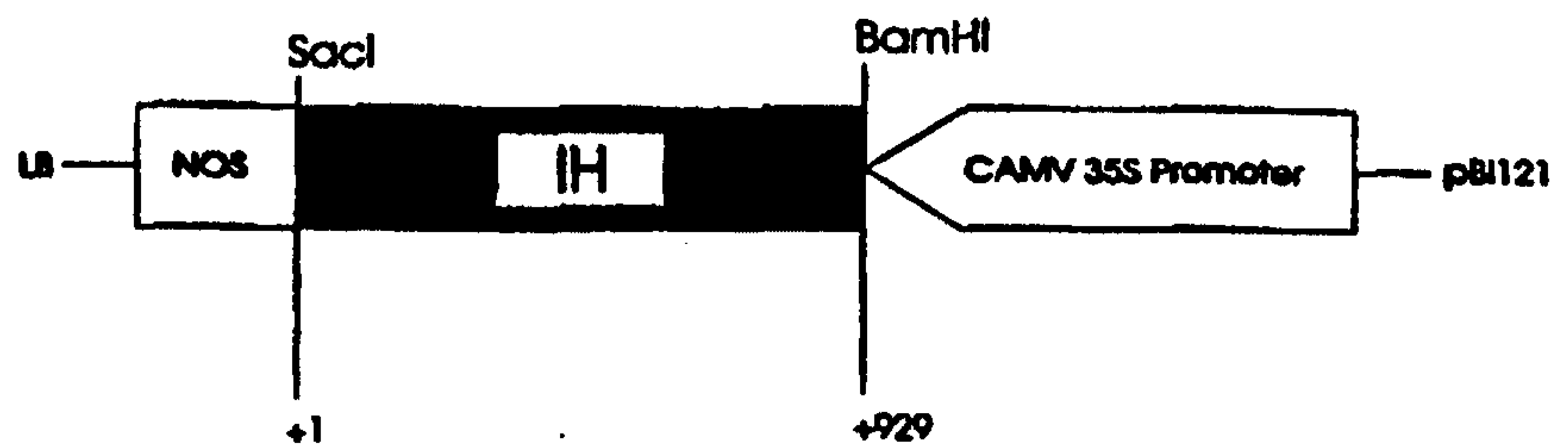
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/82, A01H 5/00 // C12N 9/10		A1	(11) International Publication Number: WO 98/35051
			(43) International Publication Date: 13 August 1998 (13.08.98)
(21) International Application Number: PCT/CA98/00055		(74) Agent: McKAY-CAREY, Mary, Jane; McKay-Carey & Company, 2125 Commerce Place, 10155 - 102nd Street, Edmonton, Alberta T5J 4G8 (CA).	
(22) International Filing Date: 5 February 1998 (05.02.98)			
(30) Priority Data: 60/036,946 10 February 1997 (10.02.97) US 08/868,786 4 June 1997 (04.06.97) US		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): HER MAJESTY THE QUEEN IN RIGHT OF CANADA as represented by THE DEPARTMENT OF AGRICULTURE AND AGRI-FOOD CANADA [CA/CA]; P.O. Box 3000, Main, Lethbridge, Alberta T1J 4B1 (CA).			
(72) Inventors; and (75) Inventors/Applicants (for US only): KAWCHUK, Lawrence, Michael [CA/CA]; 905 - 22 Avenue, Coaldale, Alberta T1M 1B5 (CA). ARMSTRONG, John, David [CA/CA]; 2422 - 12 Avenue South, Lethbridge, Alberta T1K 0P5 (CA). LYNCH, Dermot, Roborg [CA/CA]; 3821 Glacier Avenue South, Lethbridge, Alberta T1K 3N9 (CA). KNOWLES, Norman, Richard [CA/CA]; 3507 Hillview Crescent, Edmonton, Alberta T6L 1P4 (CA).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: TRANSGENIC POTATOES HAVING REDUCED LEVELS OF ALPHA GLUCAN L- OR H-TYPE TUBER PHOSPHORYLASE ACTIVITY WITH REDUCED COLD-SWEETENING

(57) Abstract

Potato plants which exhibit reduced levels of α glucan L-type tuber phosphorylase (GLTP) or α glucan H-type tuber phosphorylase (GHTP) enzyme activity within the potato tuber are provided. The conversion of starches to sugars in potato tubers, particularly when stored at temperatures below 7 °C, is reduced in tubers exhibiting reduced GLTP or GHTP enzyme activity. Reducing cold-sweetening in potatoes allows for potato storage at cooler temperatures, resulting in prolonged dormancy, reduced incidence of disease, and increased storage life. Methods for producing potato plants which produce tubers exhibiting reduced GLTP or GHTP enzyme activity are also provided. Reduction of GLTP or GHTP activity within the potato tuber may be accomplished by such techniques as suppression of gene expression using homologous antisense RNA, the use of co-suppression, regulatory silencing sequences, chemical and protein inhibitors, or the use of site-directed mutagenesis or the isolation of alternative alleles to obtain GLTP or GHTP variants with reduced starch affinity or activity.



1
2
3
4
5 TRANSGENIC POTATOES HAVING REDUCED LEVELS OF
6 ALPHA GLUCAN L- OR H-TYPE TUBER PHOSPHORYLASE ACTIVITY
7 WITH REDUCED COLD-SWEETENING
8
9

10
11 FIELD OF THE INVENTION
12

13 The invention relates to the inhibition of the accumulation of sugars in potatoes by
14 reducing the level of α glucan L-type tuber phosphorylase or α glucan H-type tuber
15 phosphorylase enzyme activity in the potato plant.
16

17 BACKGROUND OF THE INVENTION
18

19 Plant stresses caused by a wide variety of factors including disease, environment, and
20 storage of potato tubers (*Solanum tuberosum*) represent major determinants of tuber quality.
21 Dormancy periods between harvesting and sprouting are critical to maintaining quality
22 potatoes. Processing potatoes are usually stored between 7 and 12°C. Cold storage at 2 to
23 6°C, versus storage at 7 to 12°C, provides the greatest longevity by reducing respiration,
24 moisture loss, microbial infection, heating costs, and the need for chemical sprout inhibitors
25 (Burton, 1989). However, low temperatures lead to cold-induced sweetening, and the
26 resulting high sugar levels contribute to an unacceptable brown or black color in the fried
27 product (Coffin et al., 1987, Weaver et al., 1978). The sugars that accumulate are
28 predominantly glucose, fructose, and sucrose. It is primarily the glucose and fructose
29 (reducing sugars) that react with free amino groups when heated during the various cooking
30 processes such as frying via the Maillard reaction, resulting in the formation of brown
31 pigments (Burton, 1989, Shallenberger et al., 1959). Sucrose produces a black colouration
32 when fried due to caramelization and charring. The ideal reducing sugar content is generally
33 accepted to be 0.1% of tuber fresh weight with 0.33% as the upper limit and higher levels of

1 reducing sugars are sufficient to cause the formation of brown and black pigments that
2 results in an unacceptable fried product (Davies and Viola, 1992). Although the
3 accumulation of reducing sugars can be slowed in higher temperature (7 to 12°C) storage,
4 this increases microbial infection and the need to use sprout inhibitors. Given the negative
5 environmental and health risks associated with chemical use, development of pathogens
6 resistant to pesticides, and the fact that use of current sprout inhibitors may soon be
7 prohibited, a need exists for potato varieties that can withstand stress and long-term cold
8 storage without the use of chemicals, without the accumulation of reducing sugars, and with
9 greater retention of starch.

10 Carbohydrate metabolism is a complex process in plant cells. Manipulation of a
11 number of different enzymatic processes may potentially affect the accumulation of reducing
12 sugars during cold storage. For example, inhibition of starch breakdown would reduce the
13 buildup of free sugar. Other methods may also serve to enhance the cold storage properties of
14 potatoes through reduction of sugar content, including the resynthesis of starch using reducing
15 sugars, removal of sugars through glycolysis and respiration, or conversion of sugars into
16 other forms that would not participate in the Maillard reaction. However, many of the
17 enzymatic processes are reversible, and the role of most of the enzymes involved in
18 carbohydrate metabolism is poorly understood. The challenge remains to identify an enzyme
19 that will deliver the desired result, achieve function at low temperatures, and still retain the
20 product qualities desired by producers, processors, and consumers.

21 It has been suggested that phosphofructokinase (PFK) has an important role in the
22 cold-induced sweetening process (Kruger and Hammond, 1988, ap Rees et al., 1988, Dixon et
23 al., 1981, Claassen et al., 1991). ap Reese et al. (1988) suggested that cold treatment had a
24 disproportionate effect on different pathways in carbohydrate metabolism in that glycolysis
25 was more severely reduced due to the cold-sensitivity of PFK. The reduction in PFK activity
26 would then lead to an increased availability of hexose-phosphates for sucrose production. It
27 was disclosed in European Patent 0438904 (Burrell et al., July 31, 1991) that increasing PFK
28 activity reduces sugar accumulation during storage by removing hexoses through glycolysis
29 and further metabolism. A PFK enzyme from *E. coli* was expressed in potato tubers and the
30 report claimed to increase PFK activity and to reduce sucrose content in tubers assayed at
31 harvest. However it has been shown that pyrophosphate:fructose 6-phosphate

1 phosphotransferase (PFP) remains active at low temperatures (Claassen et al., 1991). PFP
2 activity can supply fructose 6-phosphate for glycolysis just as PFK can, since the two
3 enzymes catalyse the same reaction. Therefore, the efficacy of this strategy for improving
4 cold storage quality of potato tubers remains in doubt. Furthermore, removal of sugars
5 through glycolysis and further metabolism would not be a preferred method of enhancing
6 storage properties of potato tubers because of the resultant loss of valuable dry matter through
7 respiration.

8 It has also been suggested that ADPglucose pyrophosphorylase (ADPGPP) has an
9 important role in the cold-induced sweetening process. It was disclosed in International
10 Application WO 94/28149 (Barry, *et al.*, filed May 18, 1994) that increasing ADPGPP
11 activity reduces sugar accumulation during storage by re-synthesising starch using reducing
12 sugars. An ADPGPP enzyme from *E. coli* was expressed in potato tubers under the control of
13 a cold-induced promoter and the report claimed to increase ADPGPP activity and lower
14 reducing sugar content in tubers assayed at harvest and after cold temperature storage.
15 However, this strategy does not eliminate starch catabolism but instead increases the rate of
16 starch resynthesis. Thus, catabolism of sugars through glycolysis and respiration occurs and
17 re-incorporation into starch is limited. Up regulation of ADPGPP would not be a preferred
18 method of enhancing storage properties of potato tubers because of the resultant loss of
19 valuable dry matter through respiration. Again, a method involving the reduction of
20 catabolism of starch would be preferable as dry matter would be retained.

21 The degradation of starch is believed to involve several enzymes including α -amylase
22 (endoamylase), β -amylase (exoamylase), amyloglucosidase, and α -glucan phosphorylase
23 (starch phosphorylase). By slowing starch catabolism, accumulation of reducing sugars
24 should be prevented and the removal of sugars through glycolysis and further metabolism
25 would be minimized.

26 Three different isozymes of α glucan phosphorylase have been described. The tuber
27 L-type α 1,4 glucan phosphorylase (EC 2.4.1.1) isozyme (GLTP) (Nakano and Fukui, 1986)
28 has a low affinity for highly branched glucans, such as glycogen, and is localized in
29 amyoplasts. The monomer consists of 916 amino acids and sequence comparisons with
30 phosphorylases from rabbit muscle and *Escherichia coli* revealed a high level of homology,
31 51% and 40% amino acids, respectively. The nucleotide sequence of the GLTP gene and the

1 amino acid sequence of the GLTP enzyme are shown in SEQ ID NO: 1 and SEQ ID NO: 2,
2 respectively. The H-type tuber α -glucan phosphorylase isozyme H (GHTP) (Mori et al.,
3 1991) has a high affinity for glycogen and is localized in the cytoplasm. The gene encodes
4 for 838 amino acids and shows 63% sequence homology with the tuber L-type phosphorylase
5 but lacks the 78-residue insertion and 50-residue amino-terminal extension found in the L-
6 type polypeptide. The nucleotide sequence of the GHTP gene and the amino acid sequence of
7 the GHTP enzyme are shown in SEQ ID NO: 3 and SEQ ID NO: 4, respectively. A third
8 isozyme has been reported (Sonnewald et al., 1995) that consists of 974 amino acids and is
9 highly homologous to the tuber L-type phosphorylase with 81% identity over most of the
10 polypeptide. However, the regions containing the transit peptide and insertion sequence are
11 highly diverse. This isozyme is referred to as the leaf L-type phosphorylase since the mRNA
12 accumulates equally in leaf and tuber, whereas the mRNA of the tuber L-type phosphorylase
13 accumulates strongly in potato tubers and only weakly in leaf tissues. The tuber L-type
14 phosphorylase is mainly present in the tubers and the leaf L-type phosphorylase is more
15 abundant in the leaves (Sonnewald et al., 1995). The nucleotide sequence of the leaf L-type
16 phosphorylase gene and the amino acid sequence of the leaf L-type phosphorylase enzyme are
17 shown in SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

18 The role of the various starch degrading enzymes is not clear, however, and
19 considerable debate has occurred over conflicting results. For example, reduced expression
20 of the leaf L-type phosphorylase (Sonnewald et al., 1995) had no significant influence on
21 starch accumulation. Sonnewald *et al.* (1995) reported that constitutive expression of an
22 antisense RNA specific for the leaf L-type gene resulted in a strong reduction of α glucan
23 phosphorylase L-type activity in leaf tissue, but had no effect in potato tuber tissue. Since the
24 antisense repression of the α glucan phosphorylase activity had no significant influence on
25 starch accumulation in leaves of transgenic potato plants, the authors concluded that starch
26 breakdown was not catalysed by phosphorylases. Considering the high level of sequence
27 homology between identified α glucan phosphorylase isozymes, a similar negative response
28 would be expected with the H-type (GHTP) and L-type tuber (GLTP) isozymes.

29 In view of the foregoing, there remains a need for potato plants which produce tubers
30 exhibiting reduced conversion of starches to sugars during propagation and during storage at
31 ambient and reduced temperatures, particularly at temperatures below 7°C.

SUMMARY OF THE INVENTION

The inventors have found that surprisingly, reduction of the level of α glucan L-type tuber phosphorylase (GLTP) or α glucan H-type tuber phosphorylase (GHTP) enzyme activity within the potato tuber results in a substantial reduction in the accumulation of sugars in the tuber during propagation and storage, relative to wildtype potatoes, particularly at storage temperatures below 10°C, and specifically at 4°C. It is remarkable that, given the complexity of carbohydrate metabolism in the tuber, reduction in the activity of a single enzyme is effective in reducing sugar accumulation in the tuber. The inventors' discovery is even more surprising in light of the previously discussed work of Sonnewald *et al.* (1995) wherein it was reported that reduced expression of the leaf L-type phosphorylase had no significant influence on starch accumulation in leaves of potato plants.

The present invention provides tremendous commercial advantages. Tubers in which cold-induced sweetening is inhibited or reduced may be stored at cooler temperatures without producing high levels of reducing sugars in the tuber which cause unacceptable darkening of fried potato products. Cold storage of tubers storage results in longer storage life, prolonged dormancy by limiting respiration and delaying sprouting, and lower incidence of disease.

Reduction in GLTP or GHTP activity in potato plants and tubers can be accomplished by any of a number of known methods, including, without limitation, antisense inhibition of GLTP or GHTP mRNA, co-suppression, site-directed mutagenesis of wildtype GLTP or GHTP genes, chemical or protein inhibition, or plant breeding programs.

Thus, in broad terms, the invention provides modified potato plants having a reduced level of α glucan L-type tuber phosphorylase (GLTP) or α glucan H-type tuber phosphorylase (GHTP) activity in tubers produced by the plants, relative to that of tubers produced by an unmodified potato plant. In a preferred embodiment, the invention provides a potato plant transformed with an expression cassette having a plant promoter sequence operably linked to a DNA sequence which, when transcribed in the plant, inhibits expression of an endogenous GLTP gene or GHTP gene. As will be discussed in detail hereinafter, the aforementioned DNA sequence may be inserted in the expression cassette in either a sense or antisense orientation. Potato plants of the present invention could have reduced activity levels of either

1 one of GLTP or GHTP independently, or could have reduced activity levels of both GLTP
2 and GHTP.

3 As discussed above, the inventors have found that reduction of activity levels of
4 GLTP or GHTP enzymes in potato plants results in potato tubers in which sugar
5 accumulation, particularly over long storage periods at temperatures below 10°C, is reduced.
6 Therefore, the invention further extends to methods for reducing sugar production in tubers
7 produced by a potato plant comprising reducing the level of activity of GLTP or GHTP in the
8 potato plant. In a preferred embodiment, such methods involve introducing into the potato
9 plant an expression cassette having a plant promoter sequence operably linked to a DNA
10 sequence which, when transcribed in the plant, inhibits expression of an endogenous GLTP
11 gene or GHTP gene. As above, the DNA sequence may be inserted in the expression cassette
12 in either a sense or antisense orientation.

13 As described in detail in the examples herein, improvements in cold-storage
14 characteristics have been observed in the potato variety Desiree transformed by the methods
15 of the present invention. A direct measure of improved cold-storage characteristics is a
16 reduction in the level of GLTP or GHTP enzyme activity detected in potatoes after harvest
17 and cold-storage. Transformed potato varieties have been developed wherein the total α
18 glucan phosphorylase activity measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers
19 of plants stored at 4°C for 189 days is as much as 70% lower than the total α glucan
20 phosphorylase activity in tubers of untransformed plants stored under the same conditions.

21 Another relatively direct measure of improved cold-storage characteristics is a
22 reduction in sweetening of potatoes observed after a period of cold-storage. Transformed
23 potato varieties have been developed wherein the sum of the concentrations of glucose and
24 fructose in tubers stored at 4°C for 91 days is 39% lower than the sum of the concentrations
25 of glucose and fructose in tubers of an untransformed plant stored under the same conditions.

26 Yet another measure of improved cold-storage characteristics, demonstrating a
27 practical advantage of the present invention, is a reduction in darkening of a potato chip
28 during processing (cooking). As discussed hereinbefore, the accumulation of sugars in
29 potatoes during cold-storage contributes to unacceptable darkening of the fried product.
30 Reduced darkening upon frying can be quantified as a measure of the reflectance, or chip
31 score, of the fried potato chip. Techniques for measuring chip scores are discussed herein.

1 Transformed potato varieties of the present invention have been developed wherein the chip
2 score for tubers of plants stored at 4°C for 124 days was as much as 89% higher than the chip
3 scores for tubers of untransformed plants stored under the same conditions.

4 By reducing GLTP and/or GHTP activity in tubers of potato plants, thereby inhibiting
5 sugar accumulation during cold-temperature storage, the present invention allows for storage
6 of potatoes at cooler temperatures than would be possible with wildtype potatoes of the same
7 cultivar. As discussed above, storage of potatoes at cooler temperatures than those
8 traditionally used could result in increased storage life, increased dormancy through reduced
9 respiration and sprouting, and reduced incidence of disease. It will be apparent to those
10 skilled in the art that such additional benefits also constitute improved cold-storage
11 characteristics and may be measured and quantified by known techniques.
12

13 BRIEF DESCRIPTION OF THE DRAWINGS

14

15 In drawings illustrating embodiments of the invention:

16 Figure 1 is a schematic diagram of the tuber L-type α glucan phosphorylase antisense
17 sequence inserted into the pBI121 transformation vector;

18 Figure 2 is a schematic diagram of the tuber H-type α glucan phosphorylase antisense
19 sequence inserted into the pBI121 transformation vector;

20 Figure 3 shows the basic structure of the three isolated isoforms of glucan
21 phosphorylase. The transit peptide (TS) and insertion sequence (IS) are characteristic of the
22 L-type phosphorylases and are not found in the H-type phosphorylase. The percentages
23 indicate the nucleic acid sequence homologies between the isoforms;

24 Figure 4 is a schematic diagram of carbohydrate interconversions in potatoes
25 (Sowokinos 1990);

26 Figure 5 is a comparison of the amino acid sequences of the three isoforms of
27 phosphorylase found in potato for the region targeted by the antisense GLTP construct used in
28 the Examples herein. Highlighted amino acids are identical. The leaf L-type α glucan
29 phosphorylase amino acid sequence is on top (amino acids 21 - 238 of SEQ ID NO: 6), the
30 tuber L-type α glucan phosphorylase amino acid sequence is in the middle (amino acids 49 -

1 266 of SEQ ID NO: 2), and tuber H-type α glucan phosphorylase amino acid sequence is on
2 the bottom (amino acids 46 - 264 of SEQ ID NO: 4);

3 Figure 6A and 6B are a comparison of the nucleotide sequences of the three isoforms
4 of phosphorylase found in potato for the region targeted by the antisense GLTP construct used
5 in the Examples herein. Highlighted nucleotides are identical. The leaf L-type α glucan
6 phosphorylase nucleotide sequence is on top (nucleotides 389 - 1045 of SEQ ID NO: 5), the
7 tuber L-type α glucan phosphorylase nucleotide sequence is in the middle (nucleotides 338 -
8 993 of SEQ ID NO: 1), and tuber H-type α glucan phosphorylase nucleotide sequence is on
9 the bottom (nucleotides 147 - 805 of SEQ ID NO: 3);

10 Figure 7 is a northern blot of polyadenylated RNA isolated from potato tubers of wild
11 type and lines 3,4,5, and 9 transformed with the tuber L-type α glucan phosphorylase. The
12 blot was probed with a radiolabelled probe specific for the tuber L-type α glucan
13 phosphorylase;

14 Figure 8 is a northern blot of total RNA isolated from potato tubers of wild type and
15 lines 1 and 2 transformed with the H-type α -glucan phosphorylase. The blot was probed with
16 a radio labelled probe specific for the H-type α -glucan phosphorylase;

17 Figure 9 shows the dried product obtained from (A) wild type and tuber L-type α
18 glucan phosphorylase transformants (B) ATL1 (C) ATL3 (D) ATL4 (E) ATL5 (F) ATL9 field
19 grown tubers following 86 days storage at 4°C ("ATL" = antisense tuber L-type
20 transformant);

21 Figure 10 shows the activity gel and western blot of L-type and H-type isozymes of α
22 1,4 glucan phosphorylase extracted from wild type tubers and tubers transformed with the
23 antisense construct for the L-type isoform; and

24 Figure 11 shows the activity gel and western blot of L-type and H-type isozymes of α
25 1,4 glucan phosphorylase extracted from wild type tubers and transformed with the antisense
26 construct for the H-type isoform..

27 DESCRIPTION OF THE PREFERRED EMBODIMENT

28
29
30 Potato plants having a reduced level of α glucan L-type tuber phosphorylase (GLTP)
31 or α glucan H-type tuber phosphorylase (GHTP) activity in tubers produced by the plants

1 relative to that of tubers produced by unmodified potato plants are provided. In the
2 exemplified case, reduction in α glucan phosphorylase activity is accomplished by
3 transforming a potato plant with an expression cassette having a plant promoter sequence
4 operably linked to a DNA sequence which, when transcribed in the plant, inhibits expression
5 of an endogenous GLTP gene or GHTP gene. Although, in the exemplified case, the DNA
6 sequence is inserted in the expression cassette in the antisense orientation, a reduction in α
7 glucan phosphorylase activity can be achieved with the DNA sequence inserted in the
8 expression cassette in either a sense or antisense orientation.
9

10 1 Homology Dependent Silencing

11 The control of gene expression using sense or antisense gene fragments is standard
12 laboratory practice and is well documented in the literature. Antisense and sense suppression
13 are both gene sequence homology-dependent phenomena that may be described as
14 "homology-dependent silencing" phenomena.

15 A review of scientific research articles published during 1996 reveals several hundred
16 reports of homology-dependent silencing in transgenic plants. The mechanisms underlying
17 homology-dependent silencing are not fully understood, but the characteristics of the
18 phenomena have been studied in many plant genes and this body of work has been
19 extensively reviewed (Meyer and Saedler 1996, Matzke and Matzke 1995, Jorgensen 1995,
20 Weintraub 1990, Van der Krol *et al.* 1988) Homology-dependent silencing appears to be a
21 general phenomenon that may be used to control the activity of many endogenous genes.
22 Examples of genes exhibiting reduced expression after the introduction of homologous
23 sequences include dihydroflavanol reductase (Van der Krol 1990), polygalacturonidase
24 (Smith *et al.* 1990), phytoene synthase (Fray and Grierson 1993), pectinesterase (Seymour *et*
25 *al.* 1993), phenylalanine ammonia-lyase (De Carvalho *et al.* 1992), β -1,3-glucanase (Hart *et*
26 *al.* 1992), chitinase (Dorlhac *et al.* 1994) nitrate reductase (Napoli *et al.* 1990), and chalcone
27 synthase (14). Transformation of Russet Burbank potato plants with either sense- or
28 antisense- constructs of the potato leafroll virus coat protein gene has been reported to confer
29 resistance to potato leafroll virus infection (Kawchuk *et al.* 1991). The transfer of a
30 homologous sense or antisense sequence usually generates transformants with reduced
31 endogenous gene expression. As discussed in detail in the examples herein, transformed

1 potato plants exhibiting phenotypes indicating reduced GLTP or GHTP expression can be
2 readily identified.

3 In the antisense suppression technique, a gene construct or expression cassette is
4 assembled which, when inserted into a plant cell, results in expression of an RNA which is of
5 complementary sequence to the mRNA produced by the target gene. It is theorized that the
6 complementary RNA sequences form a duplex thereby inhibiting translation to protein. The
7 theory underlying both sense and antisense inhibition has been discussed in the literature,
8 including in *Antisense Research and Applications* (CRC Press, 1993) pp. 125-148. The
9 complementary sequence may be equivalent in length to the whole sequence of the target
10 gene, but a fragment is usually sufficient and is more convenient to work with. For instance,
11 Cannon *et al.* (1990) reveals that an antisense sequence as short as 41 base pairs is sufficient
12 to achieve gene inhibition. United States Patent No. 5,585,545 (Bennett *et al.*, December 17,
13 1996) describes gene inhibition by an antisense sequence of only 20 base pairs. There are
14 many examples in the patent literature of patents including descriptions and claims to
15 methods for suppressing gene expression through the introduction of antisense sequences to
16 an organism, including, for example, United States Patent No. 5,545,815 (Fischer *et al.*,
17 August 13, 1996) and United States Patent No. 5,387,757 (Bridges *et al.*, February 7, 1995).

18 Sense-sequence homology-dependent silencing is conducted in a similar manner to
19 antisense suppression, except that the nucleotide sequence is inserted in the expression
20 cassette in the normal sense orientation. A number of patents, including United States patents
21 5,034,323, 5,231,020 and 5,283,184, disclose the introduction of sense sequences leading to
22 suppression of gene expression.

23 Both forms of homology-dependent silencing, sense- and antisense-suppression, are
24 useful for accomplishing the down-regulation of GLTP or GHTP of the present invention. It
25 is recognized in the art that both techniques are equally useful strategies for gene suppression.
26 For instance, both US Patent No. 5,585,545 (Bennett *et al.*, December 17, 1996) and US
27 Patent No. 5,451,514 (Boudet *et al.*, September 15, 1995) claim methods for inhibiting gene
28 expression or recombinant DNA sequences useful in methods for suppressing gene
29 expression drawn to both sense- and antisense-suppression techniques.

30
31

2 Alternate Techniques for Reducing GHTP and/or GLTP Activity in Tubers

Although homology-dependent silencing is a preferred technique for the down-regulation of GLTP or GHTP in potato plants of the present invention, there are several commonly used alternative strategies available to reduce the activity of a specific gene product which will be understood by those skilled in the art to bear application in the present invention. Insertion of a related gene or promoter into a plant can induce rapid turnover of homologous endogenous transcripts, a process referred to as co-suppression and believed to have many similarities to the mechanism responsible for antisense RNA inhibition (Jorgensen, 1995; Brusslan and Tobin, 1995). Various regulatory sequences of DNA can be altered (promoters, polyadenylation signals, post-transcriptional processing sites) or used to alter the expression levels (enhancers and silencers) of a specific mRNA. Another strategy to reduce expression of a gene and its encoded protein is the use of ribozymes designed to specifically cleave the target mRNA rendering it incapable of producing a fully functional protein (Hasseloff and Gerlach, 1988). Identification of naturally occurring alleles or the development of genetically engineered alleles of an enzyme that have been identified to be important in determining a particular trait can alter activity levels and be exploited by classical breeding programs (Ortiz and Huaman, 1994). Site-directed mutagenesis is often used to modify the activity of an identified gene product. The structural coding sequence for a phosphorylase enzyme can be mutagenized in *E. coli* or another suitable host and screened for reduced starch phosphorolysis. Alternatively, naturally occurring alleles of the phosphorylase with reduced affinity and/or specific activity may be identified. Additionally, the activity of a particular enzyme can be altered using various inhibitors. These procedures are routinely used and can be found in text books such as Sambrook *et al.* (1989).

3 Variants of GLTP and GHTP Enzymes and Sequences Used for Homology Dependent Silencing

As discussed in the background of the invention, and in greater detail by Nakano *et al.* (1986), Mori *et al.* (1991), and Sonnewald *et al.* (1990), there are three known α glucan phosphorylase isozymes that occur in potato plants. The present invention relates to down-regulation of the GLTP and/or GHTP isozymes. While it is believed that the GLTP and GHTP genes of all known commercial potato varieties are substantially identical, it is

1 expected that the principles and techniques of the present invention would be effective in
2 potato plants having variant full length polynucleotide sequences or subsequences which
3 encode polypeptides having the starch catabolizing enzymatic activity of the described GLTP
4 and GHTP enzymes. The terms "GLTP" and "GHTP", as used herein and in the claims, are
5 intended to cover the variants described above. The foregoing variants may include GLTP
6 and GHTP nucleotide sequence variants that differ from those exemplified but still encode
7 the same polypeptide due to codon degeneracy, as well as variants which encode proteins
8 capable of recognition by antibodies raised against the GLTP and GHTP amino acid
9 sequences set forth in SEQ ID NO's. 2 and 4.

10 Similarly, those skilled in the art will recognize that homology dependent silencing of
11 GLTP and/or GHTP in potato plants may be accomplished with sense or antisense sequences
12 other than those exemplified. First, the region of the GLTP or GHTP cDNA sequence from
13 which the antisense sequence is derived is not essential. Second, as described hereinabove,
14 the length of the antisense sequence used may vary considerably. Further, the sense or
15 antisense sequence need not be identical to that of the target GLTP or GHTP gene to be
16 suppressed. As described in the Examples herein, the inventors have observed that
17 transformation of potato plants with antisense DNA sequences derived from the GHTP gene
18 not only substantially suppresses GHTP gene activity, but causes some degree of suppression
19 of GLTP gene activity. The GHTP and GLTP genes antisense sequences have 56.8%
20 sequence identity. The sequence identity between the GLTP antisense sequence and the
21 corresponding leaf type α glucan phosphorylase sequence described by Sonnewald *et al.*
22 (1990) is 71.3%. In the inventors' research to date, the same phenomenon of cross-
23 downregulation has not been observed when potato plants are transformed with antisense
24 DNA sequences derived from the GLTP gene. Nevertheless, these results clearly indicate that
25 absolute sequence identity between the target endogenous α glucan phosphorylase gene and
26 the recombinant DNA is not essential given that GLTP activity was suppressed by an
27 antisense sequence having about 57% sequence identity with the target GLTP sequence.

28 Thus, it will be understood by those skilled in the art that sense or antisense sequences
29 other than those exemplified herein and other than those having absolute sequence identity
30 with the target endogenous GLTP or GHTP gene will be effective to cause suppression of the
31 endogenous GLTP or GHTP gene when introduced into potato plant cells. Useful sense or

1 antisense sequences may differ from the exemplified antisense sequences or from other
2 sequences derived from the endogenous GHTP or GLTP gene sequences by way of
3 conservative amino acid substitutions or differences in the percentage of matched nucleotides
4 or amino acids over portions of the sequences which are aligned for comparison purposes.

5 United States Patent 5,585,545 (Bennett *et al.*, December 17, 1996) provides a helpful
6 discussion regarding techniques for comparing sequence identity for polynucleotides and
7 polypeptides, conservative amino acid substitutions, and hybridization conditions indicative
8 of degrees of sequence identity. Relevant parts of that discussion are summarized herein.

9 Percentage of sequence identity for polynucleotides and polypeptides may be
10 determined by comparing two optimally aligned sequences over a comparison window,
11 wherein the portion of the polynucleotide or polypeptide sequence in the comparison window
12 may include additions or deletions (i.e., gaps) as compared to the reference sequence (which
13 does not comprise additions or deletions) for optimal alignment of the two sequences. The
14 percentage is calculated by: (a) determining the number of positions at which the identical
15 nucleic acid base or amino acid residue occurs in both sequences to yield the number of
16 matched positions; (b) dividing the number of matched positions by the total number of
17 positions in the window of comparison; and, (c) multiplying the result by 100 to yield the
18 percentage of sequence identity. Optimal alignment of sequences for comparison may be
19 conducted by computerized implementations of known algorithms (e.g., GAP, BESTFIT,
20 FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer
21 Group (GCG), 575 Science Dr., Madison, WI, or BlastN and BlastX available from the
22 National Center for Biotechnology Information), or by inspection.

23 Polypeptides which are substantially similar share sequences as noted above
24 except that residue positions which are not identical may differ by conservative amino acid
25 changes. Conservative amino acid substitutions refer to the interchangeability of residues
26 having similar side chains. For example, a group of amino acids having aliphatic side chains
27 is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having
28 aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having
29 amide-containing side chains is asparagine and glutamine; a group of amino acids having
30 aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids

1 having basic side chains is lysine, arginine, and histidine; and a group of amino acids having
2 sulfur-containing side chains is cysteine and methionine.

3 Another indication that nucleotide sequences are substantially identical is if two
4 molecules specifically hybridize to each other under stringent conditions. Stringent
5 conditions are sequence dependent and will be different in different circumstances.
6 Generally, stringent conditions are selected to be about 10°C lower than the thermal melting
7 point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the
8 temperature (under defined ionic strength and pH) at which 50% of the target sequence
9 hybridizes to a perfectly matched probe. The T_m of a hybrid, which is a function of both the
10 length and the base composition of the probe, can be calculated as described in Sambrook *et*
11 *al.* (1989). Typically, stringent conditions for a Southern blot protocol involve washing at
12 65°C with 0.2XSSC. For preferred oligonucleotide probes, washing conditions are typically
13 about at 42°C in 6XSSC.

14 15 4 General Methods

16 Various methods are available to introduce and express foreign DNA sequences in
17 plant cells. In brief, the steps involved in preparing antisense α glucan phosphorylase cDNAs
18 and introducing them into a plant cell include: (1) isolating mRNA from potato plants and
19 preparing cDNA from the mRNA; (2) screening the cDNA for the desired sequences; (3)
20 linking a promoter to the desired cDNAs in the opposite orientation for expression of the
21 phosphorylase genes; (4) transforming suitable host plant cells; and (5) selecting and
22 regenerating cells which transcribe the inverted sequences.

23 In the exemplified case, DNA derived from potato GLTP and GHTP genes is used to
24 create expression cassettes having a plant promoter sequence operably linked to an antisense
25 DNA sequence which, when transcribed in the plant, inhibits expression of an endogenous
26 GLTP gene or GHTP gene. *Agrobacterium tumefaciens* is used as a vehicle for transmission
27 of the DNA to stem explants of potato plant shoots. A plant regenerated from the
28 transformed explants transcribes the antisense DNA which inhibits activity of the enzyme.

29 The recombinant DNA technology described herein involves standard laboratory
30 techniques that are well known in the art and are described in standard references such as
31 Sambrook *et al.* (1989). Generally, enzymatic reactions involving DNA ligase, DNA

1 polymerase, restriction endonucleases and the like are performed according to the
2 manufacturer's specifications.

3 4 5 Preparation of GHTP and GLTP cDNA

5 cDNA is prepared from isolated potato tuber mRNA by reverse transcription. A
6 primer is annealed to the mRNA, providing a free 3' end that can be used for extension by the
7 enzyme reverse transcriptase. The enzyme engages in the usual 5'-3' elongation, as directed
8 by complementary base pairing with the mRNA template to form a hybrid molecule,
9 consisting of a template RNA strand base-paired with the complementary cDNA strand.
10 After degradation of the original mRNA, a DNA polymerase is used to synthesize the
11 complementary DNA strand to convert the single-stranded cDNA into a duplex DNA.

12 After DNA amplification, the double stranded cDNA is inserted into a vector for
13 propagation in *E. coli*. Typically, identification of clones harbouring the desired cDNA's
14 would be performed by either nucleic acid hybridization or immunological detection of the
15 encoded protein, if an expression vector is used. In the exemplified case, the matter is
16 simplified in that the DNA sequences of the GLTP and GHTP genes are known, as are the
17 sequences of suitable primers (Brisson *et al.*, 1990; Fukui *et al.*, 1991). The primers used
18 hybridize within the GLTP and GHTP genes. Thus, it is expected that the amplified cDNA's
19 prepared represent portions of the GLTP and GHTP genes without further analysis. *E. coli*
20 transformed with pUC19 plasmids carrying the phosphorylase DNA insert were detected by
21 color selection. Appropriate *E. coli* strains transformed with plasmids which do not carry
22 inserts grow as blue colonies. Strains transformed with pBluescript plasmids carrying inserts
23 grow as white colonies. Plasmids isolated from transformed *E. coli* were sequenced to
24 confirm the sequence of the phosphorylase inserts.

25 26 6 Vector Construction

27 The cDNAs prepared can be inserted in the antisense or sense orientation into
28 expression cassette in expression vectors for transformation of potato plants to inhibit the
29 expression of the GLTP and/or GHTP genes in potato tubers.

30 As in the exemplified case, which involves antisense suppression, the desired
31 recombinant vector will comprise an expression cassette designed for initiating transcription

1 of the antisense cDNAs in plants. Additional sequences are included to allow the vector to be
2 cloned in a bacterial or phage host.

3 The vector will preferably contain a prokaryote origin of replication having a broad
4 host range. A selectable marker should also be included to allow selection of bacterial cells
5 bearing the desired construct. Suitable prokaryotic selectable markers include resistance to
6 antibiotics such as ampicillin.

7 Other DNA sequences encoding additional functions may also be present in the
8 vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations,
9 T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

10 For expression in plants, the recombinant expression cassette will contain in
11 addition to the desired sequence, a plant promoter region, a transcription initiation site (if
12 the sequence to be transcribed lacks one), and a transcription termination sequence. Unique
13 restriction enzyme sites at the 5' and 3' ends of the cassette are typically included to allow for
14 easy insertion into a pre-existing vector. Sequences controlling eukaryotic gene
15 expression are well known in the art.

16 Transcription of DNA into mRNA is regulated by a region of DNA referred to as the
17 promoter. The promoter region contains sequence of bases that signals RNA polymerase to
18 associate with the DNA, and to initiate the transcription of mRNA using one of the DNA
19 strands as a template to make a corresponding complementary strand of RNA. Promoter
20 sequence elements include the TATA box consensus sequence (TATAAT), which is usually
21 20 to 30 base pairs (bp) upstream (by convention -30 to -20 bp relative to the transcription
22 start site) of the transcription start site. In most instances the TATA box is required for
23 accurate transcription initiation. The TATA box is the only upstream promoter element that
24 has a relatively fixed location with respect to the start point.

25 The CAAT box consensus sequence is centered at -75, but can function at distances
26 that vary considerably from the start point and in either orientation.

27 Another common promoter element is the GC box at -90 which contains the
28 consensus sequence GGGCGG. It may occur in multiple copies and in either orientation.

29 Other sequences conferring tissue specificity, response to environmental signals, or
30 maximum efficiency of transcription may also be found in the promoter region. Such
31 sequences are often found within 400 bp of transcription initiation size, but may extend as far

1 as 2000 bp or more. In heterologous promoter/structural gene combinations, the promoter is
2 preferably positioned about the same distance from the heterologous transcription start site as
3 it is from the transcription start site in its natural setting. However, some variation in this
4 distance can be accommodated without loss of promoter function.

5 The particular promoter used in the expression cassette is not critical to the
6 invention. Any of a number of promoters which direct transcription in plant cells is suitable.
7 The promoter can be either constitutive or inducible.

8 A number of promoters which are active in plant cells have been described in the
9 literature. These include the nopaline synthase (NOS) and octopine synthase (OCS)
10 promoters (which are carried on tumour-inducing plasmids of *Agrobacterium tumefaciens*),
11 the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S and 35S and
12 the figwort mosaic virus 35S-promoters, the light-inducible promoter from the small subunit
13 of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide),
14 and the chlorophyll a/b binding protein gene promoter, etc. All of these promoters have been
15 used to create various types of DNA constructs which have been expressed in plants; see, e.g.,
16 PCT WO8402913.

17 The CaMV 35S promoter used in the Examples herein, has been shown to be highly
18 active and constitutively expressed in most tissues (Bevan *et al.*, 1986). A number of other
19 genes with tuber-specific or enhanced expression are known, including the potato tuber
20 ADPGPP genes, large and small subunits (Muller *et al.*, 1990). Other promoters which are
21 contemplated to be useful in this invention include those that show enhanced or specific
22 expression in potato tubers, that are promoters normally associated with the expression of
23 starch biosynthetic or modification enzyme genes, or that show different patterns of
24 expression, for example, or are expressed at different times during tuber development.
25 Examples of these promoters include those for the genes for the granule-bound and other
26 starch synthases, the branching enzymes (Blennow *et al.*, 1991; WO 9214827; WO 9211375),
27 disproportionating enzyme (Takaha *et al.*, 1993) debranching enzymes, amylases, starch
28 phosphorylases (Nakano *et al.*, 1989; Mori *et al.*, 1991), pectin esterases (Ebbelaar *et al.*,
29 1993), the 40 kD glycoprotein; ubiquitin, aspartic proteinase inhibitor (Stukerlj *et al.*, 1990),
30 the carboxypeptidase inhibitor, tuber polyphenol oxidases (Shahar *et al.*, 1992; GenBank
31 Accession Numbers M95196 and M95197), putative trypsin inhibitor and other tuber cDNAs

(Stiekema *et al.*, 1988), and for amylases and sporamins (Yoshida *et al.*, 1992; Ohta *et al.*, 1991).

In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. In the exemplified case the nopaline synthase NOS 3' terminator sequence (Bevan *et al.* 1983) was used.

Polyadenylation sequences are also commonly added to the vector construct if the mRNA encoded by the structural gene is to be efficiently translated (Alber and Kawasaki, 1982). Polyadenylation is believed to have an effect on stabilizing mRNAs. Polyadenylation sequences include, but are not limited to the *Agrobacterium* octopine synthase signal (Gielen *et al.*, 1984) or the nopaline synthase signal (Depicker *et al.*, 1982).

The vector will also typically contain a selectable marker gene by which transformed plant cells can be identified in culture. Typically, the marker gene encodes antibiotic resistance. These markers include resistance to G418, hygromycin, bleomycin, kanamycin, and gentamycin. In the exemplified case, the marker gene confers resistance to kanamycin. After transforming the plant cells, those cells containing the vector will be identified by their ability to grow in a medium containing the particular antibiotic.

7 Transformation of Plant Cells

Although in the exemplified case potato plant shoot stem explants were transformed via inoculation with *Agrobacterium tumefaciens* carrying the antisense sequence linked to a binary vector, direct transformation techniques which are known in the art can also be used to transfer the recombinant DNA. The vector can be microinjected directly into plant cells. Alternatively, nucleic acids may be introduced to the plant cell by high velocity ballistic penetration by small particles having the nucleic acid of interest embedded within the matrix of the particles or on the surface. Fusion of protoplasts with lipid-surfaced bodies such as minicells, cells or lysosomes carrying the DNA of interest can be used. The DNA may also be introduced into plant cells by electroporation, wherein plant protoplasts are electroporated in the presence of plasmids carrying the expression cassette.

1 In contrast to direct transformation methods, the exemplified case uses vectored
2 transformation using *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens* is a Gram-
3 negative soil bacteria which causes a neoplastic disease known as crown gall in
4 dicotyledonous plants. Induction of tumours is caused by tumour-inducing plasmids known
5 as Ti plasmids. Ti plasmids direct the synthesis of opines in the infected plant. The opines
6 are used as a source of carbon and/or nitrogen by the Agrobacteria.

7 The bacterium does not enter the plant cell, but transfers only part of the Ti plasmid, a
8 portion called T-DNA, which is stably integrated into the plant genome, where it expresses
9 the functions needed to synthesize opines and to transform the plant cell. *Vir* (virulence)
10 genes on the Ti plasmid, outside of the T-DNA region, are necessary for the transfer of the T-
11 DNA. The *vir* region, however, is not transferred. In fact, the *vir* region, although required
12 for T-DNA transfer, need not be physically linked to the T-DNA and may be provided on a
13 separate plasmid.

14 The tumour-inducing portions of the T-DNA can be interrupted or deleted without
15 loss of the transfer and integration functions, such that normal and healthy transformed plant
16 cells may be produced which have lost all properties of tumour cells, but still harbour and
17 express certain parts of T-DNA, particularly the T-DNA border regions. Therefore, modified
18 Ti plasmids, in which the disease causing genes have been deleted, may be used as vectors for
19 the transfer of the sense and antisense gene constructs of the present invention into potato
20 plants (see generally Winnacker, 1987).

21 Transformation of plants cells with Agrobacterium and regeneration of whole plants
22 typically involves either co-cultivation of Agrobacterium with cultured isolated protoplasts or
23 transformation of intact cells or tissues with Agrobacterium. In the exemplified case, stem
24 explants from potato shoot cultures are transformed with Agrobacterium.

25 Alternatively, cauliflower mosaic virus (CaMV) may be used as a vector for
26 introducing sense or antisense DNA into plants of the *Solanaceae* family. For instance,
27 United States Patent No. 4,407,956 (Howell, October 4, 1983) teaches the use of cauliflower
28 mosaic virus DNA as a plant vehicle.

8 Selection and Regeneration of Transformed Plant Cells

After transformation, transformed plant cells or plants carrying the antisense or sense DNA must be identified. A selectable marker, such as antibiotic resistance, is typically used. In the exemplified case, transformed plant cells were selected by growing the cells on growth medium containing kanamycin. Other selectable markers will be apparent to those skilled in the art. For instance, the presence of opines can be used to identify transformants if the plants are transformed with *Agrobacterium*.

Expression of the foreign DNA can be confirmed by detection of RNA encoded by the inserted DNA using well known methods such as Northern blot hybridization. The inserted DNA sequence can itself be identified by Southern blot hybridization or the polymerase chain reaction, as well (See, generally, Sambrook *et al.* (1989)).

Generally, after it is determined that the transformed plant cells carry the recombinant DNA, whole plants are regenerated. In the exemplified case, stem and leaf explants of potato shoot cultures were inoculated with a culture of *Agrobacterium tumefaciens* carrying the desired antisense DNA and a kanamycin marker gene. Transformants were selected on a kanamycin-containing growth medium. After transfer to a suitable medium for shoot induction, shoots were transferred to a medium suitable for rooting. Plants were then transferred to soil and hardened off. The plants regenerated in culture were transplanted and grown to maturity under greenhouse conditions.

9 Analysis of GHTP and GLTP Activity Levels in Transformed Tubers

Following regeneration of potato plants transformed with antisense DNA sequences derived from the GHTP and GLTP genes, the biochemistry of transformed tuber tissue was analyzed several ways. The *in vitro* activity of α glucan phosphorylase in the phosphorolytic direction was assayed according to the methods of Steup (1990) (Table 1). The activity of the enzyme in the synthetic direction and the amount of enzyme protein were compared after electrophoretic separation of the enzyme isoforms on a glycogen-containing, polyacrylamide gel (Figure 7). Starch synthesis by the tuber L-type and H-type isoforms was determined by iodine staining of the gel after incubation with glucose-1-phosphate and a starch primer (Steup, 1990). Western analysis was performed by blotting the protein from an identical unincubated native gel to nitrocellulose and probing with polyclonal antibodies specific for

tuber type L and type H glucan phosphorylase isoforms. Levels of reducing sugars (glucose and fructose) in tuber tissues were quantified by HPLC (Tables 2, 3 and 4). The extent of Maillard reaction, which is proportional to the concentration of reducing sugars in tubers was examined by determining chip scores after frying (Table 5 and Figure 9).

10 Definitions

As used herein and in the claims, the term:

- "about three months", "about four months" and "about six months" refer, respectively, to periods of time of three months plus or minus two weeks, four months plus or minus two weeks, and six months plus or minus two weeks;
- "antisense orientation" refers to the orientation of nucleic acid sequence from a structural gene that is inserted in an expression cassette in an inverted manner with respect to its naturally occurring orientation. When the sequence is double stranded, the strand that is the template strand in the naturally occurring orientation becomes the coding strand, and vice versa;
- "chip score" of a tuber means the reflectance measurement recorded by an Agtron model E-15-FP Direct Reading Abridged Spectrophotometer (Agtron Inc. 1095 Spice Island Drive #100, Sparks Nevada 89431) of a center cut potato chip fried at 205°F in soybean oil for approximately 3 minutes until bubbling stops;
- "cold storage" or "storage at reduced temperature" or variants thereof, shall mean holding at temperatures less than 10°C, that may be achieved by refrigeration or ambient temperatures;
- "endogenous", as it is used with reference to α glucan phosphorylase genes of a potato plant, shall mean a naturally occurring gene that was present in the genome of the potato plant prior to the introduction of an expression cassette carrying a DNA sequence derived from an α glucan phosphorylase gene;
- "expression" refers to the transcription and translation of a structural gene so that a protein is synthesized;
- "heterologous sequence" or "heterologous expression cassette" is one that originates from a foreign species, or, if from the same species, is substantially modified from its original form;

- WO 98/35051

PCT/CA98/00055

1 - "improved cold-storage characteristics" includes, without limitation, improvements in
2 chip score and reduction in sugar accumulation in tubers measured at harvest or after a period
3 of storage below 10°C, and further includes improvements, advantages and benefits which
4 may result from the storage of potatoes at cooler temperatures than those traditionally used,
5 such as, without limitation, increased storage life of potatoes, increased dormancy through
6 reduced respiration and sprouting of potatoes, and reduced incidence of disease. Unless
7 further qualified by a specific measure or test, an improvement in a cold-storage characteristic
8 refers to a difference in the described characteristic relative to that in a control, wildtype or
9 unmodified potato plant;

10 - "modified" or variants thereof, when used to describe potato plants or tubers, is used
11 to distinguish a potato plant or tuber that has been altered from its naturally occurring state
12 through: the introduction of a nucleotide sequence from the same or a different species,
13 whether in a sense or antisense orientation, whether by recombinant DNA technology or by
14 traditional cross-breeding methods including the introduction of modified structural or
15 regulatory sequences; modification of a native nucleotide sequence by site-directed
16 mutagenesis or otherwise; or the treatment of the potato plant with chemical or protein
17 inhibitors. An "unmodified" potato plant or tuber means a control, wildtype or naturally
18 occurring potato plant or tuber that has not been modified as described above;

19 - "nucleic acid sequence" or "nucleic acid segment" refer to a single or double-stranded
20 polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It
21 includes both self-replicating plasmids, infectious polymers of DNA or RNA and non-
22 functional DNA or RNA;

23 - "operably linked" refers to functional linkage between a promoter and a second
24 sequence, wherein the promoter sequence initiates transcription of RNA corresponding to the
25 second sequence;

26 - "plant " includes whole plants, plant organs (e.g. leaves, stems, roots, etc.) seeds and
27 plant cells;

28 - "promoter" refers to a region of DNA upstream from the structural gene and involved
29 in recognition and binding RNA polymerase and other proteins that initiate transcription. A
30 "plant promoter" is a promoter capable of initiating transcription in plant cells;

WO 98/35051

PCT/CA98/00055

- 1 - "reduced activity" or variants thereof, when used in reference to the level of GLTP or
2 GHTP enzyme activity in a potato tuber includes reduction of GLTP or GHTP enzyme
3 activity resulting from reduced expression of the GLTP or GHTP gene product, reduced
4 substrate affinity of the GLTP or GHTP enzyme, and reduced catalytic activity of the GLTP
5 or GHTP enzyme;
- 6 - "reduced" or variants thereof, may be used herein with reference to, without
7 limitation, activity levels of GLTP or GHTP enzyme in potato tubers, accumulation of sugars
8 in potato tubers and darkening of potato chips upon frying. Unless further qualified by a
9 specific measure or test, reduced levels or reduced activity refers to a demonstrable
10 statistically significant difference in the described characteristic relative to that in a control,
11 wildtype or unmodified potato plant;
- 12 - "stress" or variants thereof, when used in relation to stresses experienced by potato
13 plants and tubers, includes the effects of environment, fertility, moisture, temperature,
14 handling, disease, atmosphere and aging that impact upon plant or tuber quality and which
15 may be experienced by potato plants through all stages of their life cycle and by tubers at all
16 stages of the growth and development cycle and during subsequent harvesting, transport,
17 storage and processing;
- 18 - "stress resistance" or variants thereof, shall mean reduced effects of temperature,
19 aging, disease, atmosphere, physical handling, moisture, chemical residues, environment,
20 pests and other stresses;
- 21 - "suitable host" refers to a microorganism or cell that is compatible with a recombinant
22 plasmid, DNA sequence or recombinant expression cassette and will permit the plasmid to
23 replicate, to be incorporated into its genome, or to be expressed; and
- 24 - "uninterrupted" refers to a DNA sequence (e.g. cDNA) containing an open reading
25 frame that lacks intervening, untranslated sequences.
- 26

27 EXAMPLE 1

28 This example describes the reduction of GHTP and/or GLTP activity in tubers of
29 potato plants by transforming potato plants with expression cassettes containing DNA
30 sequences derived from the GLTP and GHTP gene sequences linked to the promoter in the
31 antisense orientation.

1 A Isolation of Potato Tuber mRNA

2 Potato total RNA was purified at 4 °C using autoclaved reagents from 1g of tuber
3 tissue ground to a fine powder under liquid nitrogen with a mortar and pestle. The powder
4 was transferred to a 30ml corex tube and 3 volumes were added of 100 mM Tris-Cl, pH 8.0,
5 100 mM NaCl, and 10 mM EDTA (10x TNE) containing 0.2% (w/v) SDS and 0.5% (v/v) 2-
6 mercaptoethanol. An equal volume of phenol-chloroform (1:1) was added and the sample
7 gently vortexed before centrifugation at 4 °C in a SS34 rotor at 8,000 rpm for 5 min. The
8 organic phase was reextracted with 0.5 volume of 10x TNE containing 0.2% (w/v) SDS and
9 0.5% (v/v) 2-mercaptoethanol and the combined aqueous phases were extracted with
10 chloroform. Nucleic acids were precipitated from the aqueous phase with sodium acetate and
11 absolute ethanol, pelleted by centrifugation, and resuspended in 3 ml of 1x TNE. An equal
12 volume of 5 M LiCl was added and the sample stored at -20 °C for at 4 h before centrifuging
13 at 8,000 rpm in a SS34 rotor at 4 °C for 10 min. The RNA pellet was washed with 70%
14 ethanol, dried, and resuspended in DEPC-treated water.

15 Poly (A⁺) RNA was isolated using oligo (dT) cellulose (Boehringer Mannheim)
16 column chromatography. Poly (A⁺) RNA was isolated from total RNA resuspended in
17 RNase free water. Columns were prepared using an autoclaved Bio-Rad Poly-Prep™ 10 ml
18 column to which was added 50 mg of oligo (dT) cellulose suspended in 1 ml of loading
19 buffer B which contains 20 mM Tris-Cl, pH 7.4, 0.1 M NaCl, 1 mM EDTA, and 0.1% (w/v)
20 SDS. The column was washed with 3 volumes of 0.1 M NaOH with 5 mM EDTA and then
21 DEPC-treated water until the pH of effluent was less than 8, as determined with pH paper.
22 The column was then washed with 5 volumes of loading buffer A containing 40 mM Tris-Cl,
23 pH 7.4, 1 M NaCl, 1 mM EDTA, and 0.1% (w/v) SDS.

24 RNA samples were heated to 65 °C for 5 min at which time 400 µl of loading buffer
25 A, prewarmed to 65 °C, was added. The sample was mixed and allowed to cool at room
26 temperature for 2 min before application to the column. Eluate was collected, heated to 65 °C
27 for 5 min, cooled to room temperature for 2 min, and reapplied to the column. This was
28 followed by a 5 volume washing with loading buffer A followed by a 4 volume wash with
29 loading buffer B. Poly (A⁺) RNA was eluted with 3 volumes of 10 mM Tris-Cl, pH 7.4, 1
30 mM EDTA, and 0.05% (w/v) SDS. Fractions were collected and those containing RNA were
31 identified in an ethidium bromide plate assay, a petri dish with 1% agarose made with TAE

1 containing EtBr. RNA was precipitated, resuspended in 10 μ l, and a 1 μ l aliquot quantitated
2 with a spectrophotometer.

3 4 B Isolation of GLTP and GHTP DNA Sequences

5 The nucleotide sequences utilized in the development of the antisense construct were
6 randomly selected from the 5' sequence of GLTP (SEQ ID NO: 1) and GHTP (SEQ ID NO:
7 3). DNA sequences used to develop the antisense constructs were obtained using reverse
8 transcription-polymerase chain reaction. GLTP (SPL1 and SPL2)- and GHTP (SPH1 and
9 SPH2)-specific primers were designed according to the published sequences (Brisson et al.
10 1990, Fukui et al. 1991) with minor modifications to facilitate restriction with enzymes:

11 SPL1 Primer: 5'ATTCGAAAAGCTCGAGATTGTCATAGA3' (SEQ ID NO: 7) (additional
12 CG creates Xho I site);

13 SPL2 Primer: 5'GTGTGCTCTCGAGCATTGAAAGC3' (SEQ ID NO: 8) (changed C to G to
14 create Xho I site);

15 SPH1 Primer: 5'GTTTATTTTCCATCGATGGAAGGTGGTG3' (SEQ ID NO: 9) (added
16 CGAT to create Cla I site);

17 SPH2 Primer: 5'ATAATATCCTGAATCGATGCACTGC3' (SEQ ID NO: 10) (changed G to
18 T to create Cla I site).

19 Reverse transcription was performed in a volume of 15 μ l containing 1 x PCR buffer
20 (10 mM Tris-Cl pH 8.2, 50 mM KCl, 0.001% gelatin, 1.5 mM MgCl₂), 670 μ M of each
21 dNTP, 6 μ g of total potato tuber cv. Russet Burbank RNA, 1 mM each primer (SPH1 and
22 SPL2, or SPH1 and SPH2) and 200 U of Maloney murine leukemia virus reverse
23 transcriptase (BRL). The reaction was set at 37°C for 30 minutes, then heat-killed at 94°C for
24 5 minutes and snap cooled on ice. To the reverse transcription reaction was added 2.5 U Taq
25 DNA polymerase (BRL) in 35 μ l of 1 x PCR buffer. DNA amplification was done in a Perkin
26 Elmer 480 programmed for 30 cycles with a 1 min 94°C denaturation step, a 1 min 56 °C
27 (SPL1 and SPL2) or 58°C (SPH1 and SPH2) annealing step, and a 2 min 72°C extension
28 step. PCR was completed with a final 10 min extension at 72°C.

C Construction of SP Vectors for Phosphorylase Inhibition

To express the antisense constructs in plant cells, it was necessary to fuse the genes to the proper plant regulatory regions. This was accomplished by cloning the antisense DNA into a plasmid vector that contained the needed sequences.

Amplified DNA was blunt ended and cloned into a pUC19 vector at the *Sma*I site. The recombinant plasmid was transformed into sub-cloning efficiency *E. coli* DH5 α cells (BRL). The transformed cells were plated on LB (15 g/l Bactotryptone, 5 g/l yeast extract, 10 g/l NaCl, pH 7.3, and solidified with 1.5% agar) plates that contained ampicillin at 100 ug/ml. Selection of bacteria containing plasmids with inserted plant phosphorylase sequence was accomplished using color selection. The polylinker and T3 and T7 RNA polymerase promoter sequences are present in the N-terminal portion of the *lacZ* gene fragment. pUC19 plasmids without inserts in the polylinker grow as blue colonies in appropriate bacterial strains such as DH5 α . Color selection was made by spreading 100 μ l of 2% X-gal (prepared in dimethyl formamide) on LB plates containing 50 μ g/ml ampicillin 30 minutes prior to plating the transformants. Colonies containing plasmids without inserts will be blue after incubation for 12 to 18 hours at 37C and colonies with plasmids containing inserts will remain white. An isolated plasmid was sequenced to confirm the sequence of the phosphorylase inserts. Sequences were determined using the ABI Prism™ Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems, Foster City, CA), M13 universal and reverse primers, and an ABI automated DNA sequencer. The engineered plasmid was purified by the rapid alkaline extraction procedure from a 5 ml overnight culture (Birnboim and Doly, 1979). Orientation of the SPL and SPH fragments in pUC19 was determined by restriction enzyme digestion. The recombinant pUC19 vectors and the binary vector pBI121 (Clontech) were restricted, run on a agarose gel and the fragments purified by gel separation as described by Thuring et al (1975).

Ligation fused the antisense sequence to the binary vector pBI121. The ligation contained pBI121 vector that had been digested with *Bam*HI and *Sac*I, along with the SPL or SPH phosphorylase DNA product, that had been cut from the pUC19 subclone with *Bam*HI and *Sac*I. Ligated DNA was transformed into SCE *E. coli* DH5 α cells, and the transformed cells were plated on LB plates containing ampicillin. The nucleotide sequences of the antisense DNA SPL and SPH are nucleotides 338 to 993 of SEQ ID NO: 1 and nucleotides

1 147 to 799 of SEQ ID NO: 3, respectively. Selection of pBI121 with phosphorlyase inserts
2 was done with CAMV and NOS specific primers.

3 Samples 1 and 2 representing the tuber L-type and tuber H-type phosphorylase DNA
4 fragments were picked from a plate after overnight growth. These samples were inoculated
5 into 5 ml of LB media and grown overnight at 37°C. Plasmids were isolated by the rapid
6 alkaline extraction procedure, and the DNA was electroporated into *Agrobacterium*
7 *tumefaciens*.

8 Constructs were engineered into the pBI121 vector that contains the CaMV 35S
9 promoter (Kay et al. 1987) and the NOS 3' terminator (Bevan et al. 1983) sequence. The
10 pBI121 plasmid is made up of the following well characterized segments of DNA. A 0.93 kb
11 fragment isolated from transposon Tn7 which encodes bacterial spectinomycin/streptomycin
12 (Spc/Str) resistance and is a determinant for selection in *E. coli* and *Agrobacterium*
13 *tumefaciens* (Fling et al., 1985). This is joined to a chimeric kanamycin resistance gene
14 engineered for plant expression to allow selection of the transformed tissue. The chimeric
15 gene consists of the 0.35 kb cauliflower mosaic virus 35S promoter (P-35S) (Odell et al.,
16 1985), the 0.83 kb neomycin phosphotransferase type II gene (NPTII), and the 0.26 kb 3' non-
17 translated region of the nopaline synthase gene (NOS 3') (Fraley et al., 1983). The next
18 segment is a 0.75 kb origin of replication from the RK2 plasmid (ori-V) (Stalker et al., 1981).
19 It is joined to a 3.1 kb *SalI* to *PvuI* segment of pBR322 which provides the origin of
20 replication for maintenance in *E. coli* (ori-322) and the *bom* site for the conjugational transfer
21 in the *Agrobacterium tumefaciens* cells. Next is a 0.36 kb *PvuI* fragment from the pTiT37
22 plasmid which contains the nopaline-type T-DNA right border region (Fraley et al., 1985).
23 The antisense sequence was engineered for expression in the tuber by placing the gene under
24 the control of a constitutive tissue non-specific promoter.

25

26 D Plant Transformation/Regeneration

27 The SPL and SPH vectors were transformed into the Desiree potato cultivar according
28 to de Block (1988). To transform "Desiree" potatoes, sterile shoot cultures of "Desiree" were
29 maintained in test tubes containing 8 ml of S1 (Murashige and Skoog (MS) medium
30 supplemented with 2% sucrose and 0.5 g/l MES pH 5.7, solidified with 6 g/l Phytagar). When
31 plantlets reached approximately 5 cm in length, leaf pieces were excised with a single cut

1 along the base and inoculated with a 1:10 dilution of an overnight culture of Agrobacterium
2 tumefaciens. The stem explants were co-cultured for 2 days at 20°C on S1 medium (De
3 Block 1988). Following co-culture, the explants were transferred to S4 medium (MS medium
4 without sucrose, supplemented with 0.5 g/l MES pH 5.7, 200 mg/l glutamine, 0.5 g/l PVP, 20
5 g/l mannitol, 20 g/l glucose, 40 mg/l adenine, 1 mg/l trans zeatin, 0.1 mg/l NAA, 1 g/l
6 carbenicillin, 50 mg/l kanamycin, solidified with 6 g/l phytagar) for 1 week and then 2 weeks
7 to induce callus formation.

8 After 3 weeks, the explants were transferred to S6 medium (S4 without NAA and with
9 half the concentration (500 mg/l) of carbenicillin). After another two weeks, the explants
10 were transferred to S8 medium (S6 with only 250 mg/l carbenicillin and 0.01 mg/l gibberellic
11 acid, GA3) to promote shoot formation. Shoots began to develop approximately 2 weeks
12 after transfer to S8 shoot induction medium. These shoots were excised and transferred to
13 vials of S1 medium for rooting. After about 6 weeks of multiplication on the rooting
14 medium, the plants were transferred to soil and are gradually hardened off.

15 Desiree plants regenerated in culture were transplanted in 1 gallon pots and were
16 grown to maturity under greenhouse conditions. Tubers were harvested and allowed to
17 suberize at room temperature for two days. All tubers greater than 2 cm in length were
18 collected and stored at 4°C under high humidity.

20 E Field Trials

21 Untransformed controls, plants expressing the SPL construct, and plants expressing
22 the SPH construct were propagated in field trials in a single replicate randomized design. All
23 plants were grown side by side in the same field and exposed to similar pesticide, fertilizer,
24 and irrigation regimes. Tubers were harvested and stored at 10°C for 2 weeks before
25 randomly selecting a fraction of the tubers from each line to be placed in storage at 4°C.

27 F Sugar Analysis

28 Tubers were stored at 4°C and were not allowed to recondition at room temperature
29 prior to sugar analysis. An intact longitudinal slice (1 cm thick, width variable and equal to
30 the outside dimensions of the tuber) was cut from the central portion of each tuber, thus
31 representing all of the tuber's tissues. At each harvest, the central slices from four tubers per

clone (3 replicates) were collectively diced into 1-cm cubes and 15 g was randomly selected from the pooled tissue for analysis. Glucan phosphorylase (see below) and sugars were extracted with 15 mL of Tris buffer (50 mM, pH 7.0) containing 2 mM sodium bisulfite, 2 mM EDTA, 0.5 mM PMSF and 10% (w/w) glycerol with a polytron homogenizer at 4°C. The extracts were centrifuged at 4°C (30,000 g, 30 min) and reducing sugars (glucose and fructose) were measured on a 10-fold dilution of the supernatant using a Spectra Physics™ high performance liquid chromatograph interfaced to a refractive index detector. The separation was performed at 80°C on a 30 x 0.78 cm Aminex™ HPX 87C column (Biorad) using 0.6 ml/min water as the mobile phase. Calibration of the instrument was via authentic standards of d-glucose and d-fructose.

H Analysis of α -Glucan Phosphorylase Activity

Tubers stored at 4°C were not allowed to warm prior to extraction and analysis of α glucan phosphorylase activity and isozymes. The *in vitro* activity of glucan phosphorylase in the phosphorolytic direction was assayed as described by Steup (1990). Briefly, samples of extracts obtained for sugar analysis (see above) were added to a reaction medium which coupled starch phosphorolysis to the reduction of NADP through the sequential actions of phosphoglucomutase and glucose-6-phosphate dehydrogenase. The rate of reduction of NADP during the reaction is stoichiometric with the rate of production of glucose-1-phosphate from the starch substrate. Reduction of NADP was followed at 340 nm in a Varian Cary double-beam spectrophotometer. Protein levels in extracts were determined according to Bradford (1976).

Glucan phosphorylase activity gels were run essentially according to Steup (1990). Proteins were separated on native polyacrylamide gels (8.5 %) containing 1.5 % glycogen. Following electrophoresis at 80 V for 15 h (4°C), the gels were incubated (1-2 h) at 37°C in 0.1 M citrate-NaOH buffer (pH 6.0) containing 20 mM glucose-1-P and 0.05% (w/v) hydrolyzed potato starch. Gels were then rinsed and stained with an iodine solution. For Western blot analysis, proteins were electrophoresed on glycogen-containing polyacrylamide gels as described above. The proteins were electroblotted to nitrocellulose and blots were probed with polyclonal antibodies raised against GHTP and GLTP.

1 Immunoblots were developed with alkaline phosphatase conjugated anti-rabbit secondary
2 antibodies (Sigma).

3 4 H Chip Color Determination

5 Five transgenic potato lines expressing the GLTP antisense sequence, two transgenic
6 lines expressing the GHTP antisense sequence, non-transgenic Desiree control lines, and two
7 control lines transformed with the pBI121 vector T-DNA, were grown under field conditions
8 in Alberta, Canada. Tubers were harvested and stored at 10°C and 4°C. Chip color was
9 determined for all potato lines by taking center cuts from representative samples from each
10 line and frying at 205°F in soybean oil for approximately 3 minutes until bubbling stops.

11 12 I Results

13 All tubers were harvested from plants of the same cultivar (Desiree), the same age,
14 and grown side by side under identical growth conditions. Northern analysis of tubers
15 showed a considerable reduction of endogenous GLTP transcript in transgenic plants
16 expressing the homologous antisense transcript (Figure 7). Glucan phosphorylase assays
17 showed that activities ($\mu\text{mol NADPH mg}^{-1} \text{ protein h}^{-1}$) were reduced (Table 1) at harvest and
18 for at least six months following harvest in transgenic plants expressing the GLTP antisense
19 DNA. The results tabulated in Table 1 show that α glucan phosphorylase activity in tubers
20 stored at 4°C for 189 days was reduced from approximately 16% to 70% in various
21 transformed potato varieties relative to the wildtype control strain. Activity gels and western
22 blot analysis showed specific reduced expression of homologous enzymes and lower
23 reduction of expression for heterologous enzymes (Figures 10 and 11). This specificity for
24 homologous products may result from differences between the phosphorylases (Figures 6A
25 and 6B).

26 Analysis of tubers at harvest (0 days) shows that those expressing the antisense GLTP
27 transcript have up to 5-fold less reducing sugars than control tubers (Table 2). Furthermore,
28 after 91 days storage at 4°C, transformed tubers contained 28-39% lower reducing sugar
29 concentrations than the wildtype control strain. Concentrations of glucose and fructose were
30 reduced significantly in tubers expressing the antisense GLTP transcript (Tables 3 and 4).
31 These results suggest that reduced GLTP activity slows the catabolism of starch into reducing

sugars in tubers, while in the control tubers the sugars continue to accumulate. The correlation between total phosphorylase activity and the concentration of reducing sugars is not direct, suggesting that certain isozymes of phosphorylase may play a more important role in the catabolism of starch, that specific levels of reduced expression of particular phosphorylase isozymes may be more optimum than others, and/or that there may be unidentified interactions involved in the lower reducing sugar levels.

Transgenic potato plants expressing the antisense GLTP or GHTP transcript have been grown under field conditions and their tubers stored at 4°C. Chip color, which correlated with sugar content, was determined prior to cold storage and after 86 and 124 days of cold storage. The chip color of tubers from all transgenic plants expressing the antisense GLTP transcript was significantly improved (lighter) relative to that of control tubers (darker) stored under identical conditions (Table 5 and Figure 9). Chip scores of tubers from "Desiree" potato plants expressing the GLTP transcript were improved by at least 4.3 points and 8.9 points as determined with an Agtron model E-15-FP Direct Reading Abridged Spectrophotometer (Agtron Inc. 1095 Spice Island Drive #100, Sparks Nevada 89431) following storage at 10°C and 4°C, respectively, for 86 days. Chip scores of GLTP transformants measured after 124 days of storage at 4°C were improved by 44% to 89% relative to wildtype (Table 5).

The Desiree cultivar is not a commercially desirable potato for chipping due to its high natural sugar content and propensity to sweeten rapidly in cold storage. Nevertheless, significant improvements in fried chip color were noted with the transformed "Desiree" potatoes. It is expected that superior color lightening would be achieved if the methods of the invention were applied to commercial processing potato varieties.

Analysis of tubers stored at 10°C and 4°C shows that those expressing the antisense GHTP transcript sometimes provided chips that fried lighter than control tubers, indicating a lower buildup of reducing sugars (Table 5). Results showing heterologous and homologous reduction in phosphorylase activity (Figures 10 and 11) indicate that the improvement may be a result of reducing one or both tuber phosphorylases. However, these results suggest that the L-type phosphorylase plays a more important role in the catabolism of starch into reducing sugars.

- 1 Further, the results show that the difference in reducing sugar levels (Table 2) and
- 2 chip scores (Table 5) between tubers wildtype plants and those expressing tuber

1 phosphorylase antisense RNA, are sustained during long-term storage. As shown in Table 5,
2 the chip scores are approximately the same at 86 days and 124 days. No further increases in
3 reducing sugar concentrations were evident after 49 and 91 days storage at 4°C (Table 2).
4 This equilibrium in sugar concentration was probably associated with the kinetics of the tuber
5 phosphorylases. The capability of maintaining lower sugar levels has the potential of
6 extending the period of storage by at least several months. Presently, processing potatoes are
7 usually stored for a maximum of three to six months at 10°C to 12°C before the sugar
8 accumulation reaches levels that reduce quality. Fresh product must be imported until the
9 present season potatoes become available. Extending the storage period of potatoes by many
10 months may reduce import costs.

11 Table 6 provides a summary of the percentage improvement in various improved tuber
12 cold-storage characteristics of tubers of potato plants transformed with antisense DNA
13 derived from the GLTP gene sequence (ATL3 - ATL9), and from the GHTP gene sequence
14 (ATH1 and ATH2) relative to untransformed control plants. It is apparent from the results
15 summarized in Table 6 that substantial improvements in tuber cold-storage characteristics
16 may be consistently obtained through the methods of the present invention. Particularly
17 noteworthy are the percentage chip score improvements over wildtype observed after storage
18 at 4°C for approximately four months (124 days). Relative chip score improvements of up to
19 89% relative to wildtype were observed. Improved chip scores reflect the commercial utility
20 of the invention. That is, by reducing cold-induced sweetening, tubers can be stored at cooler
21 temperatures, without causing unacceptable darkening of fried potato products.

22 The reduction in sugar accumulation of transformed potato lines relative to wildtype,
23 both at harvest and after 91 day storage, also demonstrates significant advantages of the
24 invention. Reduced sugar accumulation relates to the observed chip score improvements, and
25 also reflects improved specific gravity of tubers, another important commercial measure of
26 tuber quality.

27 Even at harvest, substantial improvements in chip score and reduced sugar
28 accumulation were noted for transformed lines relative to wildtype. Thus, the benefits of the
29 invention are not limited to improvements that arise only after extended periods of cold
30 storage, but that are present at the time of harvest. In this sense, the invention is not limited
31 only to improvements in cold-storage characteristics but encompasses improvements in tuber

1 quality characteristics such as chip score or sugar accumulation which are present at the time
2 of harvest, resulting in earlier maturity.

3 Turning to specific improvements summarized in Table 6, it can be seen that GLTP-
4 type transformants (ATL3 - ATL9) exhibited up to a 66%, 70% and 69% reduction in α
5 glucan phosphorylase activity relative to wildtype, at harvest, and after storage for 91 and 189
6 days, respectively. Most also exhibited improvements in excess of 10% and 30% relative to
7 wildtype at harvest and after storage for 91 and 189 days. After storage for 91 and 189 days,
8 the GHTP-type transformants (ATH1 and ATH2) exhibited, respectively, up to 28% and 39%
9 relative improvement over wildtype and generally showed at least 10% improvement.

10 The GLTP-type transformants exhibited up to 80% and 39% reduction of sugar
11 accumulation relative to wildtype at harvest and at 91 days, respectively. At harvest, all
12 GLTP-type transformants exhibited at least 10% and at least 30% relative improvement. At
13 91 days, all GLTP-type transformants exhibited at least 10% and most exhibited at least 30%
14 relative improvement.

15 The GLTP-type transformants exhibited up to 46%, 89% and 89% chip score
16 improvement relative to wildtype at harvest, and after storage for 86 days and 124 days,
17 respectively. Almost all exhibited at least 10% and 30% relative improvement at harvest, and
18 after storage for 86 and 124 days. At least one of the GHTP-type transformants exhibited at
19 least 5% and at least 10% improvement relative to wildtype at harvest, and after storage for
20 86 and 124 days. After 124 days storage, at least one of the GHTP-type transformants
21 exhibited up to 25% relative improvement in chip score.

22 The results clearly demonstrate that substantial improvements in tuber cold-storage
23 characteristics may be readily obtained through the methods of the invention. Results will
24 vary due to, among other things, the location within the plant genome where the recombinant
25 antisense or sense DNA is inserted, and the number of insertion events that occur. It is
26 important to note that despite the variability in the results amongst the various transformed
27 lines, there was little variation in the results amongst the samples within a single transformed
28 potato line (see footnotes to Tables 1 to 5). Results are presented in Table 6 for all potato
29 plant lines which were successfully transformed with the GHTP or GLTP antisense DNA.
30 Therefore, all transformants show at least some improvement in one or more cold-storage
31 characteristics such as increased chip score (lighter color on cooking) and reduced sugar

-WO 98/35051

PCT/CA98/00055

1 accumulation, and most show very substantial improvements. Given the large proportion of
2 positive transformants observed in the examples herein, it is expected that, using the cold-
3 storage characteristic testing procedures described in the examples, potato plants transformed
4 through the methods of the invention can be readily screened to identify transformed lines
5 exhibiting significantly improved cold-storage characteristics. By applying the techniques
6 disclosed herein to commercially important potato varieties, it will be possible to readily
7 create and select transformants having significantly improved cold-storage characteristics.
8 Those transformants showing the greatest relative improvements over wildtype controls can
9 be used in the development of new commercial potato varieties.

10

WO 98/35051

PCT/CA98/00055

Table 1

Effects of an antisense transcript on glucan phosphorylase activity measured in enzyme extracts from field grown "Desiree" tubers.

Glucan Phosphorylase Activity Storage Period at 4C (days)					
Clone	0	49	91	140	189
$\mu\text{mol NADPH mg}^{-1} \text{protein h}^{-1}$					
Wt ^a	10.50	11.83	9.94	11.90	13.04
ATL3	4.90	4.86	4.49	4.73	4.88
ATL4	11.45	7.17	8.09	11.32	10.99
ATL5	3.58	3.56	2.97	4.59	4.79
ATL9	3.59	3.88	3.84	4.72	3.98
LSD _{0.05} ^b	1.97	2.94	1.59	2.34	2.58
LSD _{0.01}	2.87	4.28	2.31	3.41	3.75
Clone ^c		0.01 ^d			
WT vs. ATL's		0.01			
Days		NS			
Clone x Days		0.05			
WT		11.49	8.90	12.66	13.66
ATH-1		10.40	9.69	10.79	10.10
ATH-2		6.46	6.40	6.56	8.38
LSD _{0.05} ^b		2.02	0.41	3.00	NS
LSD _{0.01}		4.78	0.95	NS	NS
Clone ^c		0.01			
WT vs. ATH's		0.01			
Days		0.05			
Clone x Days		NS			

^aWT, wild type untransformed tubers. ^bLSD, least significant difference at 0.05 or 0.01 level for each storage period. ^cSources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

WO 98/35051

PCT/CA98/00055

Table 2

Effects of an antisense GLTP transcript on low temperature induced sweetening of field grown "Desiree" tubers.

Clone	Reducing Sugars (glucose + fructose) Storage Period at 4C (days)		
	0	49	91
	mg g ⁻¹ fresh weight		
Wt ^a	5.63	31.8	28.0
ATL3	1.88	17.3	17.3
ATL4	1.11	14.3	20.1
ATL5	1.51	18.3	17.0
ATL9	1.36	17.3	18.5
WT vs. ATL's ^b	0.01	0.01	0.05
Clone ^c		0.01 ^d	
Days		0.01	
Clone x Days		NS	

^aWT, wild type untransformed tubers. ^bOrthogonal comparisons for ANOVA's at each storage period. ^csources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

WO 98/35051

PCT/CA98/00055

Table 3

Effects of an antisense GLTP transcript on low temperature induced fructose accumulation of field grown "Desiree" tubers.

Clone	Fructose Storage Period at 4C (days)		
	0	49	91
	mg g ⁻¹ fresh weight		
Wt ^a	3.53	15.10	12.20
ATL3	1.21	8.40	8.79
ATL4	0.79	7.22	8.56
ATL5	0.61	10.00	8.09
ATL9	0.54	8.38	8.72
WT vs. ATL's ^b	0.01	0.01	NS
Clone ^c		0.01 ^d	
Days		0.01	
Clone x Days		NS	

^aWT, wild type untransformed tubers. ^bOrthogonal comparisons for ANOVA's at each storage period. ^cSources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

WO 98/35051

PCT/CA98/00055

Table 4

Effects of an antisense GLTP transcript on low temperature induced glucose accumulation of field grown "Desiree" tubers.

Clone	Glucose Storage Period at 4C (days)		
	0	49	91
	mg g ⁻¹ fresh weight		
Wt ^a	2.10	16.60	15.90
ATL3	0.68	8.94	8.49
ATL4	0.32	7.07	11.06
ATL5	1.05	8.33	8.91
ATL9	0.83	8.87	9.78
WT vs. ATL's ^b	0.01	0.01	0.05
Clone ^c		0.01 ^d	
Days		0.01	
Clone x Days		NS	

^aWT, wild type untransformed tubers. ^bOrthogonal comparisons for ANOVA's at each storage period. ^csources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

Table 5

Average chip color of field grown "Desiree" tubers. The chip color rating was assigned using an Agtron meter similar to that used by industry to measure color of fried potatoes. In this index, the higher the number the lighter the chip product but color does not represent a linear relationship to the index.

Storage Temperature, Period, and Agtron Reading ^a				
	Harvest	86 days	86 days	124 days
		10C	4C	4C
Wt ^b	26	25.3	15.4	17.1
ATL3 ^c	25	37.4	26.7	30.8
ATL4	35	43.7	29.1	32.3
ATL5	36	29.6	24.7	24.6
ATL9	38	38.7	24.3	26.6
ATH1 ^d	26	49.7	17.5	21.4
ATH2	29	31.2	15.6	15.9
GMP1 ^e	31		15.7	15.7
GMP2	35		16.7	16.6

^aAgtron Inc. 1095 Spice Island Drive #100, Sparks Nevada 89431. Agtron model E-15-FP (Direct Reading Abridged Spectrophotometer). Measures ratio of reflectance in two spectral modes, near infrared and green. Results represent the measurement of 6 to 8 chips from 3 randomly selected tubers approximately 3 to 4 cm in diameter.

^bWT, negative control, wild type untransformed tubers.

^cATL, tubers transformed with the tuber L-type α glucan phosphorylase.

^dATH, tubers transformed with the tuber H-type α glucan phosphorylase.

^eGMP, negative control, tubers transformed with pBI121 T-DNA.

WO 98/35051

PCT/CA98/00055

Summary of Results

Table 6

Sample	% Reduction of α glucan phosphorylase activity relative to wildtype			% Reduction of Sugar Accumulation relative to wildtype		% Chip Score Improvement relative to wildtype		
	at harvest	91 days	189 days	at harvest	91 days	at harvest	86 days	124 days
ATL 3	53	55	63	67	38	-4	73	80
ATL 4	-9	19	16	80	28	35	89	89
ATL 5	66	70	63	73	39	38	60	44
ATL 9	66	61	69	76	34	46	58	56
ATH 1	n/a	-9	26	n/a	n/a	0	14	25
ATH 2	n/a	28	39	n/a	n/a	12	1	-7

REFERENCES

- Alber and Kawasaki (1982) Mol. And Appl. Genet. 1:419-434.
- ap Rees *et al.* (1988) Symp. Soc. Exp. Biol. 42:377-393.
- Bevan *et al.* (1983) Nature (London) 304:184-187.
- Bevan *et al.* (1986) Nucleic Acids Res. 14 (11):4625-4638.
- Birnboim *et al.* (1979) Nucleic Acids Res. 7:1513-1523.
- Blennow *et al.* (1991) Phytochemistry 30:437-444.
- Bradford, M.M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 243-254.
- Brisson *et al.* (1989) The Plant Cell 1:559-566.
- Brusslan and Tobin (1995) Plant Molecular Biology 27:809-813.
- Burton, W.G. (1989) The Potato. Longman Scientific and Technical,
- Cannon *et al.* (1990) Plant Molecular Biology 15:39-47
- Claassen *et al.* (1991) Plant Physiol. 95:1243-1249.
- Coffin *et al.* (1987) J. Food Sci. 52:639-645.
- Davies and Viola (1992) Postharvest News and Information 3:97-100.
- De Block, M. (1988) Theoretical and Applied Genetics 76:767-774.
- De Carvalho *et al.* (1992) EMBO J. 11:2595-2602.
- Depicker *et al.* (1982) Mol. And Appl. Genet. 1:561-573.
- Dixon *et al.* (1981) Phytochemistry 20:969-972.
- Dorlhac *et al.* 1994 Mol. Gen. Genetic. 243:613-621.
- Ebbelaar *et al.* (1993) Int. Symp. on Gen. Manip. of Plant Metabolism and Growth, 29-31 March, Norwich UK Abstract #9.
- Ecker and Davis (1986) Proc. Natl. Acad. Sci. 83: 5373-5376.
- Fling *et al.* (1985) Nucleic Acids Research 13 no. 19, 7095-7106.
- Fraley, *et al.* (1983) Proc Natl Acad Sci USA 80, 4803-4807.
- Fraley *et al.* (1985) Bio/Technology 3, 629-635.
- Fray and Grierson 1993 Plant Mol. Biol. 22:589-602.
- Gielen *et al.* (1984) EMBO J. 3:835-846.
- Hasseloff, J. And W.L. Gerlach (1988) Nature 334:585-591.

- 1 Hart *et al.* (1992) Mol. Gen. Genetic. 235:179-188.
- 2 Jorgensen, R.A. (1995) Science 268: 686-691.
- 3 Kawchuk *et al.* (1990) Molecular Plant-Microbe Interactions 3:301-307.
- 4 Kawchuk *et al.* (1991) Mol. Plant Microbe-Inter. 4:247-253.
- 5 Kay *et al.* (1987) Science 236:1299-1302.
- 6 Kruger, N.J. and Hammond, J.B.W. (1988) Plant Physiol. 86:645-648.
- 7 Laemmli, U.K. (1970) Nature (London) 227:680-685.
- 8 Lin *et al.* (1988) Plant Physiol. 86:1131-1135.
- 9 Loiselle *et al.* (1990) American Potato Journal 67:633-646.
- 10 Lynch *et al.* (1992) Can., J. Plant Sci. 72: 535-543.
- 11 Matzke and Matzke (1995) Plant Physiol. 107:679.
- 12 Meyer and Saedler (1996) Annu. Rev. Plant Physiol. 47:23-48.
- 13 Mori *et al.* (1991) J. Biol. Chem. 266:18446-18453.
- 14 Muller, *et al.* (1990) Mol. Gen. Genet. 224:136-146.
- 15 Nakano *et al.* (1989) J. Biochem. 106:691-695.
- 16 Nakano, K. and Fukui, T. (1986) J. Biol. Chem. 266:8230-8256.
- 17 Napoli *et al.* (1990) Plant Cell 2:279-289.
- 18 Odell, *et al.* (1985) Nature 313, 810-812.
- 19 Ohta *et al.* (1991) Mol. Gen. Genet. 225:369-378.
- 20 Ortiz, R. and Huaman, Z. (1994) In:Potato Genetics. Bradshaw, J.E. and Mackay G.R. (eds.)
- 21 Sambrook *et al.* (1989) Molecular Cloning, A Laboratory Manual. 2nd Ed. Cold Spring
- 22 Harbor Laboratory Press, N.Y.
- 23 Seymour *et al.* 1993 Plant Mol. Biol. 23:1-9.
- 24 Shahr *et al.* (1992) Plant Cell 4:135-147.
- 25 Shallenberger *et al.* (1959) Agric. and Food Chem. 7:274-277.
- 26 Smith *et al.* (1988) Nature 334:724-726.
- 27 Smith *et al.* (1990) Mol. Gen. Genet. 244:447-481.
- 28 Sonnewald *et al.* (1995) Plant Molecular Biology 27:567-576.
- 29 Sowokinos, J. (1990) In: The molecular and Cellular Biology of the Potato. M.A. Mayo and
- 30 W.D. Parks (eds.).
- 31 Stalker *et al.* (1981) Mol Gen Genet 181, 8-12.

- 1 Steup, M. (1990) Starch Degrading Enzymes *in* "Methods in Plant Biochemistry" Vol 3.
2 P.M. Dey and J.B. Harborne, eds. Academic Press, London
3 Stiekema *et al.* (1988) Plant Mol. Biol. 11:255-269.
4 Stukerlj *et al.* (1990) Nucl. Acids Res. 18:46050.
5 Takaha *et al.*, (1993) J. Biol. Chem. 268:1391-1396.
6 Thuring *et al.* (1975) Analytical Biochemistry 66:213-220.
7 Van der Krol *et al* (1988) Gene 72:45-50.
8 Van der Krol (1990) Plant Cell 2:291-299.
9 Weaver *et al.* (1978) Am. Pot. J. 55:83-93.
10 Weintraub (1990) Scientific American 1:34-40.
11 Winnacker, Ernst L. (1987) From Genes to Clones. VCH Verlagsgesellschaft mbH, Federal
12 Republic of Germany
13 Yoshida *et al.* (1992) Geneg 10:255-259.
14

15 All publications mentioned in this specification are indicative of the level of skill in
16 the art to which this invention pertains. Although the foregoing invention has been described
17 in some detail by way of illustration and example for purposes of clarity of understanding, it
18 will be obvious that certain changes and modifications may be practised within the scope of
19 the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Her Majesty the Queen in Right of Canada as Represented
by the Department of Agriculture and Agri-Food Canada
- (ii) TITLE OF INVENTION: Potatoes Having Improved Quality
Characteristics and Methods for Their Production
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: McKay-Carey & Company
 - (B) STREET: 2125 Commerce Place, 10155-102nd Street
 - (C) CITY: Edmonton
 - (D) STATE: Alberta
 - (E) COUNTRY: Canada
 - (F) ZIP: T5J 4G8
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: WO
 - (B) FILING DATE: 10-FEB-1998
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/036,946
 - (B) FILING DATE: 10-FEB-1997
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/868,786
 - (B) FILING DATE: 04-JUN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: McKay-Carey, Mary Jane
 - (B) REGISTRATION NUMBER: 3790
 - (C) REFERENCE/DOCKET NUMBER: 24002W00
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (403) 424-0222
 - (B) TELEFAX: (403) 421-0834

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3101 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 44..2944

(D) OTHER INFORMATION: /product= "potato alpha-glucan
L-type tuber phosphorylase"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 194..2941

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 44..193

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATCACTCTCA	TTCGAAAAGC	TAGATTTGCA	TAGAGAGCAC	AAA	ATG	GCG	ACT	GCA		55						
					Met	Ala	Thr	Ala								
					-50											
AAT	GGA	GCA	CAC	TTG	TTC	AAC	CAT	TAC	AGC	TCC	AAT	TCC	AGA	TTC	ATC	103
Asn	Gly	Ala	His	Leu	Phe	Asn	His	Tyr	Ser	Ser	Asn	Ser	Arg	Phe	Ile	
-45					-40					-35						
CAT	TTC	ACT	TCT	AGA	AAC	ACA	AGC	TCC	AAA	TTG	TTC	CTT	ACC	AAA	ACC	151
His	Phe	Thr	Ser	Arg	Asn	Thr	Ser	Ser	Lys	Leu	Phe	Leu	Thr	Lys	Thr	
-30					-25					-20					-15	
TCC	CAT	TTT	CGG	AGA	CCC	AAA	CGC	TGT	TTC	CAT	GTC	AAC	AAT	ACC	TTG	199
Ser	His	Phe	Arg	Arg	Pro	Lys	Arg	Cys	Phe	His	Val	Asn	Asn	Thr	Leu	
			-10					-5						1		
AGT	GAG	AAA	ATT	CAC	CAT	CCC	ATT	ACT	GAA	CAA	GGT	GGT	GAG	AGC	GAC	247
Ser	Glu	Lys	Ile	His	His	Pro	Ile	Thr	Glu	Gln	Gly	Gly	Glu	Ser	Asp	
	5					10					15					
CTG	AGT	TCT	TTT	GCT	CCT	GAT	GCC	GCA	TCT	ATT	ACC	TCA	AGT	ATC	AAA	295
Leu	Ser	Ser	Phe	Ala	Pro	Asp	Ala	Ala	Ser	Ile	Thr	Ser	Ser	Ile	Lys	
20					25				30							
TAC	CAT	GCA	GAA	TTC	ACA	CCT	GTA	TTC	TCT	CCT	GAA	AGG	TTT	GAG	CTC	343
Tyr	His	Ala	Glu	Phe	Thr	Pro	Val	Phe	Ser	Pro	Glu	Arg	Phe	Glu	Leu	
35					40				45					50		

CCT	AAG	GCA	TTC	TTT	GCA	ACA	GCT	CAA	AGT	GTT	CGT	GAT	TCG	CTC	CTT	391
Pro	Lys	Ala	Phe	Phe	Ala	Thr	Ala	Gln	Ser	Val	Arg	Asp	Ser	Leu	Leu	
				55					60					65		
ATT	AAT	TGG	AAT	GCT	ACG	TAT	GAT	ATT	TAT	GAA	AAG	CTG	AAC	ATG	AAG	439
Ile	Asn	Trp	Asn	Ala	Thr	Tyr	Asp	Ile	Tyr	Glu	Lys	Leu	Asn	Met	Lys	
			70					75					80			
CAA	GCG	TAC	TAT	CTA	TCC	ATG	GAA	TTT	CTG	CAG	GGT	AGA	GCA	TTG	TTA	487
Gln	Ala	Tyr	Tyr	Leu	Ser	Met	Glu	Phe	Leu	Gln	Gly	Arg	Ala	Leu	Leu	
		85					90					95				
AAT	GCA	ATT	GGT	AAT	CTG	GAG	CTT	ACT	GGT	GCA	TTT	GCG	GAA	GCT	TTG	535
Asn	Ala	Ile	Gly	Asn	Leu	Glu	Leu	Thr	Gly	Ala	Phe	Ala	Glu	Ala	Leu	
	100					105					110					
AAA	AAC	CTT	GGC	CAC	AAT	CTA	GAA	AAT	GTG	GCT	TCT	CAG	GAA	CCA	GAT	583
Lys	Asn	Leu	Gly	His	Asn	Leu	Glu	Asn	Val	Ala	Ser	Gln	Glu	Pro	Asp	
115					120				125						130	
GCT	GCT	CTT	GGA	AAT	GGG	GGT	TTG	GGA	CGG	CTT	GCT	TCC	TGT	TTT	CTG	631
Ala	Ala	Leu	Gly	Asn	Gly	Gly	Leu	Gly	Arg	Leu	Ala	Ser	Cys	Phe	Leu	
				135					140					145		
GAC	TCT	TTG	GCA	ACA	CTA	AAC	TAC	CCA	GCA	TGG	GGC	TAT	GGA	CTT	AGG	679
Asp	Ser	Leu	Ala	Thr	Leu	Asn	Tyr	Pro	Ala	Trp	Gly	Tyr	Gly	Leu	Arg	
			150					155					160			
TAC	AAG	TAT	GGT	TTA	TTT	AAG	CAA	CGG	ATT	ACA	AAA	GAT	GGT	CAG	GAG	727
Tyr	Lys	Tyr	Gly	Leu	Phe	Lys	Gln	Arg	Ile	Thr	Lys	Asp	Gly	Gln	Glu	
		165					170					175				
GAG	GTG	GCT	GAA	GAT	TGG	CTT	GAA	ATT	GGC	AGT	CCA	TGG	GAA	GTT	GTG	775
Glu	Val	Ala	Glu	Asp	Trp	Leu	Glu	Ile	Gly	Ser	Pro	Trp	Glu	Val	Val	
	180					185					190					
AGG	AAT	GAT	GTT	TCA	TAT	CCT	ATC	AAA	TTC	TAT	GGA	AAA	GTC	TCT	ACA	823
Arg	Asn	Asp	Val	Ser	Tyr	Pro	Ile	Lys	Phe	Tyr	Gly	Lys	Val	Ser	Thr	
195					200					205					210	
GGA	TCA	GAT	GGA	AAG	AGG	TAT	TGG	ATT	GGT	GGA	GAG	GAT	ATA	AAG	GCA	871
Gly	Ser	Asp	Gly	Lys	Arg	Tyr	Trp	Ile	Gly	Gly	Glu	Asp	Ile	Lys	Ala	
				215					220					225		
GTT	GCG	TAT	GAT	GTT	CCC	ATA	CCA	GGG	TAT	AAG	ACC	AGA	ACC	ACA	ATC	919
Val	Ala	Tyr	Asp	Val	Pro	Ile	Pro	Gly	Tyr	Lys	Thr	Arg	Thr	Thr	Ile	
			230					235					240			
AGC	CTT	CGA	CTG	TGG	TCT	ACA	CAG	GTT	CCA	TCA	GCG	GAT	TTT	GAT	TTA	967
Ser	Leu	Arg	Leu	Trp	Ser	Thr	Gln	Val	Pro	Ser	Ala	Asp	Phe	Asp	Leu	
		245					250					255				
TCT	GCT	TTC	AAT	GCT	GGA	GAG	CAC	ACC	AAA	GCA	TGT	GAA	GCC	CAA	GCA	1015
Ser	Ala	Phe	Asn	Ala	Gly	Glu	His	Thr	Lys	Ala	Cys	Glu	Ala	Gln	Ala	
	260					265					270					

AAC	GCT	GAG	AAG	ATA	TGT	TAC	ATA	CTC	TAC	CCT	GGG	GAT	GAA	TCA	GAG	1063
Asn	Ala	Glu	Lys	Ile	Cys	Tyr	Ile	Leu	Tyr	Pro	Gly	Asp	Glu	Ser	Glu	
275					280					285					290	
GAG	GGA	AAG	ATC	CTT	CGG	TTG	AAG	CAA	CAA	TAT	ACC	TTA	TGC	TCG	GCT	1111
Glu	Gly	Lys	Ile	Leu	Arg	Leu	Lys	Gln	Gln	Tyr	Thr	Leu	Cys	Ser	Ala	
				295				300						305		
TCT	CTC	CAA	GAT	ATT	ATT	TCT	CGA	TTT	GAG	AGG	AGA	TCA	GGT	GAT	CGT	1159
Ser	Leu	Gln	Asp	Ile	Ile	Ser	Arg	Phe	Glu	Arg	Arg	Ser	Gly	Asp	Arg	
			310					315					320			
ATT	AAG	TGG	GAA	GAG	TTT	CCT	GAA	AAA	GTT	GCT	GTG	CAG	ATG	AAT	GAC	1207
Ile	Lys	Trp	Glu	Glu	Phe	Pro	Glu	Lys	Val	Ala	Val	Gln	Met	Asn	Asp	
		325					330					335				
ACT	CAC	CCT	ACA	CTT	TGT	ATC	CCT	GAG	CTG	ATG	AGA	ATA	TTG	ATA	GAT	1255
Thr	His	Pro	Thr	Leu	Cys	Ile	Pro	Glu	Leu	Met	Arg	Ile	Leu	Ile	Asp	
	340					345					350					
CTG	AAG	GGC	TTG	AAT	TGG	AAT	GAA	GCT	TGG	AAT	ATT	ACT	CAA	AGA	ACT	1303
Leu	Lys	Gly	Leu	Asn	Trp	Asn	Glu	Ala	Trp	Asn	Ile	Thr	Gln	Arg	Thr	
355				360					365						370	
GTG	GCC	TAC	ACA	AAC	CAT	ACT	GTT	TTG	CCT	GAG	GCA	CTG	GAG	AAA	TGG	1351
Val	Ala	Tyr	Thr	Asn	His	Thr	Val	Leu	Pro	Glu	Ala	Leu	Glu	Lys	Trp	
				375				380						385		
AGT	TAT	GAA	TTG	ATG	CAG	AAA	CTC	CTT	CCC	AGA	CAT	GTC	GAA	ATC	ATT	1399
Ser	Tyr	Glu	Leu	Met	Gln	Lys	Leu	Leu	Pro	Arg	His	Val	Glu	Ile	Ile	
			390					395					400			
GAG	GCG	ATT	GAC	GAG	GAG	CTG	GTA	CAT	GAA	ATT	GTA	TTA	AAA	TAT	GGT	1447
Glu	Ala	Ile	Asp	Glu	Glu	Leu	Val	His	Glu	Ile	Val	Leu	Lys	Tyr	Gly	
		405					410					415				
TCA	ATG	GAT	CTG	AAC	AAA	TTG	GAG	GAA	AAG	TTG	ACT	ACA	ATG	AGA	ATC	1495
Ser	Met	Asp	Leu	Asn	Lys	Leu	Glu	Glu	Lys	Leu	Thr	Thr	Met	Arg	Ile	
	420					425					430					
TTA	GAA	AAT	TTT	GAT	CTT	CCC	AGT	TCT	GTT	GCT	GAA	TTA	TTT	ATT	AAG	1543
Leu	Glu	Asn	Phe	Asp	Leu	Pro	Ser	Ser	Val	Ala	Glu	Leu	Phe	Ile	Lys	
435					440				445						450	
CCT	GAA	ATC	TCA	GTT	GAT	GAT	GAT	ACT	GAA	ACA	GTA	GAA	GTC	CAT	GAC	1591
Pro	Glu	Ile	Ser	Val	Asp	Asp	Asp	Thr	Glu	Thr	Val	Glu	Val	His	Asp	
				455				460						465		
AAA	GTT	GAA	GCT	TCC	GAT	AAA	GTT	GTG	ACT	AAT	GAT	GAA	GAT	GAC	ACT	1639
Lys	Val	Glu	Ala	Ser	Asp	Lys	Val	Val	Thr	Asn	Asp	Glu	Asp	Asp	Thr	
			470					475					480			
GGT	AAG	AAA	ACT	AGT	GTG	AAG	ATA	GAA	GCA	GCT	GCA	GAA	AAA	GAC	ATT	1687
Gly	Lys	Lys	Thr	Ser	Val	Lys	Ile	Glu	Ala	Ala	Ala	Glu	Lys	Asp	Ile	
		485					490					495				

GAC	AAG	AAA	ACT	CCC	GTG	AGT	CCG	GAA	CCA	GCT	GTT	ATA	CCA	CCT	AAG	1735
Asp	Lys	Lys	Thr	Pro	Val	Ser	Pro	Glu	Pro	Ala	Val	Ile	Pro	Pro	Lys	
	500					505					510					
AAG	GTA	CGC	ATG	GCC	AAC	TTG	TGT	GTT	GTG	GGC	GGC	CAT	GCT	GTT	AAT	1783
Lys	Val	Arg	Met	Ala	Asn	Leu	Cys	Val	Val	Gly	Gly	His	Ala	Val	Asn	
515					520					525					530	
GGA	GTT	GCT	GAG	ATC	CAT	AGT	GAA	ATT	GTG	AAG	GAG	GAG	GTT	TTC	AAT	1831
Gly	Val	Ala	Glu	Ile	His	Ser	Glu	Ile	Val	Lys	Glu	Glu	Val	Phe	Asn	
				535					540					545		
GAC	TTC	TAT	GAG	CTC	TGG	CCG	GAA	AAG	TTC	CAA	AAC	AAA	ACA	AAT	GGA	1879
Asp	Phe	Tyr	Glu	Leu	Trp	Pro	Glu	Lys	Phe	Gln	Asn	Lys	Thr	Asn	Gly	
			550					555					560			
GTG	ACT	CCA	AGA	AGA	TGG	ATT	CGT	TTC	TGC	AAT	CCT	CCT	CTT	AGT	GCC	1927
Val	Thr	Pro	Arg	Arg	Trp	Ile	Arg	Phe	Cys	Asn	Pro	Pro	Leu	Ser	Ala	
		565					570					575				
ATC	ATA	ACT	AAG	TGG	ACT	GGT	ACA	GAG	GAT	TGG	GTC	CTG	AAA	ACT	GAA	1975
Ile	Ile	Thr	Lys	Trp	Thr	Gly	Thr	Glu	Asp	Trp	Val	Leu	Lys	Thr	Glu	
	580					585					590					
AAG	TTG	GCA	GAA	TTG	CAG	AAG	TTT	GCT	GAT	AAT	GAA	GAT	CTT	CAA	AAT	2023
Lys	Leu	Ala	Glu	Leu	Gln	Lys	Phe	Ala	Asp	Asn	Glu	Asp	Leu	Gln	Asn	
595					600					605					610	
GAG	TGG	AGG	GAA	GCA	AAA	AGG	AGC	AAC	AAG	ATT	AAA	GTT	GTC	TCC	TTT	2071
Glu	Trp	Arg	Glu	Ala	Lys	Arg	Ser	Asn	Lys	Ile	Lys	Val	Val	Ser	Phe	
				615					620					625		
CTC	AAA	GAA	AAG	ACA	GGG	TAT	TCT	GTT	GTC	CCA	GAT	GCA	ATG	TTT	GAT	2119
Leu	Lys	Glu	Lys	Thr	Gly	Tyr	Ser	Val	Val	Pro	Asp	Ala	Met	Phe	Asp	
			630					635					640			
ATT	CAG	GTA	AAA	CGC	ATT	CAT	GAG	TAC	AAG	CGA	CAA	CTG	TTA	AAT	ATC	2167
Ile	Gln	Val	Lys	Arg	Ile	His	Glu	Tyr	Lys	Arg	Gln	Leu	Leu	Asn	Ile	
		645					650					655				
TTC	GGC	ATC	GTT	TAT	CGG	TAT	AAG	AAG	ATG	AAA	GAA	ATG	ACA	GCT	GCA	2215
Phe	Gly	Ile	Val	Tyr	Arg	Tyr	Lys	Lys	Met	Lys	Glu	Met	Thr	Ala	Ala	
	660					665					670					
GAA	AGA	AAG	ACT	AAC	TTC	GTT	CCT	CGA	GTA	TGC	ATA	TTT	GGG	GGA	AAA	2263
Glu	Arg	Lys	Thr	Asn	Phe	Val	Pro	Arg	Val	Cys	Ile	Phe	Gly	Gly	Lys	
675					680					685					690	
GCT	TTT	GCC	ACA	TAT	GTG	CAA	GCC	AAG	AGG	ATT	GTA	AAA	TTT	ATC	ACA	2311
Ala	Phe	Ala	Thr	Tyr	Val	Gln	Ala	Lys	Arg	Ile	Val	Lys	Phe	Ile	Thr	
				695					700					705		
GAT	GTT	GGT	GCT	ACT	ATA	AAT	CAT	GAT	CCA	GAA	ATC	GGT	GAT	CTG	TTG	2359
Asp	Val	Gly	Ala	Thr	Ile	Asn	His	Asp	Pro	Glu	Ile	Gly	Asp	Leu	Leu	
			710					715					720			

AAG GTA GTC TTT GTG CCA GAT TAC AAT GTC AGT GTT GCT GAA TTG CTA Lys Val Val Phe Val Pro Asp Tyr Asn Val Ser Val Ala Glu Leu Leu 725 730 735	2407
ATT CCT GCT AGC GAT CTA TCA GAA CAT ATC AGT ACG GCT GGA ATG GAG Ile Pro Ala Ser Asp Leu Ser Glu His Ile Ser Thr Ala Gly Met Glu 740 745 750	2455
GCC AGT GGA ACC AGT AAT ATG AAG TTT GCA ATG AAT GGT TGT ATC CAA Ala Ser Gly Thr Ser Asn Met Lys Phe Ala Met Asn Gly Cys Ile Gln 755 760 765 770	2503
ATT GGT ACA TTG GAT GGC GCT AAT GTT GAA ATA AGG GAA GAG GTT GGA Ile Gly Thr Leu Asp Gly Ala Asn Val Glu Ile Arg Glu Glu Val Gly 775 780 785	2551
GAA GAA AAC TTC TTT CTC TTT GGT GCT CAA GCT CAT GAA ATT GCA GGG Glu Glu Asn Phe Phe Leu Phe Gly Ala Gln Ala His Glu Ile Ala Gly 790 795 800	2599
CTT AGA AAA GAA AGA GCT GAC GGA AAG TTT GTA CCT GAT GAA CGT TTT Leu Arg Lys Glu Arg Ala Asp Gly Lys Phe Val Pro Asp Glu Arg Phe 805 810 815	2647
GAA GAG GTG AAG GAA TTT GTT AGA AGC GGT GCT TTT GGC TCT TAT AAC Glu Glu Val Lys Glu Phe Val Arg Ser Gly Ala Phe Gly Ser Tyr Asn 820 825 830	2695
TAT GAT GAC CTA ATT GGA TCG TTG GAA GGA AAT GAA GGT TTT GGC CGT Tyr Asp Asp Leu Ile Gly Ser Leu Glu Gly Asn Glu Gly Phe Gly Arg 835 840 845 850	2743
GCT GAC TAT TTC CTT GTG GGC AAG GAC TTC CCC AGT TAC ATA GAA TGC Ala Asp Tyr Phe Leu Val Gly Lys Asp Phe Pro Ser Tyr Ile Glu Cys 855 860 865	2791
CAA GAG AAA GTT GAT GAG GCA TAT CGC GAC CAG AAA AGG TGG ACA ACG Gln Glu Lys Val Asp Glu Ala Tyr Arg Asp Gln Lys Arg Trp Thr Thr 870 875 880	2839
ATG TCA ATC TTG AAT ACA GCG GGA TCG TAC AAG TTC AGC AGT GAC AGA Met Ser Ile Leu Asn Thr Ala Gly Ser Tyr Lys Phe Ser Ser Asp Arg 885 890 895	2887
ACA ATC CAT GAA TAT GCC AAA GAC ATT TGG AAC ATT GAA GCT GTG GAA Thr Ile His Glu Tyr Ala Lys Asp Ile Trp Asn Ile Glu Ala Val Glu 900 905 910	2935
ATA GCA TAA GAGGGGGAAG TGAATGAAAA ATAACAAAGG CACAGTAAGT Ile Ala 915	2984
AGTTTCTCTT TTTATCATGT GATGAAGGTA TATAATGTAT GTGTAAGAGG ATGATGTTAT	3044
TACCACATAA TAAGAGATGA AGAGTCTCAT TTTGCTTCAA AAAAAAAAAA AAAAAA	3101

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 966 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Thr Ala Asn Gly Ala His Leu Phe Asn His Tyr Ser Ser Asn
-50                      -45                      -40                      -35

Ser Arg Phe Ile His Phe Thr Ser Arg Asn Thr Ser Ser Lys Leu Phe
                      -30                      -25                      -20

Leu Thr Lys Thr Ser His Phe Arg Arg Pro Lys Arg Cys Phe His Val
                      -15                      -10                      -5

Asn Asn Thr Leu Ser Glu Lys Ile His His Pro Ile Thr Glu Gln Gly
      1                      5                      10

Gly Glu Ser Asp Leu Ser Ser Phe Ala Pro Asp Ala Ala Ser Ile Thr
15                      20                      25                      30

Ser Ser Ile Lys Tyr His Ala Glu Phe Thr Pro Val Phe Ser Pro Glu
                      35                      40                      45

Arg Phe Glu Leu Pro Lys Ala Phe Phe Ala Thr Ala Gln Ser Val Arg
                      50                      55                      60

Asp Ser Leu Leu Ile Asn Trp Asn Ala Thr Tyr Asp Ile Tyr Glu Lys
                      65                      70                      75

Leu Asn Met Lys Gln Ala Tyr Tyr Leu Ser Met Glu Phe Leu Gln Gly
      80                      85                      90

Arg Ala Leu Leu Asn Ala Ile Gly Asn Leu Glu Leu Thr Gly Ala Phe
95                      100                      105                      110

Ala Glu Ala Leu Lys Asn Leu Gly His Asn Leu Glu Asn Val Ala Ser
                      115                      120                      125

Gln Glu Pro Asp Ala Ala Leu Gly Asn Gly Gly Leu Gly Arg Leu Ala
                      130                      135                      140

Ser Cys Phe Leu Asp Ser Leu Ala Thr Leu Asn Tyr Pro Ala Trp Gly
                      145                      150                      155

Tyr Gly Leu Arg Tyr Lys Tyr Gly Leu Phe Lys Gln Arg Ile Thr Lys
160                      165                      170

Asp Gly Gln Glu Glu Val Ala Glu Asp Trp Leu Glu Ile Gly Ser Pro
175                      180                      185                      190

```

Trp Glu Val Val Arg Asn Asp Val Ser Tyr Pro Ile Lys Phe Tyr Gly
 195 200 205
 Lys Val Ser Thr Gly Ser Asp Gly Lys Arg Tyr Trp Ile Gly Gly Glu
 210 215 220
 Asp Ile Lys Ala Val Ala Tyr Asp Val Pro Ile Pro Gly Tyr Lys Thr
 225 230 235
 Arg Thr Thr Ile Ser Leu Arg Leu Trp Ser Thr Gln Val Pro Ser Ala
 240 245 250
 Asp Phe Asp Leu Ser Ala Phe Asn Ala Gly Glu His Thr Lys Ala Cys
 255 260 265 270
 Glu Ala Gln Ala Asn Ala Glu Lys Ile Cys Tyr Ile Leu Tyr Pro Gly
 275 280 285
 Asp Glu Ser Glu Glu Gly Lys Ile Leu Arg Leu Lys Gln Gln Tyr Thr
 290 295 300
 Leu Cys Ser Ala Ser Leu Gln Asp Ile Ile Ser Arg Phe Glu Arg Arg
 305 310 315
 Ser Gly Asp Arg Ile Lys Trp Glu Glu Phe Pro Glu Lys Val Ala Val
 320 325 330
 Gln Met Asn Asp Thr His Pro Thr Leu Cys Ile Pro Glu Leu Met Arg
 335 340 345 350
 Ile Leu Ile Asp Leu Lys Gly Leu Asn Trp Asn Glu Ala Trp Asn Ile
 355 360 365
 Thr Gln Arg Thr Val Ala Tyr Thr Asn His Thr Val Leu Pro Glu Ala
 370 375 380
 Leu Glu Lys Trp Ser Tyr Glu Leu Met Gln Lys Leu Leu Pro Arg His
 385 390 395
 Val Glu Ile Ile Glu Ala Ile Asp Glu Glu Leu Val His Glu Ile Val
 400 405 410
 Leu Lys Tyr Gly Ser Met Asp Leu Asn Lys Leu Glu Glu Lys Leu Thr
 415 420 425 430
 Thr Met Arg Ile Leu Glu Asn Phe Asp Leu Pro Ser Ser Val Ala Glu
 435 440 445
 Leu Phe Ile Lys Pro Glu Ile Ser Val Asp Asp Asp Thr Glu Thr Val
 450 455 460
 Glu Val His Asp Lys Val Glu Ala Ser Asp Lys Val Val Thr Asn Asp
 465 470 475
 Glu Asp Asp Thr Gly Lys Lys Thr Ser Val Lys Ile Glu Ala Ala Ala
 480 485 490

Glu	Lys	Asp	Ile	Asp	Lys	Lys	Thr	Pro	Val	Ser	Pro	Glu	Pro	Ala	Val	495	500	505	510
Ile	Pro	Pro	Lys	Lys	Val	Arg	Met	Ala	Asn	Leu	Cys	Val	Val	Gly	Gly	515	520	525	
His	Ala	Val	Asn	Gly	Val	Ala	Glu	Ile	His	Ser	Glu	Ile	Val	Lys	Glu	530	535	540	
Glu	Val	Phe	Asn	Asp	Phe	Tyr	Glu	Leu	Trp	Pro	Glu	Lys	Phe	Gln	Asn	545	550	555	
Lys	Thr	Asn	Gly	Val	Thr	Pro	Arg	Arg	Trp	Ile	Arg	Phe	Cys	Asn	Pro	560	565	570	
Pro	Leu	Ser	Ala	Ile	Ile	Thr	Lys	Trp	Thr	Gly	Thr	Glu	Asp	Trp	Val	575	580	585	590
Leu	Lys	Thr	Glu	Lys	Leu	Ala	Glu	Leu	Gln	Lys	Phe	Ala	Asp	Asn	Glu	595	600	605	
Asp	Leu	Gln	Asn	Glu	Trp	Arg	Glu	Ala	Lys	Arg	Ser	Asn	Lys	Ile	Lys	610	615	620	
Val	Val	Ser	Phe	Leu	Lys	Glu	Lys	Thr	Gly	Tyr	Ser	Val	Val	Pro	Asp	625	630	635	
Ala	Met	Phe	Asp	Ile	Gln	Val	Lys	Arg	Ile	His	Glu	Tyr	Lys	Arg	Gln	640	645	650	
Leu	Leu	Asn	Ile	Phe	Gly	Ile	Val	Tyr	Arg	Tyr	Lys	Lys	Met	Lys	Glu	655	660	665	670
Met	Thr	Ala	Ala	Glu	Arg	Lys	Thr	Asn	Phe	Val	Pro	Arg	Val	Cys	Ile	675	680	685	
Phe	Gly	Gly	Lys	Ala	Phe	Ala	Thr	Tyr	Val	Gln	Ala	Lys	Arg	Ile	Val	690	695	700	
Lys	Phe	Ile	Thr	Asp	Val	Gly	Ala	Thr	Ile	Asn	His	Asp	Pro	Glu	Ile	705	710	715	
Gly	Asp	Leu	Leu	Lys	Val	Val	Phe	Val	Pro	Asp	Tyr	Asn	Val	Ser	Val	720	725	730	
Ala	Glu	Leu	Leu	Ile	Pro	Ala	Ser	Asp	Leu	Ser	Glu	His	Ile	Ser	Thr	735	740	745	750
Ala	Gly	Met	Glu	Ala	Ser	Gly	Thr	Ser	Asn	Met	Lys	Phe	Ala	Met	Asn	755	760	765	
Gly	Cys	Ile	Gln	Ile	Gly	Thr	Leu	Asp	Gly	Ala	Asn	Val	Glu	Ile	Arg	770	775	780	
Glu	Glu	Val	Gly	Glu	Glu	Asn	Phe	Phe	Leu	Phe	Gly	Ala	Gln	Ala	His	785	790	795	

Glu Ile Ala Gly Leu Arg Lys Glu Arg Ala Asp Gly Lys Phe Val Pro
 800 805 810
 Asp Glu Arg Phe Glu Glu Val Lys Glu Phe Val Arg Ser Gly Ala Phe
 815 820 825 830
 Gly Ser Tyr Asn Tyr Asp Asp Leu Ile Gly Ser Leu Glu Gly Asn Glu
 835 840 845
 Gly Phe Gly Arg Ala Asp Tyr Phe Leu Val Gly Lys Asp Phe Pro Ser
 850 855 860
 Tyr Ile Glu Cys Gln Glu Lys Val Asp Glu Ala Tyr Arg Asp Gln Lys
 865 870 875
 Arg Trp Thr Thr Met Ser Ile Leu Asn Thr Ala Gly Ser Tyr Lys Phe
 880 885 890
 Ser Ser Asp Arg Thr Ile His Glu Tyr Ala Lys Asp Ile Trp Asn Ile
 895 900 905 910
 Glu Ala Val Glu Ile Ala
 915

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2655 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 12..2528
- (D) OTHER INFORMATION: /product= "potato alpha-glucan
H-type tuber phosphorylase"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 12..2525

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTTATTTTC C ATG GAA GGT GGT GCA AAA TCG AAT GAT GTA TCA GCA GCA
 Met Glu Gly Gly Ala Lys Ser Asn Asp Val Ser Ala Ala
 1 5 10

50

CCT	ATT	GCT	CAA	CCA	CTT	TCT	GAA	GAC	CCT	ACT	GAC	ATT	GCA	TCT	AAT	98
Pro	Ile	Ala	Gln	Pro	Leu	Ser	Glu	Asp	Pro	Thr	Asp	Ile	Ala	Ser	Asn	
	15					20					25					
ATC	AAG	TAT	CAT	GCT	CAA	TAT	ACT	CCT	CAT	TTT	TCT	CCT	TTC	AAG	TTT	146
Ile	Lys	Tyr	His	Ala	Gln	Tyr	Thr	Pro	His	Phe	Ser	Pro	Phe	Lys	Phe	
	30				35					40					45	
GAG	CCA	CTA	CAA	GCA	TAC	TAT	GCT	GCT	ACT	GCT	GAC	AGT	GTT	CGT	GAT	194
Glu	Pro	Leu	Gln	Ala	Tyr	Tyr	Ala	Ala	Thr	Ala	Asp	Ser	Val	Arg	Asp	
				50					55					60		
CGC	TTG	ATC	AAA	CAA	TGG	AAT	GAC	ACC	TAT	CTT	CAT	TAT	GAC	AAA	GTT	242
Arg	Leu	Ile	Lys	Gln	Trp	Asn	Asp	Thr	Tyr	Leu	His	Tyr	Asp	Lys	Val	
			65				70						75			
AAT	CCA	AAG	CAA	ACA	TAC	TAC	TTA	TCA	ATG	GAG	TAT	CTC	CAG	GGG	CGA	290
Asn	Pro	Lys	Gln	Thr	Tyr	Tyr	Leu	Ser	Met	Glu	Tyr	Leu	Gln	Gly	Arg	
		80					85					90				
GCT	TTG	ACA	AAT	GCA	GTT	GGA	AAC	TTA	GAC	ATC	CAC	AAT	GCA	TAT	GCT	338
Ala	Leu	Thr	Asn	Ala	Val	Gly	Asn	Leu	Asp	Ile	His	Asn	Ala	Tyr	Ala	
	95					100					105					
GAT	GCT	TTA	AAC	AAA	CTG	GGT	CAG	CAG	CTT	GAG	GAG	GTC	GTT	GAG	CAG	386
Asp	Ala	Leu	Asn	Lys	Leu	Gly	Gln	Gln	Leu	Glu	Glu	Val	Val	Glu	Gln	
110					115					120					125	
GAA	AAA	GAT	GCA	GCA	TTA	GGA	AAT	GGT	GGT	TTA	GGA	AGG	CTC	GCT	TCA	434
Glu	Lys	Asp	Ala	Ala	Leu	Gly	Asn	Gly	Gly	Leu	Gly	Arg	Leu	Ala	Ser	
				130				135						140		
TGC	TTT	CTT	GAT	TCC	ATG	GCC	ACA	TTG	AAC	CTT	CCA	GCA	TGG	GGT	TAT	482
Cys	Phe	Leu	Asp	Ser	Met	Ala	Thr	Leu	Asn	Leu	Pro	Ala	Trp	Gly	Tyr	
			145					150					155			
GGC	TTG	AGG	TAC	AGA	TAT	GGA	CTT	TTT	AAG	CAG	CTT	ATC	ACA	AAG	GCT	530
Gly	Leu	Arg	Tyr	Arg	Tyr	Gly	Leu	Phe	Lys	Gln	Leu	Ile	Thr	Lys	Ala	
		160					165					170				
GGG	CAA	GAA	GAA	GTT	CCT	GAA	GAT	TGG	TTG	GAG	AAA	TTT	AGT	CCC	TGG	578
Gly	Gln	Glu	Glu	Val	Pro	Glu	Asp	Trp	Leu	Glu	Lys	Phe	Ser	Pro	Trp	
	175					180					185					
GAA	ATT	GTA	AGG	CAT	GAT	GTT	GTC	TTT	CCT	ATC	AGG	TTT	TTT	GGT	CAT	626
Glu	Ile	Val	Arg	His	Asp	Val	Val	Phe	Pro	Ile	Arg	Phe	Phe	Gly	His	
190					195					200					205	
GTT	GAA	GTC	CTC	CCT	TCT	GGC	TCG	CGA	AAA	TGG	GTT	GGT	GGA	GAG	GTC	674
Val	Glu	Val	Leu	Pro	Ser	Gly	Ser	Arg	Lys	Trp	Val	Gly	Gly	Glu	Val	
				210					215					220		
CTA	CAG	GCT	CTT	GCA	TAT	GAT	GTG	CCA	ATT	CCA	GGA	TAC	AGA	ACT	AAA	722
Leu	Gln	Ala	Leu	Ala	Tyr	Asp	Val	Pro	Ile	Pro	Gly	Tyr	Arg	Thr	Lys	
			225					230					235			

AAC	ACT	AAT	AGT	CTT	CGT	CTC	TGG	GAA	GCC	AAA	GCA	AGC	TCT	GAG	GAT	770
Asn	Thr	Asn	Ser	Leu	Arg	Leu	Trp	Glu	Ala	Lys	Ala	Ser	Ser	Glu	Asp	
		240					245					250				
TTC	AAC	TTG	TTT	CTG	TTT	AAT	GAT	GGA	CAG	TAT	GAT	GCT	GCT	GCA	CAG	818
Phe	Asn	Leu	Phe	Leu	Phe	Asn	Asp	Gly	Gln	Tyr	Asp	Ala	Ala	Ala	Gln	
	255					260					265					
CTT	CAT	TCT	AGG	GCT	CAG	CAG	ATT	TGT	GCT	GTT	CTC	TAC	CCT	GGG	GAT	866
Leu	His	Ser	Arg	Ala	Gln	Gln	Ile	Cys	Ala	Val	Leu	Tyr	Pro	Gly	Asp	
270					275					280					285	
GCT	ACA	GAG	AAT	GGA	AAA	CTC	TTA	CGG	CTA	AAG	CAA	CAA	TTT	TTT	CTG	914
Ala	Thr	Glu	Asn	Gly	Lys	Leu	Leu	Arg	Leu	Lys	Gln	Gln	Phe	Phe	Leu	
				290				295						300		
TGC	AGT	GCA	TCG	CTT	CAG	GAT	ATT	ATT	GCC	AGA	TTC	AAA	GAG	AGA	GAA	962
Cys	Ser	Ala	Ser	Leu	Gln	Asp	Ile	Ile	Ala	Arg	Phe	Lys	Glu	Arg	Glu	
			305					310					315			
GAT	GGA	AAG	GGT	TCT	CAC	CAG	TGG	TCT	GAA	TTC	CCC	AAG	AAG	GTT	GCG	1010
Asp	Gly	Lys	Gly	Ser	His	Gln	Trp	Ser	Glu	Phe	Pro	Lys	Lys	Val	Ala	
		320					325					330				
ATA	CAA	CTA	AAT	GAC	ACA	CAT	CCA	ACT	CTT	ACG	ATT	CCA	GAG	CTG	ATG	1058
Ile	Gln	Leu	Asn	Asp	Thr	His	Pro	Thr	Leu	Thr	Ile	Pro	Glu	Leu	Met	
	335					340					345					
CGG	TTG	CTA	ATG	GAT	GAT	GAA	GGA	CTT	GGG	TGG	GAT	GAA	TCT	TGG	AAT	1106
Arg	Leu	Leu	Met	Asp	Asp	Glu	Gly	Leu	Gly	Trp	Asp	Glu	Ser	Trp	Asn	
350					355					360					365	
ATC	ACT	ACT	AGG	ACA	ATT	GCC	TAT	ACG	AAT	CAT	ACA	GTC	CTA	CCT	GAA	1154
Ile	Thr	Thr	Arg	Thr	Ile	Ala	Tyr	Thr	Asn	His	Thr	Val	Leu	Pro	Glu	
				370				375						380		
GCA	CTT	GAA	AAA	TGG	TCT	CAG	GCA	GTC	ATG	TGG	AAG	CTC	CTT	CCT	AGA	1202
Ala	Leu	Glu	Lys	Trp	Ser	Gln	Ala	Val	Met	Trp	Lys	Leu	Leu	Pro	Arg	
			385					390					395			
CAT	ATG	GAA	ATC	ATT	GAA	GAA	ATT	GAC	AAA	CGG	TTT	GTT	GCT	ACA	ATA	1250
His	Met	Glu	Ile	Ile	Glu	Glu	Ile	Asp	Lys	Arg	Phe	Val	Ala	Thr	Ile	
		400					405					410				
ATG	TCA	GAA	AGA	CCT	GAT	CTT	GAG	AAT	AAG	ATG	CCT	AGC	ATG	CGC	ATT	1298
Met	Ser	Glu	Arg	Pro	Asp	Leu	Glu	Asn	Lys	Met	Pro	Ser	Met	Arg	Ile	
	415					420					425					
TTG	GAT	CAC	AAC	GCC	ACA	AAA	CCT	GTT	GTG	CAT	ATG	GCT	AAC	TTG	TGT	1346
Leu	Asp	His	Asn	Ala	Thr	Lys	Pro	Val	Val	His	Met	Ala	Asn	Leu	Cys	
430					435					440					445	
GTT	GTC	TCT	TCA	CAT	ACG	GTA	AAT	GGT	GTT	GCC	CAG	CTG	CAT	AGT	GAC	1394
Val	Val	Ser	Ser	His	Thr	Val	Asn	Gly	Val	Ala	Gln	Leu	His	Ser	Asp	
				450				455						460		

ATC	CTG	AAG	GCT	GAG	TTA	TTT	GCT	GAT	TAT	GTC	TCT	GTA	TGG	CCC	ACC	1442
Ile	Leu	Lys	Ala	Glu	Leu	Phe	Ala	Asp	Tyr	Val	Ser	Val	Trp	Pro	Thr	
			465					470					475			
AAG	TTC	CAG	AAT	AAG	ACC	AAT	GGT	ATA	ACT	CCT	CGT	AGG	TGG	ATC	CGA	1490
Lys	Phe	Gln	Asn	Lys	Thr	Asn	Gly	Ile	Thr	Pro	Arg	Arg	Trp	Ile	Arg	
		480					485					490				
TTT	TGT	AGT	CCT	GAG	CTG	AGT	CAT	ATA	ATT	ACC	AAG	TGG	TTA	AAA	ACA	1538
Phe	Cys	Ser	Pro	Glu	Leu	Ser	His	Ile	Ile	Thr	Lys	Trp	Leu	Lys	Thr	
	495					500					505					
GAT	CAA	TGG	GTG	ACG	AAC	CTC	GAA	CTG	CTT	GCT	AAT	CTT	CGG	GAG	TTT	1586
Asp	Gln	Trp	Val	Thr	Asn	Leu	Glu	Leu	Leu	Ala	Asn	Leu	Arg	Glu	Phe	
510					515					520					525	
GCT	GAT	AAT	TCG	GAG	CTC	CAT	GCT	GAA	TGG	GAA	TCA	GCC	AAG	ATG	GCC	1634
Ala	Asp	Asn	Ser	Glu	Leu	His	Ala	Glu	Trp	Glu	Ser	Ala	Lys	Met	Ala	
				530					535					540		
AAC	AAG	CAG	CGT	TTG	GCA	CAG	TAT	ATA	CTG	CAT	GTG	ACA	GGT	GTG	AGC	1682
Asn	Lys	Gln	Arg	Leu	Ala	Gln	Tyr	Ile	Leu	His	Val	Thr	Gly	Val	Ser	
		545						550					555			
ATC	GAT	CCA	AAT	TCC	CTT	TTT	GAC	ATA	CAA	GTC	AAA	CGT	ATC	CAT	GAA	1730
Ile	Asp	Pro	Asn	Ser	Leu	Phe	Asp	Ile	Gln	Val	Lys	Arg	Ile	His	Glu	
		560					565					570				
TAC	AAA	AGG	CAG	CTT	CTA	AAT	ATT	CTG	GGC	GTC	ATC	TAT	AGA	TAC	AAG	1778
Tyr	Lys	Arg	Gln	Leu	Leu	Asn	Ile	Leu	Gly	Val	Ile	Tyr	Arg	Tyr	Lys	
	575					580					585					
AAG	CTT	AAG	GGA	ATG	AGC	CCT	GAA	GAA	AGG	AAA	AAT	ACA	ACT	CCT	CGC	1826
Lys	Leu	Lys	Gly	Met	Ser	Pro	Glu	Glu	Arg	Lys	Asn	Thr	Thr	Pro	Arg	
590					595					600					605	
ACA	GTC	ATG	ATT	GGA	GGA	AAA	GCA	TTT	GCA	ACA	TAC	ACA	AAT	GCA	AAA	1874
Thr	Val	Met	Ile	Gly	Gly	Lys	Ala	Phe	Ala	Thr	Tyr	Thr	Asn	Ala	Lys	
				610					615					620		
CGA	ATT	GTC	AAG	CTC	GTG	ACT	GAT	GTT	GGC	GAC	GTT	GTC	AAT	AGT	GAC	1922
Arg	Ile	Val	Lys	Leu	Val	Thr	Asp	Val	Gly	Asp	Val	Val	Asn	Ser	Asp	
			625					630					635			
CCT	GAC	GTC	AAT	GAC	TAT	TTG	AAG	GTG	GTT	TTT	GTT	CCC	AAC	TAC	AAT	1970
Pro	Asp	Val	Asn	Asp	Tyr	Leu	Lys	Val	Val	Phe	Val	Pro	Asn	Tyr	Asn	
		640					645					650				
GTA	TCT	GTG	GCA	GAG	ATG	CTT	ATT	CCG	GGA	AGT	GAG	CTA	TCA	CAA	CAC	2018
Val	Ser	Val	Ala	Glu	Met	Leu	Ile	Pro	Gly	Ser	Glu	Leu	Ser	Gln	His	
	655					660					665					
ATC	AGT	ACT	GCA	GGC	ATG	GAA	GCA	AGT	GGA	ACA	AGC	AAC	ATG	AAA	TTT	2066
Ile	Ser	Thr	Ala	Gly	Met	Glu	Ala	Ser	Gly	Thr	Ser	Asn	Met	Lys	Phe	
670					675					680					685	

GCC CTT AAT GGA TGC CTT ATC ATT GGG ACA CTA GAT GGG GCC AAT GTG	2114
Ala Leu Asn Gly Cys Leu Ile Ile Gly Thr Leu Asp Gly Ala Asn Val	
690 695 700	
GAA ATT AGG GAG GAA ATT GGA GAA GAT AAC TTC TTT CTT TTT GGT GCA	2162
Glu Ile Arg Glu Glu Ile Gly Glu Asp Asn Phe Phe Leu Phe Gly Ala	
705 710 715	
ACA GCT GAT GAA GTT CCT CAA CTG CGC AAA GAT CGA GAG AAT GGA CTG	2210
Thr Ala Asp Glu Val Pro Gln Leu Arg Lys Asp Arg Glu Asn Gly Leu	
720 725 730	
TTC AAA CCT GAT CCT CGG TTT GAA GAG GCA AAA CAA TTT ATT AGG TCT	2258
Phe Lys Pro Asp Pro Arg Phe Glu Glu Ala Lys Gln Phe Ile Arg Ser	
735 740 745	
GGA GCA TTT GGG ACG TAT GAT TAT AAT CCC CTC CTT GAA TCA CTG GAA	2306
Gly Ala Phe Gly Thr Tyr Asp Tyr Asn Pro Leu Leu Glu Ser Leu Glu	
750 755 760 765	
GGG AAC TCG GGA TAT GGT CGT GGA GAC TAT TTT CTT GTT GGT CAT GAT	2354
Gly Asn Ser Gly Tyr Gly Arg Gly Asp Tyr Phe Leu Val Gly His Asp	
770 775 780	
TTT CCG AGC TAC ATG GAT GCT CAG GCA AGG GTT GAT GAA GCT TAC AAG	2402
Phe Pro Ser Tyr Met Asp Ala Gln Ala Arg Val Asp Glu Ala Tyr Lys	
785 790 795	
GAC AGG AAA AGA TGG ATA AAG ATG TCT ATA CTG AGC ACT AGT GGG AGT	2450
Asp Arg Lys Arg Trp Ile Lys Met Ser Ile Leu Ser Thr Ser Gly Ser	
800 805 810	
GGC AAA TTT AGT AGT GAC CGT ACA ATT TCT CAA TAT GCA AAA GAG ATC	2498
Gly Lys Phe Ser Ser Asp Arg Thr Ile Ser Gln Tyr Ala Lys Glu Ile	
815 820 825	
TGG AAC ATT GCC GAG TGT CGC GTG CCT TGA GCACACTTCT GAACCTGGTA	2548
Trp Asn Ile Ala Glu Cys Arg Val Pro	
830 835	
TCTAATAAGG ATCTAATGTT CATTGTTTAC TAGCATATGA ATAATGTAAG TTCAAGCACA	2608
ACATGCTTTC TTATTTCTTA CTGCTCTCAA GAAGCAGTTA TTTGTTG	2655

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 838 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Glu	Gly	Gly	Ala	Lys	Ser	Asn	Asp	Val	Ser	Ala	Ala	Pro	Ile	Ala	
1				5					10					15		
Gln	Pro	Leu	Ser	Glu	Asp	Pro	Thr	Asp	Ile	Ala	Ser	Asn	Ile	Lys	Tyr	
			20					25					30			
His	Ala	Gln	Tyr	Thr	Pro	His	Phe	Ser	Pro	Phe	Lys	Phe	Glu	Pro	Leu	
		35					40					45				
Gln	Ala	Tyr	Tyr	Ala	Ala	Thr	Ala	Asp	Ser	Val	Arg	Asp	Arg	Leu	Ile	
	50					55					60					
Lys	Gln	Trp	Asn	Asp	Thr	Tyr	Leu	His	Tyr	Asp	Lys	Val	Asn	Pro	Lys	
65					70					75					80	
Gln	Thr	Tyr	Tyr	Leu	Ser	Met	Glu	Tyr	Leu	Gln	Gly	Arg	Ala	Leu	Thr	
				85					90					95		
Asn	Ala	Val	Gly	Asn	Leu	Asp	Ile	His	Asn	Ala	Tyr	Ala	Asp	Ala	Leu	
			100				105						110			
Asn	Lys	Leu	Gly	Gln	Gln	Leu	Glu	Glu	Val	Val	Glu	Gln	Glu	Lys	Asp	
		115					120					125				
Ala	Ala	Leu	Gly	Asn	Gly	Gly	Leu	Gly	Arg	Leu	Ala	Ser	Cys	Phe	Leu	
	130					135					140					
Asp	Ser	Met	Ala	Thr	Leu	Asn	Leu	Pro	Ala	Trp	Gly	Tyr	Gly	Leu	Arg	
145					150				155						160	
Tyr	Arg	Tyr	Gly	Leu	Phe	Lys	Gln	Leu	Ile	Thr	Lys	Ala	Gly	Gln	Glu	
				165					170					175		
Glu	Val	Pro	Glu	Asp	Trp	Leu	Glu	Lys	Phe	Ser	Pro	Trp	Glu	Ile	Val	
			180					185					190			
Arg	His	Asp	Val	Val	Phe	Pro	Ile	Arg	Phe	Phe	Gly	His	Val	Glu	Val	
		195					200					205				
Leu	Pro	Ser	Gly	Ser	Arg	Lys	Trp	Val	Gly	Gly	Glu	Val	Leu	Gln	Ala	
	210					215					220					
Leu	Ala	Tyr	Asp	Val	Pro	Ile	Pro	Gly	Tyr	Arg	Thr	Lys	Asn	Thr	Asn	
225					230					235					240	
Ser	Leu	Arg	Leu	Trp	Glu	Ala	Lys	Ala	Ser	Ser	Glu	Asp	Phe	Asn	Leu	
				245					250					255		
Phe	Leu	Phe	Asn	Asp	Gly	Gln	Tyr	Asp	Ala	Ala	Ala	Gln	Leu	His	Ser	
			260					265					270			
Arg	Ala	Gln	Gln	Ile	Cys	Ala	Val	Leu	Tyr	Pro	Gly	Asp	Ala	Thr	Glu	
		275					280					285				
Asn	Gly	Lys	Leu	Leu	Arg	Leu	Lys	Gln	Gln	Phe	Phe	Leu	Cys	Ser	Ala	
	290					295					300					

Ser	Leu	Gln	Asp	Ile	Ile	Ala	Arg	Phe	Lys	Glu	Arg	Glu	Asp	Gly	Lys	305	310	315	320
Gly	Ser	His	Gln	Trp	Ser	Glu	Phe	Pro	Lys	Lys	Val	Ala	Ile	Gln	Leu	325	330	335	
Asn	Asp	Thr	His	Pro	Thr	Leu	Thr	Ile	Pro	Glu	Leu	Met	Arg	Leu	Leu	340	345	350	
Met	Asp	Asp	Glu	Gly	Leu	Gly	Trp	Asp	Glu	Ser	Trp	Asn	Ile	Thr	Thr	355	360	365	
Arg	Thr	Ile	Ala	Tyr	Thr	Asn	His	Thr	Val	Leu	Pro	Glu	Ala	Leu	Glu	370	375	380	
Lys	Trp	Ser	Gln	Ala	Val	Met	Trp	Lys	Leu	Leu	Pro	Arg	His	Met	Glu	385	390	395	400
Ile	Ile	Glu	Glu	Ile	Asp	Lys	Arg	Phe	Val	Ala	Thr	Ile	Met	Ser	Glu	405	410	415	
Arg	Pro	Asp	Leu	Glu	Asn	Lys	Met	Pro	Ser	Met	Arg	Ile	Leu	Asp	His	420	425	430	
Asn	Ala	Thr	Lys	Pro	Val	Val	His	Met	Ala	Asn	Leu	Cys	Val	Val	Ser	435	440	445	
Ser	His	Thr	Val	Asn	Gly	Val	Ala	Gln	Leu	His	Ser	Asp	Ile	Leu	Lys	450	455	460	
Ala	Glu	Leu	Phe	Ala	Asp	Tyr	Val	Ser	Val	Trp	Pro	Thr	Lys	Phe	Gln	465	470	475	480
Asn	Lys	Thr	Asn	Gly	Ile	Thr	Pro	Arg	Arg	Trp	Ile	Arg	Phe	Cys	Ser	485	490	495	
Pro	Glu	Leu	Ser	His	Ile	Ile	Thr	Lys	Trp	Leu	Lys	Thr	Asp	Gln	Trp	500	505	510	
Val	Thr	Asn	Leu	Glu	Leu	Leu	Ala	Asn	Leu	Arg	Glu	Phe	Ala	Asp	Asn	515	520	525	
Ser	Glu	Leu	His	Ala	Glu	Trp	Glu	Ser	Ala	Lys	Met	Ala	Asn	Lys	Gln	530	535	540	
Arg	Leu	Ala	Gln	Tyr	Ile	Leu	His	Val	Thr	Gly	Val	Ser	Ile	Asp	Pro	545	550	555	560
Asn	Ser	Leu	Phe	Asp	Ile	Gln	Val	Lys	Arg	Ile	His	Glu	Tyr	Lys	Arg	565	570	575	
Gln	Leu	Leu	Asn	Ile	Leu	Gly	Val	Ile	Tyr	Arg	Tyr	Lys	Lys	Leu	Lys	580	585	590	
Gly	Met	Ser	Pro	Glu	Glu	Arg	Lys	Asn	Thr	Thr	Pro	Arg	Thr	Val	Met	595	600	605	

Ile Gly Gly Lys Ala Phe Ala Thr Tyr Thr Asn Ala Lys Arg Ile Val
 610 615 620
 Lys Leu Val Thr Asp Val Gly Asp Val Val Asn Ser Asp Pro Asp Val
 625 630 635 640
 Asn Asp Tyr Leu Lys Val Val Phe Val Pro Asn Tyr Asn Val Ser Val
 645 650 655
 Ala Glu Met Leu Ile Pro Gly Ser Glu Leu Ser Gln His Ile Ser Thr
 660 665 670
 Ala Gly Met Glu Ala Ser Gly Thr Ser Asn Met Lys Phe Ala Leu Asn
 675 680 685
 Gly Cys Leu Ile Ile Gly Thr Leu Asp Gly Ala Asn Val Glu Ile Arg
 690 695 700
 Glu Glu Ile Gly Glu Asp Asn Phe Phe Leu Phe Gly Ala Thr Ala Asp
 705 710 715 720
 Glu Val Pro Gln Leu Arg Lys Asp Arg Glu Asn Gly Leu Phe Lys Pro
 725 730 735
 Asp Pro Arg Phe Glu Glu Ala Lys Gln Phe Ile Arg Ser Gly Ala Phe
 740 745 750
 Gly Thr Tyr Asp Tyr Asn Pro Leu Leu Glu Ser Leu Glu Gly Asn Ser
 755 760 765
 Gly Tyr Gly Arg Gly Asp Tyr Phe Leu Val Gly His Asp Phe Pro Ser
 770 775 780
 Tyr Met Asp Ala Gln Ala Arg Val Asp Glu Ala Tyr Lys Asp Arg Lys
 785 790 795 800
 Arg Trp Ile Lys Met Ser Ile Leu Ser Thr Ser Gly Ser Gly Lys Phe
 805 810 815
 Ser Ser Asp Arg Thr Ile Ser Gln Tyr Ala Lys Glu Ile Trp Asn Ile
 820 825 830
 Ala Glu Cys Arg Val Pro
 835

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3171 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 87..3011

(D) OTHER INFORMATION: /product= "potato alpha-glucan
L-type leaf phosphorylase"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 330..3008

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 87..329

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTTTTTTTTT CAACATGCAC AACAATTATT TTGATTAAAT TTTGTATCTA AAAATTTAGC	60
ATTTTGAAAT TCAGTTCAGA GACATC ATG GCA ACT TTT GCT GTC TCT GGA TTG	113
Met Ala Thr Phe Ala Val Ser Gly Leu	
-81 -80 -75	
AAC TCA ATT TCA AGT ATT TCT AGT TTT AAT AAC AAT TTC AGA AGC AAA	161
Asn Ser Ile Ser Ser Ile Ser Ser Phe Asn Asn Asn Phe Arg Ser Lys	
-70 -65 -60	
AAC TCA AAC ATT TTG TTG AGT AGA AGG AGG ATT TTA TTG TTC AGT TTT	209
Asn Ser Asn Ile Leu Leu Ser Arg Arg Arg Ile Leu Leu Phe Ser Phe	
-55 -50 -45	
AGA AGA AGA AGA AGA AGT TTC TCT GTT AGC AGT GTT GCT AGT GAT CAA	257
Arg Arg Arg Arg Arg Ser Phe Ser Val Ser Ser Val Ala Ser Asp Gln	
-40 -35 -30 -25	
AAG CAG AAG ACA AAG GAT TCT TCC TCT GAT GAA GGA TTT ACA TTA GAT	305
Lys Gln Lys Thr Lys Asp Ser Ser Ser Asp Glu Gly Phe Thr Leu Asp	
-20 -15 -10	
GTT TTT CAG CCG GAC TCC ACG TCT GTT TTA TCA AGT ATA AAG TAT CAC	353
Val Phe Gln Pro Asp Ser Thr Ser Val Leu Ser Ser Ile Lys Tyr His	
-5 1 5	
GCT GAG TTC ACA CCA TCA TTT TCT CCT GAG AAG TTT GAA CTT CCC AAG	401
Ala Glu Phe Thr Pro Ser Phe Ser Pro Glu Lys Phe Glu Leu Pro Lys	
10 15 20	
GCA TAC TAT GCA ACT GCA GAG AGT GTT CGA GAT ACG CTC ATT ATA AAT	449
Ala Tyr Tyr Ala Thr Ala Glu Ser Val Arg Asp Thr Leu Ile Ile Asn	
25 30 35 40	

TGG	AAT	GCC	ACA	TAC	GAA	TTC	TAT	GAA	AAG	ATG	AAT	GTA	AAG	CAG	GCA	497
Trp	Asn	Ala	Thr	Tyr	Glu	Phe	Tyr	Glu	Lys	Met	Asn	Val	Lys	Gln	Ala	
				45					50					55		
TAT	TAC	TTG	TCT	ATG	GAA	TTT	CTT	CAG	GGA	AGA	GCT	TTA	CTC	AAT	GCT	545
Tyr	Tyr	Leu	Ser	Met	Glu	Phe	Leu	Gln	Gly	Arg	Ala	Leu	Leu	Asn	Ala	
			60					65					70			
ATT	GGT	AAC	TTG	GGG	CTA	ACC	GGA	CCT	TAT	GCA	GAT	GCT	TTA	ACT	AAG	593
Ile	Gly	Asn	Leu	Gly	Leu	Thr	Gly	Pro	Tyr	Ala	Asp	Ala	Leu	Thr	Lys	
		75					80					85				
CTC	GGA	TAC	AGT	TTA	GAG	GAT	GTA	GCC	AGG	CAG	GAA	CCG	GAT	GCA	GCT	641
Leu	Gly	Tyr	Ser	Leu	Glu	Asp	Val	Ala	Arg	Gln	Glu	Pro	Asp	Ala	Ala	
	90					95					100					
TTA	GGT	AAT	GGA	GGT	TTA	GGA	AGA	CTT	GCT	TCT	TGC	TTT	CTG	GAC	TCA	689
Leu	Gly	Asn	Gly	Gly	Leu	Gly	Arg	Leu	Ala	Ser	Cys	Phe	Leu	Asp	Ser	
105					110				115						120	
ATG	GCG	ACA	CTA	AAC	TAC	CCT	GCA	TGG	GGC	TAT	GGA	CTT	AGA	TAC	CAA	737
Met	Ala	Thr	Leu	Asn	Tyr	Pro	Ala	Trp	Gly	Tyr	Gly	Leu	Arg	Tyr	Gln	
				125					130					135		
TAT	GGC	CTT	TTC	AAA	CAG	CTT	ATT	ACA	AAA	GAT	GGA	CAG	GAG	GAA	GTT	785
Tyr	Gly	Leu	Phe	Lys	Gln	Leu	Ile	Thr	Lys	Asp	Gly	Gln	Glu	Glu	Val	
			140					145					150			
GCT	GAA	AAT	TGG	CTC	GAG	ATG	GGA	AAT	CCA	TGG	GAA	ATT	GTG	AGG	AAT	833
Ala	Glu	Asn	Trp	Leu	Glu	Met	Gly	Asn	Pro	Trp	Glu	Ile	Val	Arg	Asn	
		155					160					165				
GAT	ATT	TCG	TAT	CCC	GTA	AAA	TTC	TAT	GGG	AAG	GTC	ATT	GAA	GGA	GCT	881
Asp	Ile	Ser	Tyr	Pro	Val	Lys	Phe	Tyr	Gly	Lys	Val	Ile	Glu	Gly	Ala	
	170					175					180					
GAT	GGG	AGG	AAG	GAA	TGG	GCT	GGC	GGA	GAA	GAT	ATA	ACT	GCT	GTT	GCC	929
Asp	Gly	Arg	Lys	Glu	Trp	Ala	Gly	Gly	Glu	Asp	Ile	Thr	Ala	Val	Ala	
185					190				195						200	
TAT	GAT	GTC	CCA	ATA	CCA	GGA	TAT	AAA	ACA	AAA	ACA	ACG	ATT	AAC	CTT	977
Tyr	Asp	Val	Pro	Ile	Pro	Gly	Tyr	Lys	Thr	Lys	Thr	Thr	Ile	Asn	Leu	
				205					210					215		
CGA	TTG	TGG	ACA	ACA	AAG	CTA	GCT	GCA	GAA	GCT	TTT	GAT	TTA	TAT	GCT	1025
Arg	Leu	Trp	Thr	Thr	Lys	Leu	Ala	Ala	Glu	Ala	Phe	Asp	Leu	Tyr	Ala	
			220					225					230			
TTT	AAC	AAT	GGA	GAC	CAT	GCC	AAA	GCA	TAT	GAG	GCC	CAG	AAA	AAG	GCT	1073
Phe	Asn	Asn	Gly	Asp	His	Ala	Lys	Ala	Tyr	Glu	Ala	Gln	Lys	Lys	Ala	
		235					240					245				
GAA	AAG	ATT	TGC	TAT	GTC	TTA	TAT	CCA	GGT	GAC	GAA	TCG	CTT	GAA	GGA	1121
Glu	Lys	Ile	Cys	Tyr	Val	Leu	Tyr	Pro	Gly	Asp	Glu	Ser	Leu	Glu	Gly	
	250					255					260					

AAG	ACG	CTT	AGG	TTA	AAG	CAG	CAA	TAC	ACA	CTA	TGT	TCT	GCT	TCT	CTT	1169
Lys	Thr	Leu	Arg	Leu	Lys	Gln	Gln	Tyr	Thr	Leu	Cys	Ser	Ala	Ser	Leu	
265					270					275					280	
CAG	GAC	ATT	ATT	GCA	CGG	TTC	GAG	AAG	AGA	TCA	GGG	AAT	GCA	GTA	AAC	1217
Gln	Asp	Ile	Ile	Ala	Arg	Phe	Glu	Lys	Arg	Ser	Gly	Asn	Ala	Val	Asn	
				285					290					295		
TGG	GAT	CAG	TTC	CCC	GAA	AAG	GTT	GCA	GTA	CAG	ATG	AAT	GAC	ACT	CAT	1265
Trp	Asp	Gln	Phe	Pro	Glu	Lys	Val	Ala	Val	Gln	Met	Asn	Asp	Thr	His	
			300					305					310			
CCA	ACA	CTT	TGT	ATA	CCA	GAA	CTT	TTA	AGG	ATA	TTG	ATG	GAT	GTT	AAA	1313
Pro	Thr	Leu	Cys	Ile	Pro	Glu	Leu	Leu	Arg	Ile	Leu	Met	Asp	Val	Lys	
		315					320					325				
GGT	TTG	AGC	TGG	AAG	CAG	GCA	TGG	GAA	ATT	ACT	CAA	AGA	ACG	GTC	GCA	1361
Gly	Leu	Ser	Trp	Lys	Gln	Ala	Trp	Glu	Ile	Thr	Gln	Arg	Thr	Val	Ala	
	330					335					340					
TAC	ACT	AAC	CAC	ACT	GTT	CTA	CCT	GAG	GCT	CTT	GAG	AAA	TGG	AGC	TTC	1409
Tyr	Thr	Asn	His	Thr	Val	Leu	Pro	Glu	Ala	Leu	Glu	Lys	Trp	Ser	Phe	
345					350					355					360	
ACA	CTT	CTT	GGT	GAA	CTG	CTT	CCT	CGG	CAC	GTG	GAG	ATC	ATA	GCA	ATG	1457
Thr	Leu	Leu	Gly	Glu	Leu	Leu	Pro	Arg	His	Val	Glu	Ile	Ile	Ala	Met	
				365				370						375		
ATA	GAT	GAG	GAG	CTC	TTG	CAT	ACT	ATA	CTT	GCT	GAA	TAT	GGT	ACT	GAA	1505
Ile	Asp	Glu	Glu	Leu	Leu	His	Thr	Ile	Leu	Ala	Glu	Tyr	Gly	Thr	Glu	
			380					385					390			
GAT	CTT	GAC	TTG	TTG	CAA	GAA	AAG	CTA	AAC	CAA	ATG	AGG	ATT	CTG	GAT	1553
Asp	Leu	Asp	Leu	Leu	Gln	Glu	Lys	Leu	Asn	Gln	Met	Arg	Ile	Leu	Asp	
		395					400					405				
AAT	GTT	GAA	ATA	CCA	AGT	TCT	GTT	TTG	GAG	TTG	CTT	ATA	AAA	GCC	GAA	1601
Asn	Val	Glu	Ile	Pro	Ser	Ser	Val	Leu	Glu	Leu	Leu	Ile	Lys	Ala	Glu	
	410					415					420					
GAA	AGT	GCT	GCT	GAT	GTC	GAA	AAG	GCA	GCA	GAT	GAA	GAA	CAA	GAA	GAA	1649
Glu	Ser	Ala	Ala	Asp	Val	Glu	Lys	Ala	Ala	Asp	Glu	Glu	Gln	Glu	Glu	
425					430					435					440	
GAA	GGT	AAG	GAT	GAC	AGT	AAA	GAT	GAG	GAA	ACT	GAG	GCT	GTA	AAG	GCA	1697
Glu	Gly	Lys	Asp	Asp	Ser	Lys	Asp	Glu	Glu	Thr	Glu	Ala	Val	Lys	Ala	
				445				450						455		
GAA	ACT	ACG	AAC	GAA	GAG	GAG	GAA	ACT	GAG	GTT	AAG	AAG	GTT	GAG	GTG	1745
Glu	Thr	Thr	Asn	Glu	Glu	Glu	Glu	Thr	Glu	Val	Lys	Lys	Val	Glu	Val	
			460					465					470			
GAG	GAT	AGT	CAA	GCA	AAA	ATA	AAA	CGT	ATA	TTC	GGG	CCA	CAT	CCA	AAT	1793
Glu	Asp	Ser	Gln	Ala	Lys	Ile	Lys	Arg	Ile	Phe	Gly	Pro	His	Pro	Asn	
		475					480					485				

AAA	CCA	CAG	GTG	GTT	CAC	ATG	GCA	AAT	CTA	TGT	GTA	GTT	AGC	GGG	CAT	1841
Lys	Pro	Gln	Val	Val	His	Met	Ala	Asn	Leu	Cys	Val	Val	Ser	Gly	His	
	490					495					500					
GCA	GTT	AAC	GGT	GTT	GCT	GAG	ATT	CAT	AGT	GAA	ATA	GTT	AAG	GAT	GAA	1889
Ala	Val	Asn	Gly	Val	Ala	Glu	Ile	His	Ser	Glu	Ile	Val	Lys	Asp	Glu	
505					510					515					520	
GTT	TTC	AAT	GAA	TTT	TAC	AAG	TTA	TGG	CCA	GAG	AAA	TTC	CAA	AAC	AAG	1937
Val	Phe	Asn	Glu	Phe	Tyr	Lys	Leu	Trp	Pro	Glu	Lys	Phe	Gln	Asn	Lys	
				525					530					535		
ACA	AAT	GGT	GTG	ACA	CCA	AGA	AGA	TGG	CTA	AGT	TTC	TGT	AAT	CCA	GAG	1985
Thr	Asn	Gly	Val	Thr	Pro	Arg	Arg	Trp	Leu	Ser	Phe	Cys	Asn	Pro	Glu	
			540					545					550			
TTG	AGT	GAA	ATT	ATA	ACC	AAG	TGG	ACA	GGA	TCT	GAT	GAT	TGG	TTA	GTA	2033
Leu	Ser	Glu	Ile	Ile	Thr	Lys	Trp	Thr	Gly	Ser	Asp	Asp	Trp	Leu	Val	
		555					560					565				
AAC	ACT	GAA	AAA	TTG	GCA	GAG	CTT	CGA	AAG	TTT	GCT	GAT	AAC	GAA	GAA	2081
Asn	Thr	Glu	Lys	Leu	Ala	Glu	Leu	Arg	Lys	Phe	Ala	Asp	Asn	Glu	Glu	
	570					575					580					
CTC	CAG	TCT	GAG	TGG	AGG	AAG	GCA	AAA	GGA	AAT	AAC	AAA	ATG	AAG	ATT	2129
Leu	Gln	Ser	Glu	Trp	Arg	Lys	Ala	Lys	Gly	Asn	Asn	Lys	Met	Lys	Ile	
585					590					595					600	
GTC	TCT	CTC	ATT	AAA	GAA	AAA	ACA	GGA	TAC	GTG	GTC	AGT	CCC	GAT	GCA	2177
Val	Ser	Leu	Ile	Lys	Glu	Lys	Thr	Gly	Tyr	Val	Val	Ser	Pro	Asp	Ala	
				605					610					615		
ATG	TTT	GAT	GTT	CAG	ATC	AAG	CGC	ATC	CAT	GAG	TAT	AAA	AGG	CAG	CTA	2225
Met	Phe	Asp	Val	Gln	Ile	Lys	Arg	Ile	His	Glu	Tyr	Lys	Arg	Gln	Leu	
			620					625					630			
TTA	AAT	ATA	TTT	GGA	ATC	GTT	TAT	CGC	TAT	AAG	AAG	ATG	AAA	GAA	ATG	2273
Leu	Asn	Ile	Phe	Gly	Ile	Val	Tyr	Arg	Tyr	Lys	Lys	Met	Lys	Glu	Met	
		635					640					645				
AGC	CCT	GAA	GAA	CGA	AAA	GAA	AAG	TTT	GTC	CCT	CGA	GTT	TGC	ATA	TTT	2321
Ser	Pro	Glu	Glu	Arg	Lys	Glu	Lys	Phe	Val	Pro	Arg	Val	Cys	Ile	Phe	
	650					655					660					
GGA	GGA	AAA	GCA	TTT	GCT	ACA	TAT	GTT	CAG	GCC	AAG	AGA	ATT	GTA	AAA	2369
Gly	Gly	Lys	Ala	Phe	Ala	Thr	Tyr	Val	Gln	Ala	Lys	Arg	Ile	Val	Lys	
665					670					675					680	
TTT	ATC	ACT	GAT	GTA	GGG	GAA	ACA	GTC	AAC	CAT	GAT	CCC	GAG	ATT	GGT	2417
Phe	Ile	Thr	Asp	Val	Gly	Glu	Thr	Val	Asn	His	Asp	Pro	Glu	Ile	Gly	
				685					690					695		
GAT	CTT	TTG	AAG	GTT	GTA	TTT	GTT	CCT	GAT	TAC	AAT	GTC	AGT	GTA	GCA	2465
Asp	Leu	Leu	Lys	Val	Val	Phe	Val	Pro	Asp	Tyr	Asn	Val	Ser	Val	Ala	
			700					705					710			

GAA GTG CTA ATT CCT GGT AGT GAG TTG TCC CAG CAT ATT AGT ACT GCT Glu Val Leu Ile Pro Gly Ser Glu Leu Ser Gln His Ile Ser Thr Ala 715 720 725	2513
GGT ATG GAG GCT AGT GGA ACC AGC AAC ATG AAA TTT TCA ATG AAT GGC Gly Met Glu Ala Ser Gly Thr Ser Asn Met Lys Phe Ser Met Asn Gly 730 735 740	2561
TGC CTC CTC ATC GGG ACA TTA GAT GGT GCC AAT GTT GAG ATA AGA GAG Cys Leu Leu Ile Gly Thr Leu Asp Gly Ala Asn Val Glu Ile Arg Glu 745 750 755 760	2609
GAA GTT GGA GAG GAC AAT TTC TTT CTT TTC GGA GCT CAG GCT CAT GAA Glu Val Gly Glu Asp Asn Phe Phe Leu Phe Gly Ala Gln Ala His Glu 765 770 775	2657
ATT GCT GGC CTA CGA AAG GAA AGA GCC GAG GGA AAG TTT GTC CCG GAC Ile Ala Gly Leu Arg Lys Glu Arg Ala Glu Gly Lys Phe Val Pro Asp 780 785 790	2705
CCA AGA TTT GAA GAA GTA AAG GCG TTC ATT AGG ACA GGC GTC TTT GGC Pro Arg Phe Glu Glu Val Lys Ala Phe Ile Arg Thr Gly Val Phe Gly 795 800 805	2753
ACC TAC AAC TAT GAA GAA CTC ATG GGA TCC TTG GAA GGA AAC GAA GGC Thr Tyr Asn Tyr Glu Glu Leu Met Gly Ser Leu Glu Gly Asn Glu Gly 810 815 820	2801
TAT GGT CGT GCT GAC TAT TTT CTT GTA GGA AAG GAT TTC CCC GAT TAT Tyr Gly Arg Ala Asp Tyr Phe Leu Val Gly Lys Asp Phe Pro Asp Tyr 825 830 835 840	2849
ATA GAG TGC CAA GAT AAA GTT GAT GAA GCA TAT CGA GAC CAG AAG AAA Ile Glu Cys Gln Asp Lys Val Asp Glu Ala Tyr Arg Asp Gln Lys Lys 845 850 855	2897
TGG ACC AAA ATG TCG ATC TTA AAC ACA GCT GGA TCG TTC AAA TTT AGC Trp Thr Lys Met Ser Ile Leu Asn Thr Ala Gly Ser Phe Lys Phe Ser 860 865 870	2945
AGT GAT CGA ACA ATT CAT CAA TAT GCA AGA GAT ATA TGG AGA ATT GAA Ser Asp Arg Thr Ile His Gln Tyr Ala Arg Asp Ile Trp Arg Ile Glu 875 880 885	2993
CCT GTT GAA TTA CCT TAA AAGTTAGCCA GTTAAAGGAT GAAAGCCAAT Pro Val Glu Leu Pro 890	3041
TTTTTCCCCC TGAGGTTCTC CCATACTGTT TATTAGTACA TATATTGTCA ATTGTTGCTA	3101
CTGAAATGAT AGAAGTTTTG AATATTTACT GTCAATAAAA TACAGTTGAT TCCATTTGAA	3161
AAAAAAAAAA	3171

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 974 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Ala Thr Phe Ala Val Ser Gly Leu Asn Ser Ile Ser Ser Ile Ser
-81 -80                      -75                      -70

Ser Phe Asn Asn Asn Phe Arg Ser Lys Asn Ser Asn Ile Leu Leu Ser
-65                      -60                      -55                      -50

Arg Arg Arg Ile Leu Leu Phe Ser Phe Arg Arg Arg Arg Arg Ser Phe
                      -45                      -40                      -35

Ser Val Ser Ser Val Ala Ser Asp Gln Lys Gln Lys Thr Lys Asp Ser
                      -30                      -25                      -20

Ser Ser Asp Glu Gly Phe Thr Leu Asp Val Phe Gln Pro Asp Ser Thr
                      -15                      -10                      -5

Ser Val Leu Ser Ser Ile Lys Tyr His Ala Glu Phe Thr Pro Ser Phe
      1                      5                      10                      15

Ser Pro Glu Lys Phe Glu Leu Pro Lys Ala Tyr Tyr Ala Thr Ala Glu
                      20                      25                      30

Ser Val Arg Asp Thr Leu Ile Ile Asn Trp Asn Ala Thr Tyr Glu Phe
                      35                      40                      45

Tyr Glu Lys Met Asn Val Lys Gln Ala Tyr Tyr Leu Ser Met Glu Phe
                      50                      55                      60

Leu Gln Gly Arg Ala Leu Leu Asn Ala Ile Gly Asn Leu Gly Leu Thr
      65                      70                      75

Gly Pro Tyr Ala Asp Ala Leu Thr Lys Leu Gly Tyr Ser Leu Glu Asp
      80                      85                      90                      95

Val Ala Arg Gln Glu Pro Asp Ala Ala Leu Gly Asn Gly Gly Leu Gly
                      100                      105                      110

Arg Leu Ala Ser Cys Phe Leu Asp Ser Met Ala Thr Leu Asn Tyr Pro
                      115                      120                      125

Ala Trp Gly Tyr Gly Leu Arg Tyr Gln Tyr Gly Leu Phe Lys Gln Leu
                      130                      135                      140

Ile Thr Lys Asp Gly Gln Glu Glu Val Ala Glu Asn Trp Leu Glu Met
      145                      150                      155

Gly Asn Pro Trp Glu Ile Val Arg Asn Asp Ile Ser Tyr Pro Val Lys
      160                      165                      170                      175

```

Phe	Tyr	Gly	Lys	Val	Ile	Glu	Gly	Ala	Asp	Gly	Arg	Lys	Glu	Trp	Ala	
				180					185					190		
Gly	Gly	Glu	Asp	Ile	Thr	Ala	Val	Ala	Tyr	Asp	Val	Pro	Ile	Pro	Gly	
			195					200					205			
Tyr	Lys	Thr	Lys	Thr	Thr	Ile	Asn	Leu	Arg	Leu	Trp	Thr	Thr	Lys	Leu	
		210					215					220				
Ala	Ala	Glu	Ala	Phe	Asp	Leu	Tyr	Ala	Phe	Asn	Asn	Gly	Asp	His	Ala	
	225					230					235					
Lys	Ala	Tyr	Glu	Ala	Gln	Lys	Lys	Ala	Glu	Lys	Ile	Cys	Tyr	Val	Leu	
240					245					250					255	
Tyr	Pro	Gly	Asp	Glu	Ser	Leu	Glu	Gly	Lys	Thr	Leu	Arg	Leu	Lys	Gln	
				260					265					270		
Gln	Tyr	Thr	Leu	Cys	Ser	Ala	Ser	Leu	Gln	Asp	Ile	Ile	Ala	Arg	Phe	
			275					280					285			
Glu	Lys	Arg	Ser	Gly	Asn	Ala	Val	Asn	Trp	Asp	Gln	Phe	Pro	Glu	Lys	
		290					295					300				
Val	Ala	Val	Gln	Met	Asn	Asp	Thr	His	Pro	Thr	Leu	Cys	Ile	Pro	Glu	
	305					310					315					
Leu	Leu	Arg	Ile	Leu	Met	Asp	Val	Lys	Gly	Leu	Ser	Trp	Lys	Gln	Ala	
320					325					330					335	
Trp	Glu	Ile	Thr	Gln	Arg	Thr	Val	Ala	Tyr	Thr	Asn	His	Thr	Val	Leu	
				340					345					350		
Pro	Glu	Ala	Leu	Glu	Lys	Trp	Ser	Phe	Thr	Leu	Leu	Gly	Glu	Leu	Leu	
			355					360					365			
Pro	Arg	His	Val	Glu	Ile	Ile	Ala	Met	Ile	Asp	Glu	Glu	Leu	Leu	His	
		370					375					380				
Thr	Ile	Leu	Ala	Glu	Tyr	Gly	Thr	Glu	Asp	Leu	Asp	Leu	Leu	Gln	Glu	
	385					390					395					
Lys	Leu	Asn	Gln	Met	Arg	Ile	Leu	Asp	Asn	Val	Glu	Ile	Pro	Ser	Ser	
400					405					410					415	
Val	Leu	Glu	Leu	Leu	Ile	Lys	Ala	Glu	Glu	Ser	Ala	Ala	Asp	Val	Glu	
				420					425					430		
Lys	Ala	Ala	Asp	Glu	Glu	Gln	Glu	Glu	Gly	Lys	Asp	Asp	Ser	Lys		
			435					440				445				
Asp	Glu	Glu	Thr	Glu	Ala	Val	Lys	Ala	Glu	Thr	Thr	Asn	Glu	Glu	Glu	
	450						455					460				
Glu	Thr	Glu	Val	Lys	Lys	Val	Glu	Val	Glu	Asp	Ser	Gln	Ala	Lys	Ile	
	465					470					475					

Lys	Arg	Ile	Phe	Gly	Pro	His	Pro	Asn	Lys	Pro	Gln	Val	Val	His	Met	480	485	490	495
Ala	Asn	Leu	Cys	Val	Val	Ser	Gly	His	Ala	Val	Asn	Gly	Val	Ala	Glu		500	505	510
Ile	His	Ser	Glu	Ile	Val	Lys	Asp	Glu	Val	Phe	Asn	Glu	Phe	Tyr	Lys	515	520	525	
Leu	Trp	Pro	Glu	Lys	Phe	Gln	Asn	Lys	Thr	Asn	Gly	Val	Thr	Pro	Arg	530	535	540	
Arg	Trp	Leu	Ser	Phe	Cys	Asn	Pro	Glu	Leu	Ser	Glu	Ile	Ile	Thr	Lys	545	550	555	
Trp	Thr	Gly	Ser	Asp	Asp	Trp	Leu	Val	Asn	Thr	Glu	Lys	Leu	Ala	Glu	560	565	570	575
Leu	Arg	Lys	Phe	Ala	Asp	Asn	Glu	Glu	Leu	Gln	Ser	Glu	Trp	Arg	Lys		580	585	590
Ala	Lys	Gly	Asn	Asn	Lys	Met	Lys	Ile	Val	Ser	Leu	Ile	Lys	Glu	Lys		595	600	605
Thr	Gly	Tyr	Val	Val	Ser	Pro	Asp	Ala	Met	Phe	Asp	Val	Gln	Ile	Lys	610	615	620	
Arg	Ile	His	Glu	Tyr	Lys	Arg	Gln	Leu	Leu	Asn	Ile	Phe	Gly	Ile	Val	625	630	635	
Tyr	Arg	Tyr	Lys	Lys	Met	Lys	Glu	Met	Ser	Pro	Glu	Glu	Arg	Lys	Glu	640	645	650	655
Lys	Phe	Val	Pro	Arg	Val	Cys	Ile	Phe	Gly	Gly	Lys	Ala	Phe	Ala	Thr		660	665	670
Tyr	Val	Gln	Ala	Lys	Arg	Ile	Val	Lys	Phe	Ile	Thr	Asp	Val	Gly	Glu		675	680	685
Thr	Val	Asn	His	Asp	Pro	Glu	Ile	Gly	Asp	Leu	Leu	Lys	Val	Val	Phe	690	695	700	
Val	Pro	Asp	Tyr	Asn	Val	Ser	Val	Ala	Glu	Val	Leu	Ile	Pro	Gly	Ser	705	710	715	
Glu	Leu	Ser	Gln	His	Ile	Ser	Thr	Ala	Gly	Met	Glu	Ala	Ser	Gly	Thr	720	725	730	735
Ser	Asn	Met	Lys	Phe	Ser	Met	Asn	Gly	Cys	Leu	Leu	Ile	Gly	Thr	Leu		740	745	750
Asp	Gly	Ala	Asn	Val	Glu	Ile	Arg	Glu	Glu	Val	Gly	Glu	Asp	Asn	Phe		755	760	765
Phe	Leu	Phe	Gly	Ala	Gln	Ala	His	Glu	Ile	Ala	Gly	Leu	Arg	Lys	Glu	770	775	780	

Arg Ala Glu Gly Lys Phe Val Pro Asp Pro Arg Phe Glu Glu Val Lys
785 790 795

Ala Phe Ile Arg Thr Gly Val Phe Gly Thr Tyr Asn Tyr Glu Glu Leu
800 805 810 815

Met Gly Ser Leu Glu Gly Asn Glu Gly Tyr Gly Arg Ala Asp Tyr Phe
820 825 830

Leu Val Gly Lys Asp Phe Pro Asp Tyr Ile Glu Cys Gln Asp Lys Val
835 840 845

Asp Glu Ala Tyr Arg Asp Gln Lys Lys Trp Thr Lys Met Ser Ile Leu
850 855 860

Asn Thr Ala Gly Ser Phe Lys Phe Ser Ser Asp Arg Thr Ile His Gln
865 870 875

Tyr Ala Arg Asp Ile Trp Arg Ile Glu Pro Val Glu Leu Pro
880 885 890

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..27
- (D) OTHER INFORMATION: /function= "primer"

/label= SPL1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATTCGAAAAG CTCGAGATTT GCATAGA

27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..27

(D) OTHER INFORMATION: /function= "primer"

/label= SPL2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTATTTTC CATCGATGGA AGGTGGT

27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..23

(D) OTHER INFORMATION: /function= "primer"

/label= SPH1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGTGCTCTC GAGCATTGAA AGC

23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION: 1..25
 (D) OTHER INFORMATION: /function= "primer"
/label= SPH2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATAATATCCT GAATCGATGC ACTGC

25

1 WHAT IS CLAIMED IS:

2 1. A method for improving the cold-storage characteristics of a potato tuber, comprising
3 transforming a potato plant with an expression cassette having a plant promoter sequence
4 operably linked to a DNA sequence comprising at least 20 nucleotides from the exon of a
5 gene encoding an α glucan phosphorylase selected from the group consisting of α glucan L-
6 type tuber phosphorylase (GLTP) and α glucan H-type tuber phosphorylase (GHTP).

7 2. The method of claim 1, wherein the encoded α glucan phosphorylase is GLTP.

8 3. The method of claim 1, wherein the encoded α glucan phosphorylase is GHTP.

9 4. The method of claim 1, wherein the encoded α glucan phosphorylase comprises the
10 amino acid sequence depicted in SEQ ID NO: 2.

11 5. The method of claim 1, wherein the DNA sequence comprises at least 20 nucleotides
12 of the gene encoding an α glucan phosphorylase as depicted in SEQ ID NO:1.

13 6. The method of claim 1, wherein the encoded α glucan phosphorylase comprises the
14 amino acid sequence depicted in SEQ ID NO:4.

15 7. The method of claim 1, wherein the DNA sequence comprises at least 20 nucleotides
16 of the gene encoding an α glucan phosphorylase as depicted in SEQ ID NO:3.

17 8. The method of claim 1, wherein the DNA sequence comprises nucleotides 338 to 993
18 of SEQ ID NO: 1.

19 9. The method of claim 1, wherein the DNA sequence comprises nucleotides 147 to 799
20 of SEQ ID NO: 3.

21 10. The method of any one of claims 1, 5, 7, 8 or 9, wherein the DNA sequence is linked
22 to the promoter sequence in an antisense orientation.

23 11. The method of claim 2, wherein the sum of the concentration of glucose and fructose
24 in tubers of the plant measured at harvest is at least 10% lower than the sum of the
25 concentration of glucose and fructose in tubers of an untransformed plant measured at
26 harvest.

27 12. The method of claim 2, wherein the sum of the concentration of glucose and fructose
28 in tubers of the plant measured at harvest is at least 30% lower than the sum of the
29 concentration of glucose and fructose in tubers of an untransformed plant measured at
30 harvest.

1 13. The method of claim 2, wherein the sum of the concentration of glucose and fructose
2 in tubers of the plant measured at harvest is at least 80% lower than the sum of the
3 concentration of glucose and fructose in tubers of an untransformed plant measured at
4 harvest.

5 14. The method of claim 2, wherein the sum of the concentration of glucose and fructose
6 in tubers of the plant stored at 4°C for about three months is at least 10% lower than the sum
7 of the concentration of glucose and fructose in tubers of an untransformed plant stored under
8 the same conditions.

9 15. The method of claim 2, wherein the sum of the concentration of glucose and fructose
10 in tubers of the plant stored at 4°C for about three months is at least 30% lower than the sum
11 of the concentration of glucose and fructose in tubers of an untransformed plant stored under
12 the same conditions.

13 16. The method of claim 2, wherein the sum of the concentration of glucose and fructose
14 in tubers of the plant stored at 4°C for about three months is at least 39% lower than the sum
15 of the concentration of glucose and fructose in tubers of an untransformed plant stored under
16 the same conditions.

17 17. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
18 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant measured at harvest is at least
19 10% lower than the total α glucan phosphorylase activity in tubers of an untransformed plant
20 measured at harvest.

21 18. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
22 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant measured at harvest is at least
23 30% lower than the total α glucan phosphorylase activity in tubers of an untransformed plant
24 measured at harvest.

25 19. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
26 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant measured at harvest is at least
27 66% lower than the total α glucan phosphorylase activity in tubers of an untransformed plant
28 measured at harvest.

29 20. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
30 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about three

1 months is at least 10% lower than the total α glucan phosphorylase activity in tubers of an
2 untransformed plant stored under the same conditions.

3 21. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
4 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about three
5 months is at least 30% lower than the total α glucan phosphorylase activity in tubers of an
6 untransformed plant stored under the same conditions.

7 22. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
8 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about three
9 months is at least 70% lower than the total α glucan phosphorylase activity in tubers of an
10 untransformed plant stored under the same conditions.

11 23. The method of claim 3, wherein the total α glucan phosphorylase activity measured as
12 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about three
13 months is at least 10% lower than the total α glucan phosphorylase activity in tubers of an
14 untransformed plant stored under the same conditions.

15 24. The method of claim 3, wherein the total α glucan phosphorylase activity measured as
16 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about three
17 months is at least 28% lower than the total α glucan phosphorylase activity in tubers of an
18 untransformed plant stored under the same conditions.

19 25. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
20 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about six
21 months is at least 10% lower than the total α glucan phosphorylase activity in tubers of an
22 untransformed plant stored under the same conditions.

23 26. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
24 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about six
25 months is at least 30% lower than the total α glucan phosphorylase activity in tubers of an
26 untransformed plant stored under the same conditions.

27 27. The method of claim 2, wherein the total α glucan phosphorylase activity measured as
28 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about six
29 months is at least 69% lower than the total α glucan phosphorylase activity in tubers of an
30 untransformed plant stored under the same conditions.

1 28. The method of claim 3, wherein the total α glucan phosphorylase activity measured as
2 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about six
3 months is at least 10% lower than the total α glucan phosphorylase activity in tubers of an
4 untransformed plant stored under the same conditions.

5 29. The method of claim 3, wherein the total α glucan phosphorylase activity measured as
6 $\mu\text{mol NADPH produced mg}^{-1} \text{ protein h}^{-1}$ in tubers of the plant stored at 4°C for about six
7 months is at least 39% lower than the total α glucan phosphorylase activity in tubers of an
8 untransformed plant stored under the same conditions.

9 30. The method of claim 2, wherein a chip score for tubers of the plant measured at
10 harvest is at least 5% higher than the chip scores for tubers of an untransformed plant
11 measured at harvest.

12 31. The method of claim 2, wherein a chip score for tubers of the plant measured at
13 harvest is at least 30% higher than the chip scores for tubers of an untransformed plant
14 measured at harvest.

15 32. The method of claim 2, wherein a chip score for tubers of the plant measured at
16 harvest is at least 46% higher than the chip scores for tubers of an untransformed plant
17 measured at harvest.

18 33. The method of claim 3, wherein a chip score for tubers of the plant measured at
19 harvest is at least 5% higher than the chip scores for tubers of an untransformed plant
20 measured at harvest.

21 34. The method of claim 3, wherein a chip score for tubers of the plant measured at
22 harvest is at least 10% higher than the chip scores for tubers of an untransformed plant
23 measured at harvest.

24 35. The method of claim 2, wherein a chip score for tubers of the plant stored at 4°C for
25 about three months is at least 5% higher than the chip scores for tubers of an untransformed
26 plant stored under the same conditions.

27 36. The method of claim 2, wherein a chip score for tubers of the plant stored at 4°C for
28 about three months is at least 30% higher than the chip scores for tubers of an untransformed
29 plant stored under the same conditions.

1 37. The method of claim 2, wherein a chip score for tubers of the plant stored at 4°C for
2 about three months is at least 89% higher than the chip scores for tubers of an untransformed
3 plant stored under the same conditions.

4 38. The method of claim 3, wherein a chip score for tubers of the plant stored at 4°C for
5 about three months is at least 5% higher than the chip scores for tubers of an untransformed
6 plant stored under the same conditions.

7 39. The method of claim 3, wherein a chip score for tubers of the plant stored at 4°C for
8 about three months is at least 10% higher than the chip scores for tubers of an untransformed
9 plant stored under the same conditions.

10 40. The method of claim 2, wherein a chip score for tubers of the plant stored at 4°C for
11 about four months is at least 5% higher than the chip scores for tubers of an untransformed
12 plant stored under the same conditions.

13 41. The method of claim 2, wherein a chip score for tubers of the plant stored at 4°C for
14 about four months is at least 30% higher than the chip scores for tubers of an untransformed
15 plant stored under the same conditions.

16 42. The method of claim 2, wherein a chip score for tubers of the plant stored at 4°C for
17 about four months is at least 89% higher than the chip scores for tubers of an untransformed
18 plant stored under the same conditions.

19 43. The method of claim 3, wherein a chip score for tubers of the plant stored at 4°C for
20 about four months is at least 5% higher than the chip scores for tubers of an untransformed
21 plant stored under the same conditions.

22 44. The method of claim 3, wherein a chip score for tubers of the plant stored at 4°C for
23 about four months is at least 25% higher than the chip scores for tubers of an untransformed
24 plant stored under the same conditions.

WO 98/35051

PCT/CA98/00055

1/7

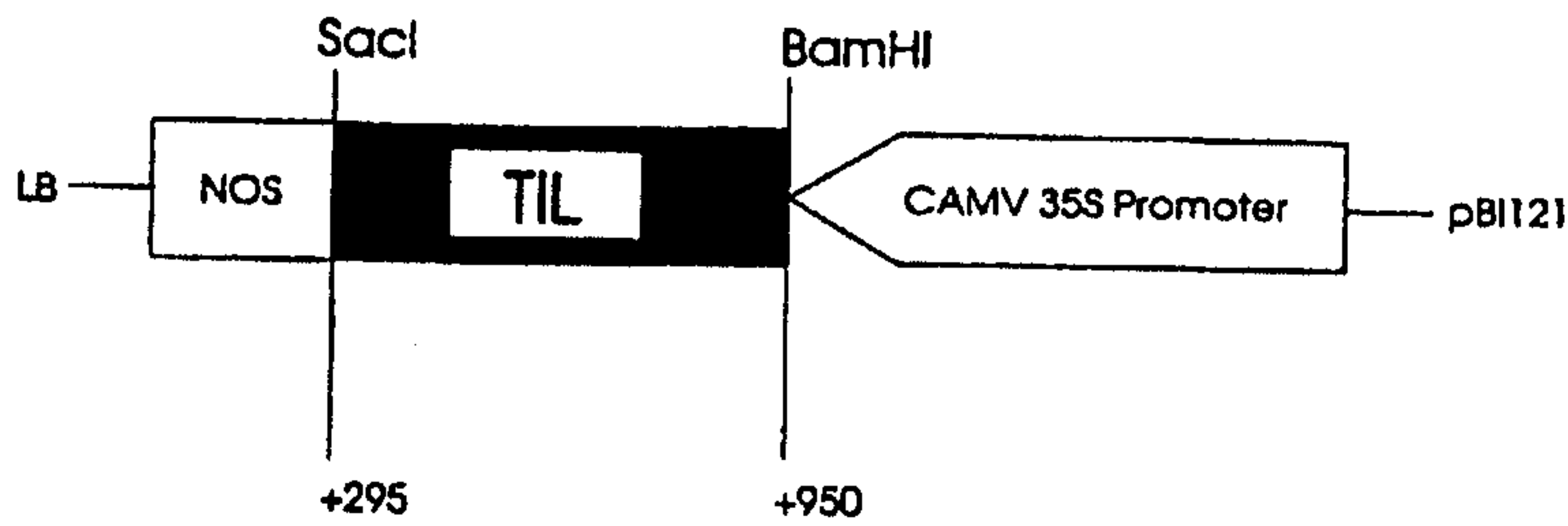


FIG.1

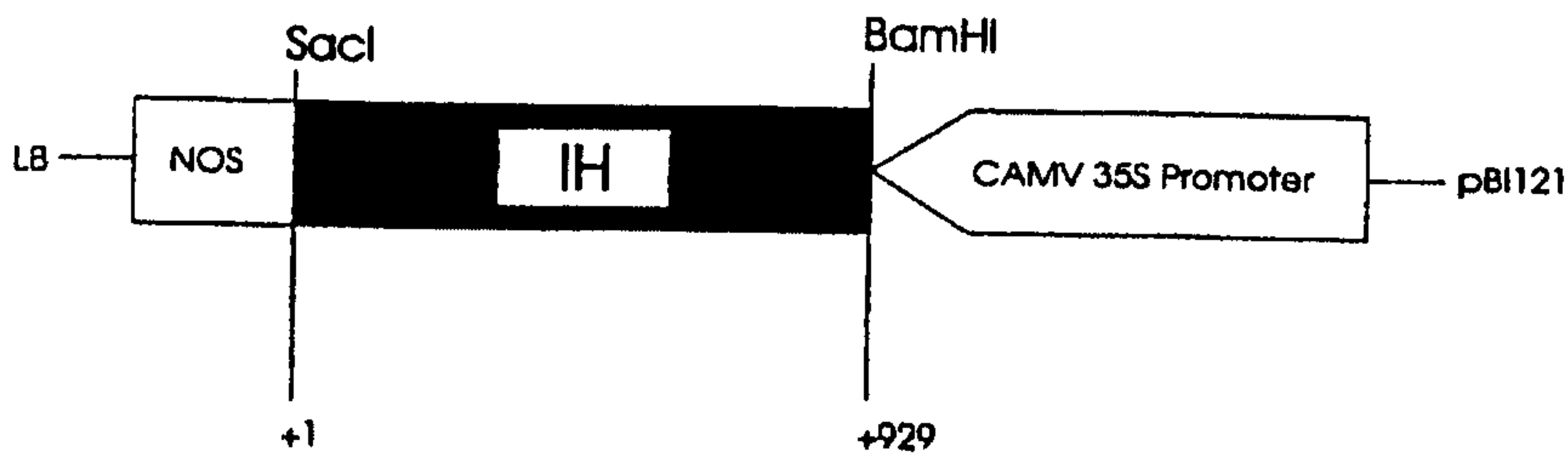


FIG.2

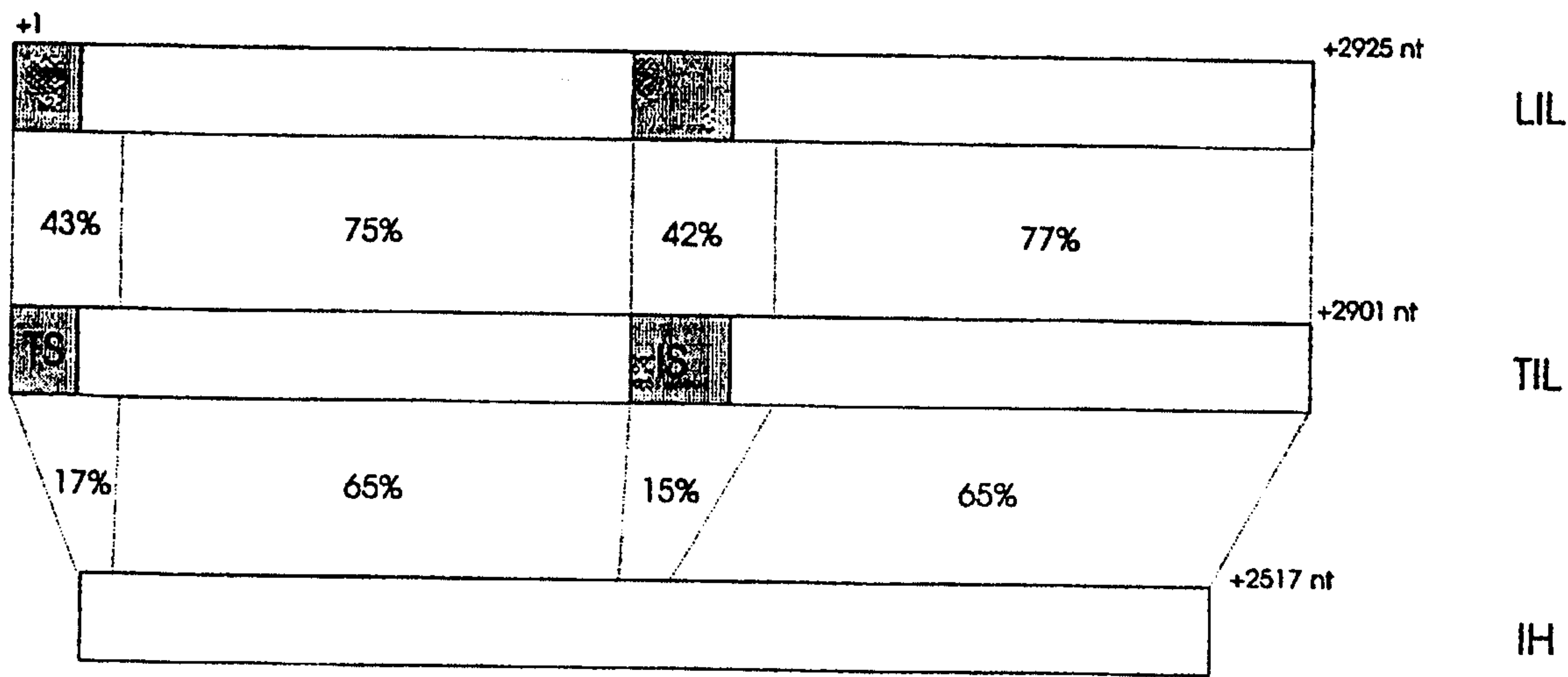
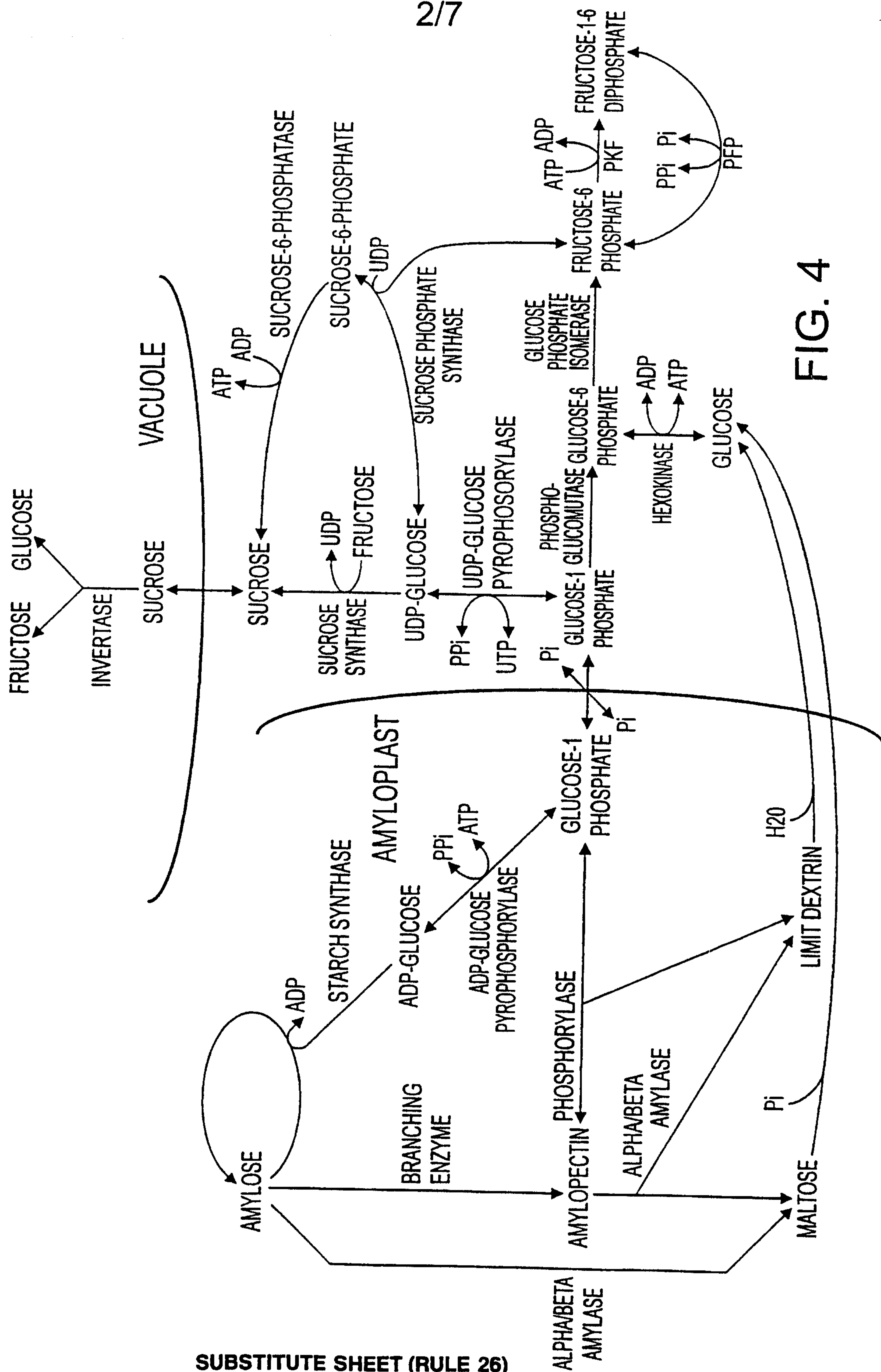


FIG.3

4
G.
L

WO 98/35051

PCT/CA98/00055

3/7

ELPKAYYA-TAESVRDTLIINWNATYEFYEKMNVKQAYYLSMEFLQGRAL	49
ELPKAFFA-TAQSVRDSLLINWNATYDIYEKLNMKQAYYLSMEFLQGRAL	49
EPLQAYYAATADSVDRDLIKQWNDTYLHYDKVNPQTYYSMEYLQGRAL	50
* . * . . * * * . * * * * * . * . * * * * * . * * * * * . * * * * *	
LNAIGNLGLTGPYADALTKLGYSLEDVARQEPDAALGNGGLGRLASCFLD	99
LNAIGNLELTGDFAEALKNLGHNLENVASQEPDAALGNGGLGRLASCFLD	99
TNAVGNLDIHNAYADALNKLGOQLEEVVEQEKDAALGNGGLGRLASCFLD	100
** . * * * * . * * . . * * * * * . * * * * * . * * * * * . * * * * *	
SMATLNYPAWGYGLRYQYGLFKQLITKDGQEEVAENWLEMGNPWEIVRND	149
SLATLNYPAWGYGLRYKYGLFKQRITKDGQEEVAEDWLEIGSPWEVVRND	149
SMATLNLPAWGYGLRYRYGLFKQLITKAGQEEVPEDWLEKFSWEIVRHD	150
* . * * * * * * * * * * . * * * * * * * * * * . * . * * * * . * * * * . * * * * . * * . *	
ISYPVKFYGKVIEGADGRKEWAGGEDITAVAYDVPIPGYKTKTTINLRLW	199
VSYPVKFYGKVSTGSDGKRYWIGGEDIKAVAYDVPIPGYKTRTTISLRLW	199
VVFPIRFFGHVEVLPSGSRKWVGGEVLQALAYDVPIPGYRTKNTNSLRLW	200
. . * . . * . * . . * . . * * * . * . * * * * * * * * . * . . * . * * * *	
TTKLAAEAFDLYAFNNGDH	218
STQVPSADFDLSAFNAGEH	218
EAKASSEDFNLFLFNDGQY	219
. * . * * * . * . .	

FIG.5

4/7

GAAC TTCCCAAGGCATACTATGCA---ACTGCAGAGAGTGTTTCGAGATAC	47
GAGCTCCCTAAGGCATTCTTTGCA---ACAGCTCAAAGTGTTTCGTGATTC	47
GAGCCACTACAAGCATACTATGCTGCTACTGCTGACAGTGTTTCGTGAT-C	49
.* * * .***.***.***. **.* * .*****.*** *	
GCTCATTATAA-ATTGGAATGCCACATACGAATTCTATGAAAAGATGAAT	96
GCTCCTTATTA-ATTGGAATGCTACGTATGATATTTATGAAAAGCTGAAC	96
GCTTGATCAAACAATGGAATGACACCTATCTTCATTATGACAAAGTTAAT	99
*** . * . * * .***** ** ** . . . ***** ** . * .**	
GTAAAGCAGGCATATTACTTGTCTATGGAATTTCTTCAGGGAAGAGCTTT	146
ATGAAGCAAGCGTACTATCTATCCATGGAATTTCTGCAGGGTAGAGCATT	146
CCAAAGCAAACATACTACTTATCAATGGAGTATCTCCAGGGGCGAGCTTT	149
.*****. * .** ** * .** *****. * .*** *****. *****.***	
ACTCAATGCTATTGGTAACTTGGGGCTAAC-CGGACCTTATGCAGATGCT	195
GTTAAATGCAATTGGTAATCTGGAGCTTAC-TGGTGACTTTGCGGAAGCT	195
GACAAATGCAGTTGGAAACTTAGA-CATCCACAATGCATATGCTGATGCT	198
. *****.*****.*** * . * . * . . . * .*****.***.***	
TTAACTAAGCTCGGATACAGTTTAGAGGATGTAGCCAGGCAGGAACCGGA	245
TTGAAAACCTTGGCCACAATCTAGAAAATGTGGCTTCTCAGGAACCAGA	245
TTAAACAAACTGGGTCAGCAGCTTGAGGAGGTCGTTGAGCAGGAAAAAGA	248
.* **.* ** ** * . . * * .** * . .***** .**	
TGCAGCTTTAGGTAATGGAGGTTTAGGAAGACTTGCTTCTTGCTTTCTGG	295
TGCTGCTCTTGGAATGGGGGTTTGGGACGGCTTGCTTCCTGTTTCTTG	295
TGCAGCATTAGGAATGGTGGTTTAGGAAGGCTCGCTTCATGCTTTCTTG	298
.* * . * .** .**.*****.*** * .** ***** ** ** * .*	
ACTCAATGGCGACACTAACTACCCTGCATGGGGCTATGGACTTAGATAC	345
ACTCTTTGGCAACACTAACTACCCAGCATGGGGCTATGGACTTAGGTAC	345
ATTCCATGGCCACATTGAACCTTCCAGCATGGGGTTATGGCTTGAGGTAC	348
* ** .***** *** * .*** . ** .***** ***** ** ** * .**.* **	
CAATATGGCCTTTTCAAACAGCTTATTACAAAAGATGGACAGGAGGAAGT	395
AAGTATGGTTTATTTAAGCAACGGATTACAAAAGATGGTCAGGAGGAGGT	395
AGATATGGACTTTTAAAGCAGCTTATCACAAAGGCTGGGCAAGAAGAAGT	398
..***** * .** **.* **.* ** *****. * **.* **.* **.* **	
TGCTGAAAATTGGCTCGAGATGGGAAATCCATGGGAAATTGTGAGGAATG	445
GGCTGAAGATTGGCTTGAAATTGGCAGTCCATGGGAAAGTTGTGAGGAATG	445
TCCTGAAGATTGGTTGGAGAAATTTAGTCCCTGGGAAATTGTAAGGCATG	448
. *****.***** * **.* . . . * .*** *****.*****.*** ***	
ATATTTTCGTATCCCGTAAAATTCTATGGGAAGGTCATTGAAGGAGCTGAT	495
ATGTTTCATATCCTATCAAATTCTATGGAAAAGTCTCTACAGGATCAGAT	495
ATGTTGTCTTTCCTATCAGGTTTTTTGGTCATGTTGAAGTCCTCCCTTCT	498
.* * .* * * .** * .***. * .** . . . * .** *	

FIG. 6A

5/7

GGGAGGAAGGAATGGGCTGGCGGAGAAGATATAACTGCTGTTGCCTATGA 545
GGAAAGAGGTATTGGATTGGTGGAGAGGATATAAAGGCAGTTGCGTATGA 545
GGCTCGCGAAAATGGGTGGTGGAGAGGTCCTACAGGCTCTTGCATATGA 548
** . * ...*.***. *** *****.*. ** .**.*.***.***.***

TGTCCCAATACCAGGATATAAAACAAAAACAACGATTAACTTCGATTGT 595
TGTTCCCATACCAGGGTATAAGACCAGAACCAATCAGCCTTCGACTGT 595
TGTGCCAATTCCAGGATACAGAACTAAAAACACTAATAGTCTTCGTCTCT 598
*** ** *.*****.*. *..** *.** *.**.*. *. *****. * *

GGACAAC-AAAGCTAGCTGCAGAAGCTTTTGATTTATATGCTTTTAACAA 644
GGTCTAC-ACAGGTTCCATCAGCGGATTTTGATTTATCTGCTTTCAATGC 644
GGGAAGCCAAAGCAAGCT-CTGAGGATTTCAACTTGTTTCTGTTTAATGA 647
..* * ** .. *..* * *** .* **.* * .** ** .

TGGAGACCATGC 656
TGGAGAGCACAC 656
TGGACAGTATGA 659
**** * * .

FIG.6B

WO 98/35051

PCT/CA98/00055

6/7

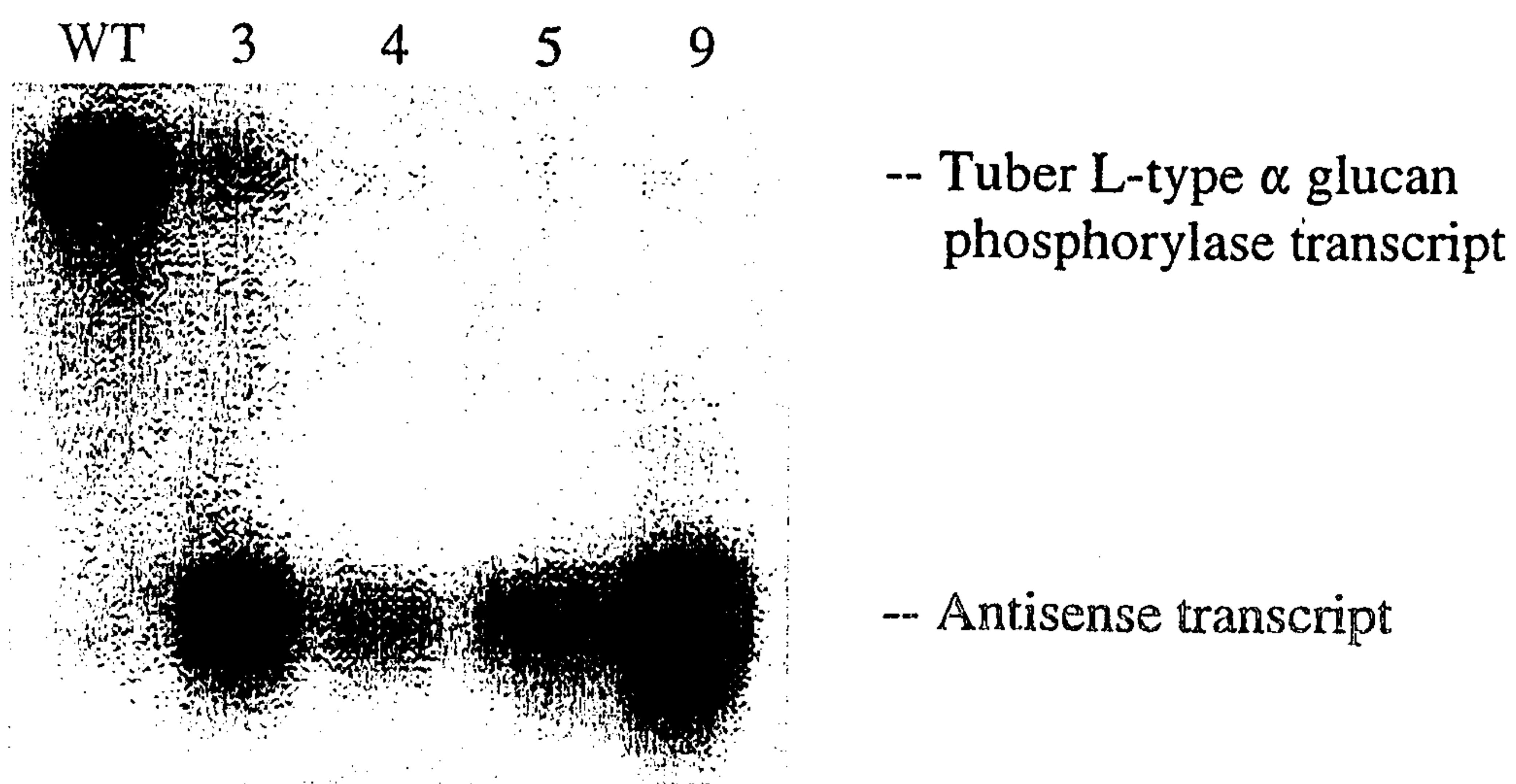


FIG.7

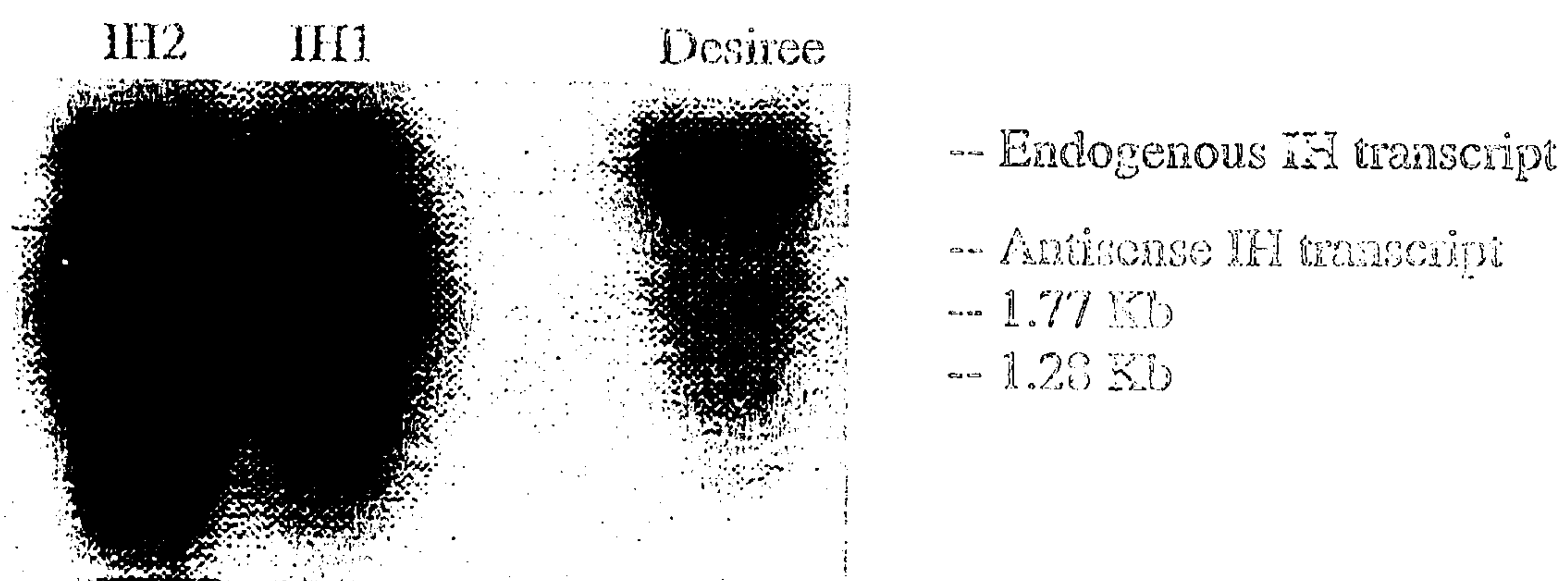


FIG.8

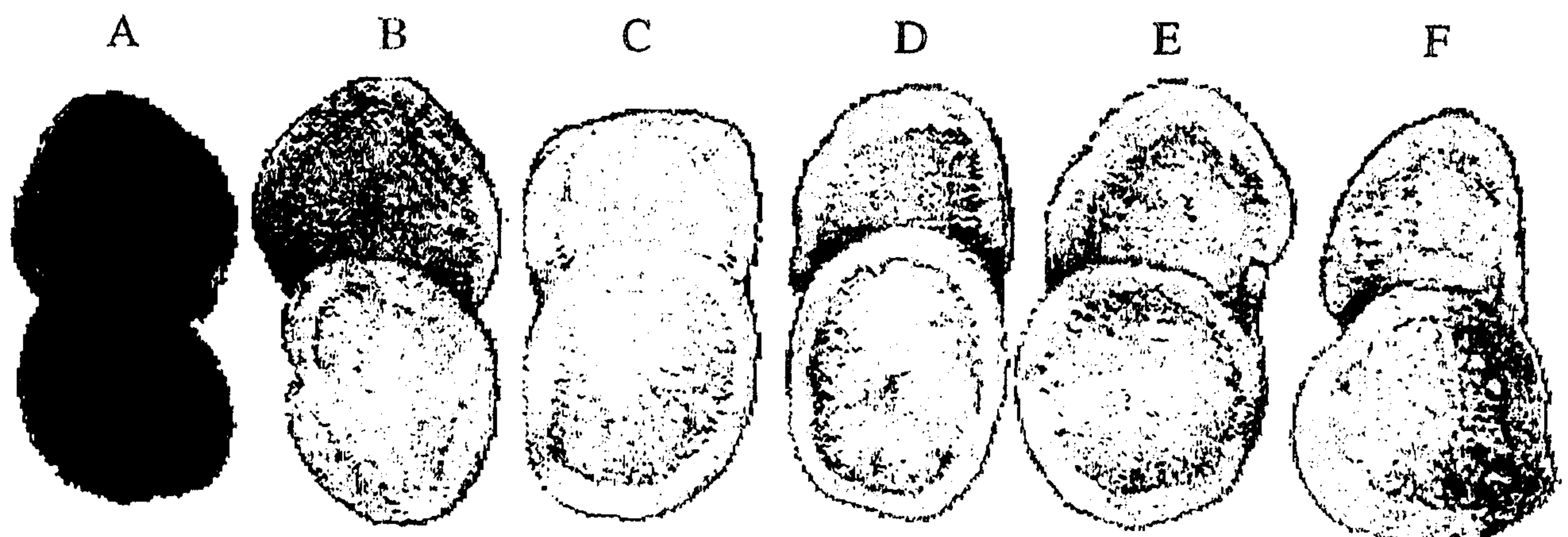


FIG.9

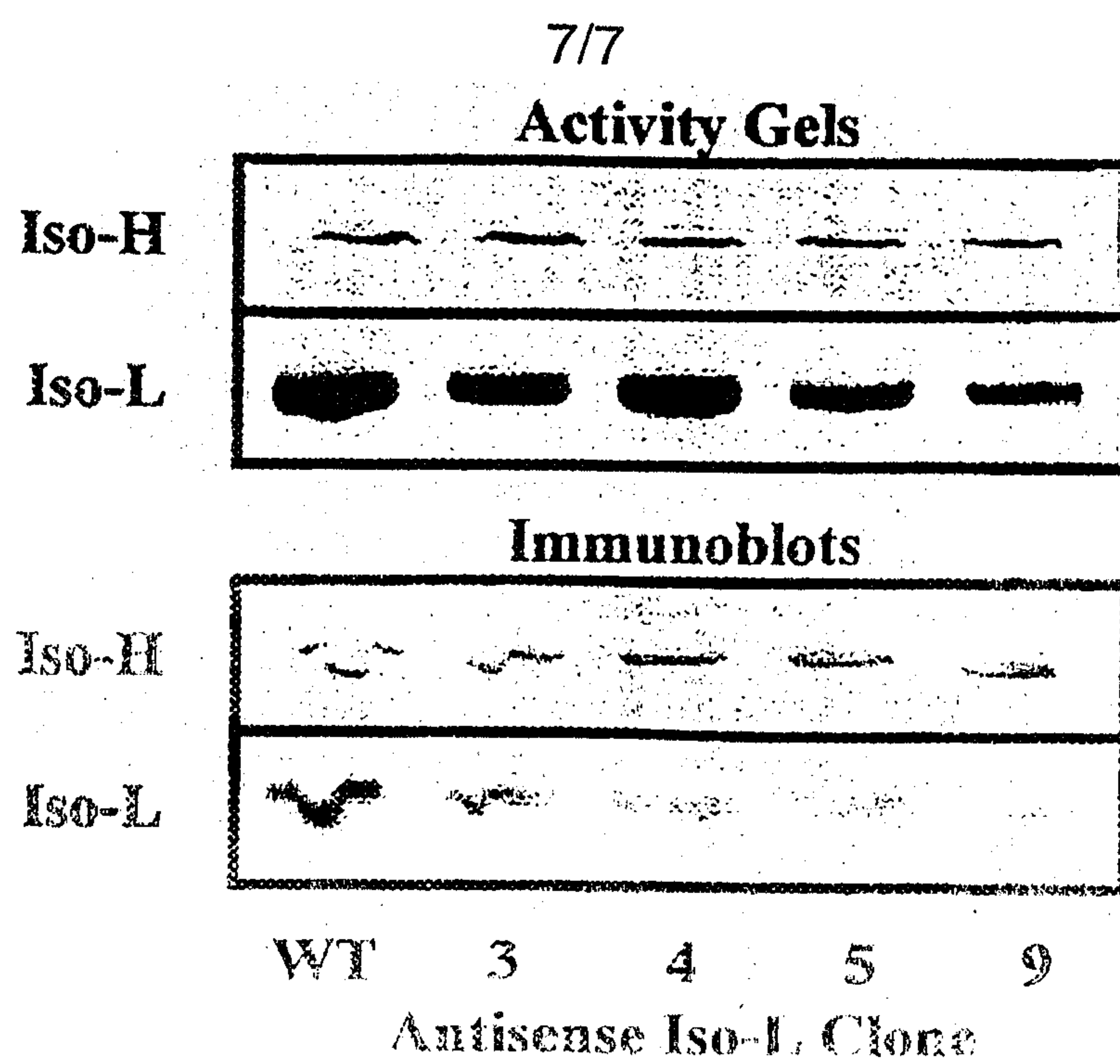


FIG.10

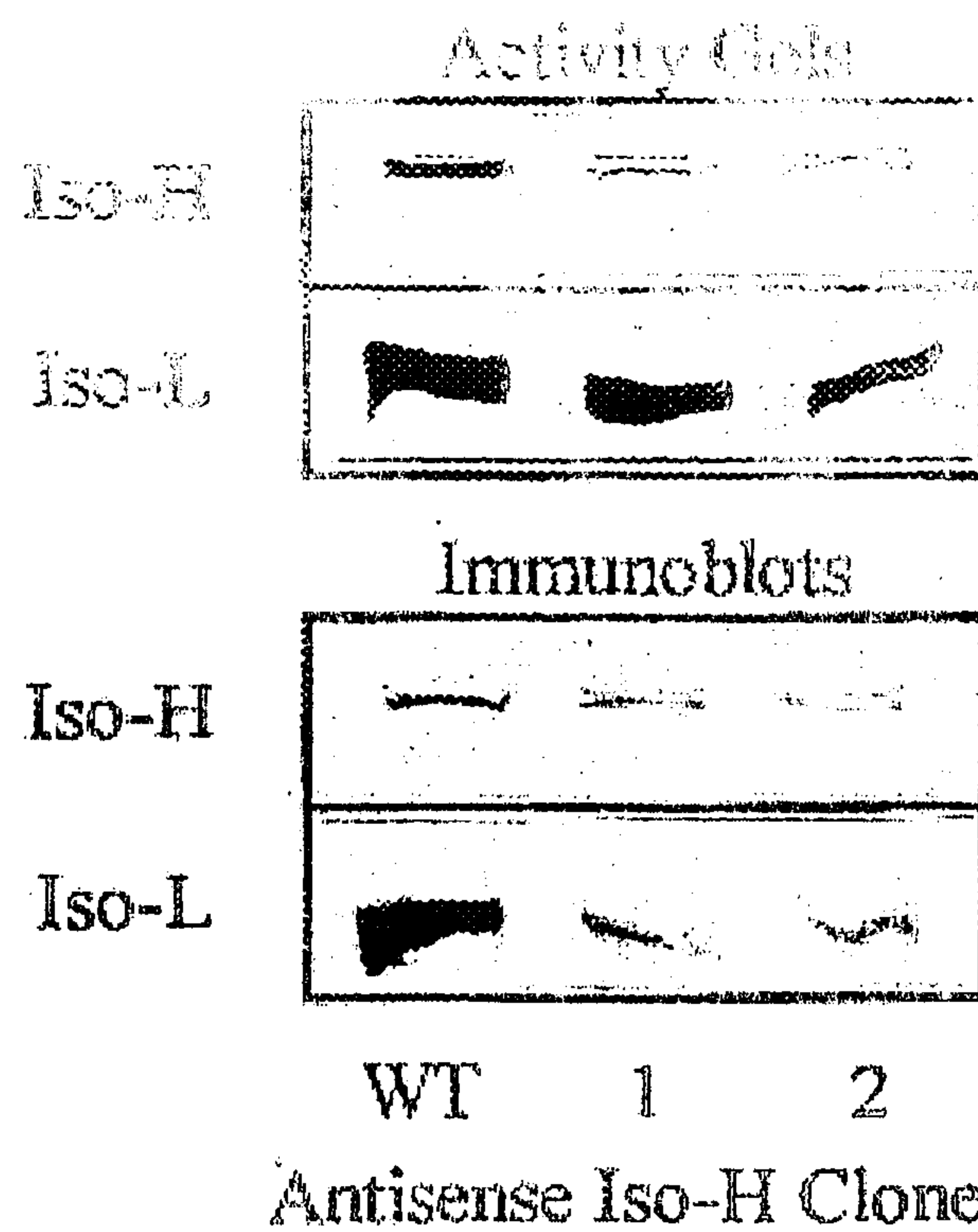


FIG.11

