



(86) Date de dépôt PCT/PCT Filing Date: 2008/12/24
(87) Date publication PCT/PCT Publication Date: 2009/07/02
(85) Entrée phase nationale/National Entry: 2010/06/23
(86) N° demande PCT/PCT Application No.: AU 2008/001906
(87) N° publication PCT/PCT Publication No.: 2009/079714
(30) Priorité/Priority: 2007/12/24 (AU2007907071)

(51) Cl.Int./Int.Cl. *C12N 15/29* (2006.01),
C12N 9/42 (2006.01)
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(54) Titre : POLYSACCHARIDE SYNTHASES (H)
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(57) **Abrégé/Abstract:**

The present invention relates generally to polysaccharide synthases. More particularly, the present invention relates to (1,3;1,4)- β -D-glucan synthases. The present invention provides, among other things, methods for influencing the level of (1,3;1,4)- β -D-glucan produced by a cell and nucleic acid and amino acid sequences which encode (1,3;1,4)- β -D-glucan synthases.

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 July 2009 (02.07.2009)

PCT

(10) International Publication Number
WO 2009/079714 A1

(51) International Patent Classification:
C12N 15/29 (2006.01) *C12N 9/42* (2006.01)

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(21) International Application Number:
PCT/AU2008/001906

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(22) International Filing Date:
24 December 2008 (24.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2007907071 24 December 2007 (24.12.2007) AU

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(54) Title: POLYSACCHARIDE SYNTHASES (H)

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POLYSACCHARIDE SYNTHASES (H)

PRIORITY CLAIM

- 5 The present invention claims priority to Australian provisional patent application 2007907071 the content of which is hereby incorporated by reference.

FIELD OF THE INVENTION

- 10 The present invention relates generally to polysaccharide synthases. More particularly, the present invention relates to (1,3;1,4)- β -D-glucan synthases.

BACKGROUND OF THE INVENTION

- 15 The various tissues of cereal grains have diverse functions during grain development, dormancy and after germination.

For example, the pericarp and seed coat tissues are concerned with the protection of the seed during development and during dormancy. However, by grain maturity, these outer grain tissues have died and the tissue residues consist almost entirely of cell wall residues. The nucellar tissue between the seed coat and the aleurone surface is involved in transfer of nutrients to the developing grain, however, at maturity this tissue has also collapsed to leave cell wall remnants. The thin walled cells of the starchy endosperm of mature grain are dead, but are packed with starch and storage protein. In contrast, the thick-walled, nucleated, aleurone cells are alive at grain maturity, and are packed with protein bodies and lipid droplets. At the interface of the starchy endosperm lies the scutellum, which functions in delivering nutrients to the developing endosperm and, during germination, transfers digestion products of the endosperm reserves to the developing embryo.

The different structure and function of each tissue type in the grain is determined, at least in part, by the cell wall composition of each of these cell types.

Non-cellulosic polysaccharides are key components in the cell walls of cereal grain tissues and include, for example, (1,3;1,4)- β -D-glucans, heteroxylans (mainly arabinoxylans), glucomannans, xyloglucans, pectic polysaccharides and callose. These non-cellulosic polysaccharides usually constitute less than 10% of the overall weight of the grain, but nevertheless are key determinants of grain quality.

Although the precise physical relationships between individual non-cellulosic polysaccharides and other wall components have not been described, it is generally considered that in the wall, microfibrils of cellulose are embedded in a matrix phase of non-cellulosic polysaccharides and protein. Wall integrity is maintained predominantly through extensive non-covalent interactions, especially hydrogen bonding, between the matrix phase and microfibrillar constituents. In the walls of some grain tissues covalent associations between heteroxylans, lignin and proteins are present. The extent of covalent associations between components also varies with the wall type and genotype.

Non-cellulosic polysaccharides, especially heteroxylans and (1,3;1,4)- β -D-glucans, constitute a relatively high proportion of the walls of the aleurone and starchy endosperm, and probably also of the scutellum. In these tissues, cellulose contents are correspondingly lower. The generally low cellulose content of these walls, together with the fact that they contain no lignin, are thought to be related to a limited requirement for structural rigidity of walls in central regions of the grain, and to a requirement to rapidly depolymerize wall components following germination of the grain.

In contrast, in the cell walls of the pericarp-seed coat, which provides a protective coat for the embryo and endosperm and which is not mobilized during germination,

cellulose and lignin contents are much higher and the concentrations of non-cellulosic polysaccharides are correspondingly lower.

(1,3;1,4)- β -D-glucans, also referred to as mixed-linkage or cereal β -glucans, are non-cellulosic polysaccharides which naturally occur in plants of the monocotyledon family Poaceae, to which the cereals and grasses belong, and in related families of the order Poales.

These non-cellulosic polysaccharides are important constituents of the walls of the starchy endosperm and aleurone cells of most cereal grains, where they can account for up to 70% - 90% by weight of the cell walls.

Barley, oat and rye grains are rich sources of (1,3;1,4)- β -D-glucan, whereas wheat, rice and maize have lower concentrations of this polysaccharide. The (1,3;1,4)- β -D-glucans are also relatively minor components of walls in vegetative tissues of cereals and grasses. Although present as a relatively minor component in vegetative tissues (1,3;1,4)- β -D-glucan) is still important in terms of, for example, the digestibility of vegetative tissue by animals and in the use of crop residues for bioethanol production.

(1,3;1,4)- β -D-glucans are important in large-scale food processing activities that include brewing and stockfeed manufacture. Moreover, the non-starchy polysaccharides of cereals, such as (1,3;1,4)- β -D-glucans, have attracted renewed interest in recent years because of their potentially beneficial effects in human nutrition.

However, despite this interest, major gaps remain in our knowledge of the genes and enzymes that control non-cellulosic polysaccharide biosynthesis, including (1,3;1,4)- β -D-glucan biosynthesis, in cereal grain.

(1,3;1,4)- β -D-glucan concentrations in grain are thought to be influenced by both genotype and environment. For example, the concentration of (1,3;1,4)- β -D-glucan in

cereal grains depends on the genotype, the position of the grain on the spike and environmental factors such as planting location, climatic conditions during development and soil nitrogen.

- 5 Identification of the genes encoding (1,3;1,4)- β -D-glucan synthases would be desirable, as this would facilitate modulation of the level of (1,3;1,4)- β -D-glucan produced by a cell, and therefore, allow the qualities of grain or vegetative tissue to be altered. Therefore, in order to enable the modulation of the level of (1,3;1,4)- β -D-glucan in a cell and associated changes in grain or vegetative tissue quality, there is a clear need to
10 identify genes that encode (1,3;1,4)- β -D-glucan synthases.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

15

SUMMARY OF THE INVENTION

- In accordance with the present invention, nucleotide sequences and corresponding amino acid sequences that encode a family of (1,3;1,4)- β -D-glucan synthases are
20 provided. In accordance with the present invention, it has been revealed that (1,3;1,4)- β -D-glucan synthases are encoded by members of the *CslH* gene family.

- As a result of the identification of nucleotide sequences and corresponding amino acid sequences that encode (1,3;1,4)- β -D-glucan synthases, the present invention provides,
25 *inter alia*, methods and compositions for modulating the level and/or activity of (1,3;1,4)- β -D-glucan synthase in a cell and/or modulating the level of (1,3;1,4)- β -D-glucan produced by the cell.

- Therefore, in a first aspect, the present invention provides a method for modulating the
30 level of (1,3;1,4)- β -D-glucan produced by a cell, the method comprising modulating the

level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell.

In some embodiments, the level and/or activity of a (1,3;1,4)- β -D-glucan synthase is modulated by modulating the expression of a *CslH* nucleic acid in the cell. Therefore, in a second aspect, the present invention provides a method for modulating the level and/or activity of a (1,3;1,4)- β -D-glucan synthase in a cell, the method comprising modulating the expression of a *CslH* nucleic acid in the cell.

In some embodiments, the present invention contemplates increasing the level of (1,3;1,4)- β -D-glucan produced by a cell, by expressing, overexpressing or introducing a *CslH* nucleic acid into the cell. Alternatively, in further embodiments the present invention also provides methods for down-regulating expression of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in a cell by knockout or knockdown of a *CslH* nucleic acid in a cell.

The present invention also facilitates the production of (1,3;1,4)- β -D-glucan in a recombinant expression system. For example, (1,3;1,4)- β -D-glucan may be recombinantly produced by introducing a *CslH* nucleic acid under the control of a promoter, into a cell, wherein the cell subsequently expresses a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase and produces (1,3;1,4)- β -D-glucan. Therefore, in a third aspect, the present invention provides a method for producing (1,3;1,4)- β -D-glucan, the method comprising transforming a cell with an isolated *CslH* nucleic acid and allowing the cell to express the isolated *CslH* nucleic acid.

In a fourth aspect, the present invention also provides (1,3;1,4)- β -D-glucan produced according to the method of the third aspect of the invention.

In a fifth aspect, the present invention also provides a cell comprising:
a modulated level and/or activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase relative to a wild type cell of the same taxon; and/or

modulated expression of a *CslH* nucleic acid relative to a wild type cell of the same taxon.

In some embodiments, the cell further comprises a modulated level of (1,3;1,4)- β -D-glucan relative to a wild type cell of the same taxon.

Furthermore, in a sixth aspect, the present invention provides a multicellular structure comprising one or more cells according to the fifth aspect of the invention.

10 The present invention also provides cereal grain comprising one or more cells according to the fifth aspect of the invention. Therefore, in a seventh aspect, the present invention provides a cereal grain comprising a modulated level of (1,3;1,4)- β -D-glucan, wherein the grain comprises one or more cells comprising a modulated level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase and/or modulated expression
15 of a *CslH* nucleic acid.

In an eighth aspect, the present invention also provides flour comprising:

flour produced by the milling of the grain of the seventh aspect of the invention; and

20 optionally, flour produced by the milling of one or more other grains.

As set out above, the present invention is predicated, in part, on the identification and isolation of *CslH* nucleotide sequences and *CslH* amino acid sequences that encode (1,3;1,4)- β -D-glucan synthases.

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Therefore, in a ninth aspect, the present invention provides an isolated *CslH* nucleic acid or a complement, reverse complement or fragment thereof

In a tenth aspect, the present invention provides a genetic construct or vector
30 comprising an isolated nucleic acid molecule of the ninth aspect of the invention.

In an eleventh aspect, the present invention provides a cell comprising the isolated nucleic acid molecule of the ninth aspect of the invention or genetic construct of the tenth aspect of the invention.

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In a twelfth aspect, the present invention provides a multicellular structure comprising one or more of the cells of the eleventh aspect of the invention.

As set out above, the present invention also provides amino acid sequences for *CslH*-
10 encoded (1,3;1,4)- β -D-glucan synthases.

Accordingly, in a thirteenth aspect, the present invention provides an isolated polypeptide comprising an amino acid sequence defining a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide or a fragment thereof.

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In a fourteenth aspect, the present invention provides an antibody or an epitope binding fragment thereof, raised against an isolated *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide as hereinbefore defined or an epitope thereof.

20 Throughout this specification, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

25 Nucleotide and amino acid sequences are referred to herein by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers < 400 > 1 (SEQ ID NO: 1), < 400 > 2 (SEQ ID NO: 2), etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided at the end of the specification.

30

TABLE 1 – Summary of Sequence Identifiers

Sequence Identifier	Sequence
SEQ ID NO: 1	<i>HvCslH1</i> coding region nucleotide sequence
SEQ ID NO: 2	HvCslH1 amino acid sequence
SEQ ID NO: 3	<i>OsCslH1</i> coding region nucleotide sequence
SEQ ID NO: 4	OsCslH1 amino acid sequence
SEQ ID NO: 5	<i>OsCslH2</i> coding region nucleotide sequence
SEQ ID NO: 6	OsCslH2 amino acid sequence
SEQ ID NO: 7	<i>OsCslH3</i> coding region nucleotide sequence
SEQ ID NO: 8	OsCslH3 amino acid sequence
SEQ ID NO: 9	<i>HvCslH1</i> genomic nucleotide sequence
SEQ ID NO: 10	<i>OsCslH1</i> genomic nucleotide sequence
SEQ ID NO: 11	<i>OsCslH2</i> genomic nucleotide sequence
SEQ ID NO: 12	<i>OsCslH3</i> genomic nucleotide sequence
SEQ ID NO: 13	H1F1 primer nucleotide sequence
SEQ ID NO: 14	H1F2 primer nucleotide sequence
SEQ ID NO: 15	HvCslH1cF1 primer nucleotide sequence
SEQ ID NO: 16	HvH1TOPOf primer nucleotide sequence
SEQ ID NO: 17	H1R1 primer nucleotide sequence
SEQ ID NO: 18	H1R2 primer nucleotide sequence
SEQ ID NO: 19	H1R5 primer nucleotide sequence
SEQ ID NO: 20	H1R6 primer nucleotide sequence
SEQ ID NO: 21	H1R7 primer nucleotide sequence
SEQ ID NO: 22	H1R10 primer nucleotide sequence
SEQ ID NO: 23	HvCslH1cR1 primer nucleotide sequence
SEQ ID NO: 24	HvH1TOPOr primer nucleotide sequence
SEQ ID NO: 25	Adaptor 1 primer nucleotide sequence
SEQ ID NO: 26	Adaptor 2 primer nucleotide sequence
SEQ ID NO: 27	AP1 primer nucleotide sequence
SEQ ID NO: 28	AP2 primer nucleotide sequence
SEQ ID NO: 29	Hv18SRTr primer nucleotide sequence

SEQ ID NO: 30	Hv18Sf primer nucleotide sequence
SEQ ID NO: 31	Hv18Sr primer nucleotide sequence
SEQ ID NO: 32	<i>Hv GAPDH</i> Q-PCR forward primer nucleotide sequence
SEQ ID NO: 33	<i>Hv GAPDH</i> Q-PCR reverse primer nucleotide sequence
SEQ ID NO: 34	<i>Hv Cyclophilin</i> Q-PCR forward primer nucleotide sequence
SEQ ID NO: 35	<i>Hv Cyclophilin</i> Q-PCR reverse primer nucleotide sequence
SEQ ID NO: 36	<i>Hv α-Tubulin</i> Q-PCR forward primer nucleotide sequence
SEQ ID NO: 37	<i>Hv α-Tubulin</i> Q-PCR reverse primer nucleotide sequence
SEQ ID NO: 38	<i>Hv HSP70</i> Q-PCR forward primer nucleotide sequence
SEQ ID NO: 39	<i>Hv HSP70</i> Q-PCR reverse primer nucleotide sequence
SEQ ID NO: 40	<i>Hv EL1a</i> Q-PCR forward primer nucleotide sequence
SEQ ID NO: 41	<i>Hv EL1a</i> Q-PCR reverse primer nucleotide sequence
SEQ ID NO: 42	<i>HvCslH1</i> Q-PCR forward primer nucleotide sequence
SEQ ID NO: 43	<i>HvCslH1</i> Q-PCR reverse primer nucleotide sequence
SEQ ID NO: 44	SJ27 primer nucleotide sequence
SEQ ID NO: 45	SJ28 primer nucleotide sequence
SEQ ID NO: 46	SJ72 primer nucleotide sequence
SEQ ID NO: 47	SJ73 primer nucleotide sequence
SEQ ID NO: 48	SJ79 primer nucleotide sequence
SEQ ID NO: 49	SJ75 primer nucleotide sequence
SEQ ID NO: 50	SJ85 primer nucleotide sequence
SEQ ID NO: 51	SJ91 primer nucleotide sequence
SEQ ID NO: 52	SJ163 primer nucleotide sequence
SEQ ID NO: 53	SJ164 primer nucleotide sequence
SEQ ID NO: 54	SJ183 primer nucleotide sequence
SEQ ID NO: 55	SJ204 primer nucleotide sequence
SEQ ID NO: 56	TUB primer nucleotide sequence
SEQ ID NO: 57	TUB2F primer nucleotide sequence
SEQ ID NO: 58	SJ107 primer nucleotide sequence
SEQ ID NO: 59	SJ82 primer nucleotide sequence
SEQ ID NO: 60	SJ94 primer nucleotide sequence

SEQ ID NO: 61	SJ95 primer nucleotide sequence
SEQ ID NO: 62	SJ97 primer nucleotide sequence
SEQ ID NO: 63	SJ93 primer nucleotide sequence
SEQ ID NO: 64	SJ44 primer nucleotide sequence
SEQ ID NO: 65	SJ38 primer nucleotide sequence
SEQ ID NO: 66	SJ96 primer nucleotide sequence
SEQ ID NO: 67	SJ37 primer nucleotide sequence
SEQ ID NO: 68	SJ244 primer nucleotide sequence
SEQ ID NO: 69	<i>HvCslH1</i> (cv. Himalaya) coding region nucleotide sequence
SEQ ID NO: 70	<i>HvCslH1</i> (cv. Himalaya) amino acid sequence
SEQ ID NO: 71	<i>HvCslH1</i> (cv. Himalaya) genomic nucleotide sequence
SEQ ID NO: 72	<i>TaCslH1-1</i> coding region nucleotide sequence
SEQ ID NO: 73	<i>TaCslH1-2</i> coding region nucleotide sequence
SEQ ID NO: 74	<i>TaCslH1-3</i> coding region nucleotide sequence
SEQ ID NO: 75	<i>TaCslH1-1</i> amino acid sequence
SEQ ID NO: 76	<i>TaCslH1-2</i> amino acid sequence
SEQ ID NO: 77	<i>TaCslH1-3</i> amino acid sequence
SEQ ID NO: 78	<i>TaCslH1-1</i> genomic nucleotide sequence
SEQ ID NO: 79	<i>TaCslH1-2</i> genomic nucleotide sequence
SEQ ID NO: 80	<i>TaCslH1-3</i> genomic nucleotide sequence

DESCRIPTION OF EXEMPLARY EMBODIMENTS

It is to be understood that following description is for the purpose of describing particular embodiments only and is not intended to be limiting with respect to the above description.

The present invention is predicated, in part, on the identification of genes which encode biosynthetic enzymes for (1,3;1,4)- β -D-glucans.

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“(1,3;1,4)- β -D-glucans” should be understood to include linear, unbranched

polysaccharides in which β -D-glucopyranosyl monomers are polymerized through both (1 $\rightarrow$ 4)- and (1 $\rightarrow$ 3)-linkages.

The ratio of (1 $\rightarrow$ 4)- to (1 $\rightarrow$ 3)-linkages in naturally occurring (1,3;1,4)- β -D-glucans is generally in the range 2.2-2.6:1, although the ratio may also be outside of this range. For example, in (1,3;1,4)- β -D-glucan from sorghum endosperm the ratio is 1.15:1. The two types of linkages are not arranged in regular, repeating sequences. Single (1 $\rightarrow$ 3)-linkages are separated by two or more (1 $\rightarrow$ 4)-linkages. Regions of two or three adjacent (1 $\rightarrow$ 4)-linkages predominate, but again there is no regularity in the arrangement of these units. The linkage sequence does not depend on preceding linkages further away than two glucose units and follows a second order Markov chain distribution. Moreover, up to 10% of the chain may consist of longer stretches of 5 to 20 adjacent (1 $\rightarrow$ 4)-linkages. Thus, cereal (1,3;1,4)- β -D-glucans may be considered as (1 $\rightarrow$ 3)- β -linked copolymers of cellotriosyl (G4G4G_{Red}), cellotetraosyl (G4G4G4G_{Red}) units and longer (1 $\rightarrow$ 4)- β -D-oligoglucosyl units.

The ratio of tri- to tetra-saccharide units in endogenous (1,3;1,4)- β -D-glucans varies between cereal species. For example, in wheat the ratio is 3.0-4.5:1, in barley 2.9-3.4:1, in rye 2.7:1 and in oats 1.8-2.3:1. Furthermore, the observed ratios may also vary according to the temperature and conditions of (1,3;1,4)- β -D-glucan extraction.

The average molecular masses reported for cereal (1,3;1,4)- β -D-glucans range from 48,000 (DP ~300) to 3,000,000 (DP ~1850), depending on the cereal species, cell wall type, extraction procedure and the method used for molecular mass determination. They are invariably polydisperse with respect to molecular mass and this is illustrated by a weight average to number average molecular mass ratio (M_w/M_n) of 1.18 for barley (1,3;1,4)- β -D-glucan. Certain barley (1,3;1,4)- β -D-glucans are also covalently-associated with small amounts of protein and have estimated molecular masses of up to 40,000,000.

The extractability of (1,3;1,4)- β -D-glucans from walls of cereal grains is a function of their degree of self-association and their association with other wall polysaccharides and proteins. In particular, extractability depends on the molecular mass and linkage distribution in the (1,3;1,4)- β -D-glucan chains. Extensive association with other
5 polymers and very high molecular masses render the (1,3;1,4)- β -D-glucans more difficult to extract from grain.

For example, a portion of the (1,3;1,4)- β -D-glucan from barley, oat and rye flours may be extracted by water at pH 7.0 and 40°C. Further fractions can be solubilised at higher
10 temperatures. The proportion of total (1,3;1,4)- β -D-glucan that is water-soluble at 40°C varies within and between species. For example, waxy (high amylose) barleys have a higher proportion of water-soluble (1,3;1,4)- β -D-glucan than normal barleys. (1,3;1,4)- β -D-glucans extracted from barley at 40°C have a slightly lower tri-/tetrasaccharide ratio (1.7:1) than those extracted at 65°C (2.0:1). Complete extraction of cereal (1,3;1,4)- β -D-
15 glucans from grain requires the use of alkaline extractants such as 4 M NaOH or aqueous Ba(OH)₂, containing NaBH₄ to prevent alkali-induced degradation from the reducing terminus. Alkali-extracted barley (1,3;1,4)- β -D-glucan fractions have higher molecular masses, higher ratios of (1→4): (1→3) linkages, more contiguously linked (1→4)-linked segments and higher tri-: tetra-saccharide ratios than their water-
20 extractable counterparts. Other extractants, such as dimethylsulphoxide, hot perchloric acid, trichloroacetic acid, N-methylmorpholino-N-oxide and dimethylacetamide-LiCl, may also be used to solubilise (1,3;1,4)- β -D-glucans, but these extractants may cause some depolymerisation or degradation of the polymer. Once extracted with hot water or alkali, the (1,3;1,4)- β -D-glucans are often soluble at neutral
25 pH and room temperature. However, upon cooling, (1,3;1,4)- β -D-glucans can aggregate and precipitate.

As mentioned above, the present invention is predicated, in part, on the identification of biosynthetic enzymes, and their encoding genes, that catalyse the synthesis of
30 (1,3;1,4)- β -D-glucan. Such enzymes are referred to herein as “(1,3;1,4)- β -D-glucan

synthases”.

The present invention arises, in part, from an analysis of expressed sequence tag libraries and other sequence databases including cellulose synthase (*CesA*) genes. More particularly, it was noted in these analyses that the *CesA* genes were in fact members of a much larger super-family of genes, which included both the *CesA* genes and the cellulose synthase-like (*Csl*) gene family.

The *Csl* gene families in most vascular plants are very large and have been divided into several groups, designated *CslA* to *CslH*. In *Arabidopsis thaliana* there are 29 known *Csl* genes and in rice about 37. Overall, the *Arabidopsis* genome is believed to contain more than 700 genes involved in cell wall metabolism. However, in general, the specific functions of these genes are poorly understood.

Furthermore, in contrast to the *CesA* genes, it has proved difficult to define the functions of the *Csl* genes. In fact, of the multiple *Csl* genes in higher plants, only the *CslA* and *CslF* groups have been assigned a function.

In accordance with the present invention, it has been revealed that members of the *CslH* gene family encode (1,3;1,4)- β -D-glucan synthases.

As a result of the identification of *CslH* nucleotide sequences, and corresponding amino acid sequences that encode (1,3;1,4)- β -D-glucan synthases, the present invention provides, *inter alia*, methods and compositions for modulating the level and/or activity of (1,3;1,4)- β -D-glucan synthase in a cell and/or modulating the level of (1,3;1,4)- β -D-glucan produced by the cell.

Therefore, in a first aspect, the present invention provides a method for modulating the level of (1,3;1,4)- β -D-glucan produced by a cell, the method comprising modulating the level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell.

The “cell” may be any suitable eukaryotic or prokaryotic cell. As such, a “cell” as referred to herein may be a eukaryotic cell including a fungal cell such as a yeast cell or mycelial fungus cell; an animal cell such as a mammalian cell or an insect cell; or a
5 plant cell. Alternatively, the cell may also be a prokaryotic cell such as a bacterial cell including an *E. coli* cell, or an archaea cell.

In some embodiments, the cell is a plant cell, a vascular plant cell, including a monocotyledonous or dicotyledonous angiosperm plant cell, or a gymnosperm plant
10 cell. In some embodiments the plant is a monocotyledonous plant cell. In some embodiments, the plant is a member of the order Poales. In some embodiments, the monocotyledonous plant cell is a cereal crop plant cell.

As used herein, the term “cereal crop plant” includes members of the Poales (grass
15 family) that produce edible grain for human or animal food. Examples of Poales cereal crop plants which in no way limit the present invention include wheat, rice, maize, millet, sorghum, rye, triticale, oats, barley, teff, wild rice, spelt and the like. However, the term cereal crop plant should also be understood to include a number of non-Poales species that also produce edible grain and are known as the pseudocereals, such
20 as amaranth, buckwheat and quinoa.

In other embodiments, the present invention also contemplates the use of other monocotyledonous plants, such as other non-cereal plants of the Poales, specifically including pasture grasses such as *Lolium* spp.
25

As set out above, the present invention is predicated, in part, on modulating the level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in a cell.

A “*CslH*-encoded (1,3;1,4)- β -D-glucan synthase” should be regarded as any *CslH*-
30 encoded protein which catalyses the synthesis of (1,3;1,4)- β -D-glucan and, optionally,

catalyses the polymerisation of glucopyranosyl monomers.

In some embodiments, the *CslH*-encoded (1,3;1,4)- β -D-glucan synthase comprises the amino acid sequence set forth in SEQ ID NO: 2 or an amino acid sequence which is at
5 least 50% identical thereto.

In some embodiments the *CslH*-encoded (1,3;1,4)- β -D-glucan synthase comprises at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least
10 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 90.5%, at least 91%, at least 91.5%, at least 92%, at least 92.5%, at least
15 93%, at least 93.5%, at least 94%, at least 94.5%, at least 95%, at least 95.5%, at least 96%, at least 96.5%, at least 97%, at least 97.5%, at least 98%, at least 98.5%, at least 99% at least 99.5% or 100% amino acid sequence identity to SEQ ID NO: 2.

When comparing amino acid sequences, the compared sequences should be compared
20 over a comparison window of at least 100 amino acid residues, at least 200 amino acid residues, at least 400 amino acid residues, at least 800 amino acid residues or over the full length of SEQ ID NO: 2. The comparison window may comprise additions or deletions (i. e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences.
25 Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms such the BLAST family of programs as, for example, disclosed by Altschul *et al.* (*Nucl. Acids Res.* 25: 3389-3402, 1997). A detailed discussion of sequence analysis can be found in Unit 19. 3 of Ausubel *et al.* (*Current Protocols in Molecular Biology*, John Wiley & Sons Inc, 1994-1998, Chapter
30 15,1998).

Examples of additional *CslH*-encoded (1,3;1,4)- β -D-glucan synthases contemplated by the present invention include *CslH*-encoded (1,3;1,4)- β -D-glucan synthase orthologs of SEQ ID NO: 2.

5

For example, barley (*Hordeum vulgare*) orthologs or allelic variants of SEQ ID NO: 2 include, for example, polypeptides comprising the amino acid sequence set forth in SEQ ID NO: 70. Rice (*Oryza sativa*) orthologs of SEQ ID NO: 2 include, for example, polypeptides comprising the amino acid sequences set forth in any of SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8. Wheat (*Triticum aestivum*) orthologs of SEQ ID NO: 2 include, for example, polypeptides comprising the amino acid sequences set forth in SEQ ID NO: 75, SEQ ID NO: 76 and SEQ ID NO: 77.

As referred to herein, modulation of the “level” of the *CslH*-encoded (1,3;1,4)- β -D-glucan synthase should be understood to include modulation of the level of *CslH* transcripts and/or *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptides in the cell. Modulation of the “activity” of the *CslH*-encoded (1,3;1,4)- β -D-glucan synthase should be understood to include modulation of the total activity, specific activity, half-life and/or stability of the *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell.

20

By “modulating” with regard to the level and/or activity of the *CslH*-encoded (1,3;1,4)- β -D-glucan synthase is intended decreasing or increasing the level and/or activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell. By “decreasing” is intended, for example, a 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% reduction in the level or activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell. By “increasing” is intended, for example, a 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 5-fold, 10-fold, 20 fold, 50-fold, 100-fold increase in the level of activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell. “Modulating” also includes introducing a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase into a cell which does not normally express the

30

introduced enzyme, or the substantially complete inhibition of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase activity in a cell that normally has such activity.

- 5 In some embodiments, the level of (1,3;1,4)- β -D-glucan produced by a cell is increased by increasing the level and/or activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell. In another embodiment, the level of (1,3;1,4)- β -D-glucan produced by a cell is decreased by decreasing the level and/or activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell.
- 10 The methods of the present invention contemplate any means known in the art by which the level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in a cell may be modulated. This includes methods such as the application of agents which modulate *CslH*-encoded (1,3;1,4)- β -D-glucan synthase activity in a cell, such as the application of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase agonist or antagonist; the
- 15 application of agents which mimic *CslH*-encoded (1,3;1,4)- β -D-glucan synthase activity in a cell; modulating the expression of a *CslH* nucleic acid which encodes *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell; or effecting the expression of an altered or mutated *CslH* nucleic acid in a cell such that a (1,3;1,4)- β -D-glucan synthase with increased or decreased specific activity, half-life and/or stability is expressed by
- 20 the cell.

In some embodiments, the level and/or activity of a (1,3;1,4)- β -D-glucan synthase is modulated by modulating the expression of a *CslH* nucleic acid in the cell.

- 25 Therefore, in a second aspect, the present invention provides a method for modulating the level and/or activity of a (1,3;1,4)- β -D-glucan synthase in a cell, the method comprising modulating the expression of a *CslH* nucleic acid in the cell.

- 30 As used herein, the term "*CslH* nucleic acid" should be understood to include to a nucleic acid molecule which:

encodes a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase as defined herein; and/or
comprises at least 50% nucleotide sequence identity to the nucleotide sequence
set forth in SEQ ID NO: 1; and/or

5 hybridises to a nucleic acid molecule comprising one or more of the nucleotide
sequence set forth in SEQ ID NO: 1 under stringent conditions.

In some embodiments the *CslH* nucleic acid comprises at least 50%, at least 51%, at
least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%,
at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least
10 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at
least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%,
at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least
85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 90.5%, at
least 91%, at least 91.5%, at least 92%, at least 92.5%, at least 93%, at least 93.5%, at least
15 94%, at least 94.5%, at least 95%, at least 95.5%, at least 96%, at least 96.5%, at least 97%,
at least 97.5%, at least 98%, at least 98.5%, at least 99% at least 99.5% or 100% sequence
identity to SEQ ID NO: 1.

When comparing nucleic acid sequences to SEQ ID NO: 1 to calculate a percentage
20 identity, the compared nucleotide sequences should be compared over a comparison
window of at least 300 nucleotide residues, at least 600 nucleotide residues, at least
1200 nucleotide residues, at least 2400 nucleotide residues or over the full length of
SEQ ID NO: 1. The comparison window may comprise additions or deletions (ie. gaps)
of about 20% or less as compared to the reference sequence (which does not comprise
25 additions or deletions) for optimal alignment of the two sequences. Optimal alignment
of sequences for aligning a comparison window may be conducted by computerized
implementations of algorithms such the BLAST family of programs as, for example,
disclosed by Altschul *et al.* (*Nucl. Acids Res.* 25: 3389-3402, 1997). A detailed discussion
of sequence analysis can be found in Unit 19. 3 of Ausubel *et al.* ("Current Protocols in
30 Molecular Biology" John Wiley & Sons Inc, 1994-1998, Chapter 15,1998).

As set out above, the *CslH* nucleic acid may also comprise a nucleic acid that hybridises to a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 1 under stringent conditions. As used herein, "stringent" hybridisation conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least 30°C. Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Stringent hybridisation conditions may be low stringency conditions, medium stringency conditions or high stringency conditions. Exemplary low stringency conditions include hybridisation with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1x to 2xSSC (20xSSC=3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary medium stringency conditions include hybridisation in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C., and a wash in 0.5x to 1xSSC at 55 to 60°C. Exemplary high stringency conditions include hybridisation in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1xSSC at 60 to 65°C. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours.

Specificity of hybridisation is also a function of post-hybridization washes, and is influenced by the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (*Anal. Biochem.* 138: 267-284, 1984), ie. $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\% \text{ GC}) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, % GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of different degrees of

complementarity. For example, sequences with $\geq 90\%$ identity can be hybridised by decreasing the T_m by about 10°C . Generally, stringent conditions are selected to be lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. For example, high stringency conditions can utilize a hybridization and/or wash at, for example, 1, 2, 3, 4 or 5°C lower than the thermal melting point (T_m); medium stringency conditions can utilize a hybridization and/or wash at, for example, 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at, for example, 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), the SSC concentration may be increased so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (*Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes*, Pt I, Chapter 2, Elsevier, New York, 1993), Ausubel *et al.*, eds. (*Current Protocols in Molecular Biology*, Chapter 2, Greene Publishing and Wiley-Interscience, New York, 1995) and Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY, 1989).

Examples of additional *CslH* nucleic acids contemplated by the present invention include nucleic acids having coding regions which are orthologs of SEQ ID NO: 1.

For example, barley (*Hordeum vulgare*) coding region orthologs or allelic variants of SEQ ID NO: 1 include, for example, nucleic acids comprising the nucleotide sequence set forth in SEQ ID NO: 69. Rice (*Oryza sativa*) coding region orthologs of SEQ ID NO: 1 include, for example, nucleic acids comprising the nucleotide sequence set forth in any of SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7. Wheat (*Triticum aestivum*) coding region orthologs of SEQ ID NO: 1 include, for example, nucleic acids comprising the

nucleotide sequence set forth in SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74.

The *CslH* nucleic acids contemplated by the present invention may also comprise one or more non-translated regions such as 3' and 5' untranslated regions and/or introns.

5 For example, the *CslH* nucleic acids contemplated by the present invention may comprise, for example, mRNA sequences, cDNA sequences or genomic nucleotide sequences

In some specific embodiments, the *CslH* nucleic acid may comprise a genomic
10 nucleotide sequence from an organism which may include one or more non-protein-coding regions and/or one or more introns. Genomic nucleotide sequences which comprise a *CslH* nucleic acid include, for example:

barley (*Hordeum vulgare*) *CslH* genomic nucleotide sequences, for example, as set forth in SEQ ID NO: 9 and/or SEQ ID NO: 71;

15 rice (*Oryza sativa*) *CslH* genomic nucleotide sequences, for example, as set forth in any one or more of SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 12; and/or

wheat (*Triticum aestivum*) *CslH* genomic nucleotide sequences, for example, as set forth in any one or more of SEQ ID NO: 78, SEQ ID NO: 79 and/or SEQ ID NO: 80.

20 As mentioned above, the present invention provides methods for modulating the expression of a *CslH* nucleic acid in a cell. The present invention contemplates any method by which the expression of a *CslH* nucleic acid in a cell may be modulated.

The term "modulating" with regard to the expression of the *CslH* nucleic acid is
25 generally intended to refer to decreasing or increasing the transcription and/or translation of a *CslH* nucleic acid. By "decreasing" is intended, for example, a 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% reduction in the transcription and/or translation of a *CslH* nucleic acid. By "increasing" is intended, for example a 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%,
30 70%, 80%, 90%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold or greater

increase in the transcription and/or translation of a *CslH* nucleic acid. Modulating also comprises introducing expression of a *CslH* nucleic acid not normally found in a particular cell; or the substantially complete inhibition (eg. knockout) of expression of a *CslH* nucleic acid in a cell that normally has such activity.

5

Methods for modulating the expression of a particular nucleic acid molecule in a cell are known in the art and the present invention contemplates any such method. Exemplary methods for modulating the expression of a *CslH* nucleic acid include: genetic modification of the cell to upregulate or downregulate endogenous *CslH* nucleic acid expression; genetic modification by transformation with a *CslH* nucleic acid; administration of a nucleic acid molecule to the cell which modulates expression of an endogenous *CslH* nucleic acid in the cell; and the like.

In some embodiments, the expression of a *CslH* nucleic acid is modulated by genetic modification of the cell. The term “genetically modified”, as used herein, should be understood to include any genetic modification that effects an alteration in the expression of a *CslH* nucleic acid in the genetically modified cell relative to a non-genetically modified form of the cell. Exemplary types of genetic modification contemplated herein include: random mutagenesis such as transposon, chemical, UV or phage mutagenesis together with selection of mutants which overexpress or underexpress an endogenous *CslH* nucleic acid; transient or stable introduction of one or more nucleic acid molecules into a cell which direct the expression and/or overexpression of *CslH* nucleic acid in the cell; site-directed mutagenesis of an endogenous *CslH* nucleic acid; introduction of one or more nucleic acid molecules which inhibit the expression of an endogenous *CslH* nucleic acid in the cell, eg. a cosuppression construct or an RNAi construct; and the like.

In one particular embodiment, the genetic modification comprises the introduction of a nucleic acid into a cell of interest.

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The nucleic acid may be introduced using any method known in the art which is suitable for the cell type being used, for example, those described in Sambrook and Russell (*Molecular Cloning – A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory Press, 2000).

5

In some embodiments of the invention wherein the cell is a plant cell, suitable methods for introduction of a nucleic acid molecule may include: *Agrobacterium*-mediated transformation, microprojectile bombardment based transformation methods and direct DNA uptake based methods. Roa-Rodriguez *et al.* (*Agrobacterium-mediated transformation of plants*, 3rd Ed. CAMBIA Intellectual Property Resource, Canberra, Australia, 2003) review a wide array of suitable *Agrobacterium*-mediated plant transformation methods for a wide range of plant species. Microprojectile bombardment may also be used to transform plant tissue and methods for the transformation of plants, particularly cereal plants, and such methods are reviewed by Casas *et al.* (*Plant Breeding Rev.* 13: 235-264, 1995). Direct DNA uptake transformation protocols such as protoplast transformation and electroporation are described in detail in Galbraith *et al.* (eds.), *Methods in Cell Biology Vol. 50*, Academic Press, San Diego, 1995). In addition to the methods mentioned above, a range of other transformation protocols may also be used. These include infiltration, electroporation of cells and tissues, electroporation of embryos, microinjection, pollen-tube pathway, silicon carbide- and liposome mediated transformation. Methods such as these are reviewed by Rakoczy-Trojanowska (*Cell. Mol. Biol. Lett.* 7: 849-858, 2002). A range of other plant transformation methods may also be evident to those of skill in the art.

25 The introduced nucleic acid may be single stranded or double stranded. The nucleic acid may be transcribed into mRNA and translated into a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase or another protein; may encode for a non-translated RNA such as an RNAi construct, cosuppression construct, antisense RNA, tRNA, miRNA, siRNA, ntRNA and the like; or may act directly in the cell. The introduced nucleic acid may be
30 an unmodified DNA or RNA or a modified DNA or RNA which may include

modifications to the nucleotide bases, sugar or phosphate backbones but which retain functional equivalency to a nucleic acid. The introduced nucleic acid may optionally be replicated in the cell; integrated into a chromosome or any extrachromosomal elements of the cell; and/or transcribed by the cell. Also, the introduced nucleic acid may be
5 either homologous or heterologous with respect to the host cell. That is, the introduced nucleic acid may be derived from a cell of the same species as the genetically modified cell (ie. homologous) or the introduced nucleic may be derived from a different species (ie. heterologous). The transgene may also be a synthetic transgene.

10 In one particular embodiment, the present invention contemplates increasing the level of (1,3;1,4)- β -D-glucan produced by a cell, by expressing, overexpressing or introducing a *CslH* nucleic acid into the cell.

By identifying *CslH* nucleotide sequences which encode (1,3;1,4)- β -D-glucan synthases,
15 in further embodiments, the present invention also provides methods for down-regulating expression of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in a cell.

For example, the identification of *CslH* genes as encoding (1,3;1,4)- β -D-glucan synthases facilitates methods such as knockout or knockdown of a *CslH*-encoded
20 (1,3;1,4)- β -D-glucan synthase in a cell using methods such as:

insertional mutagenesis of a *CslH* nucleic acid in a cell, including knockout or knockdown of a *CslH* nucleic acid in a cell, by homologous recombination with a knockout construct (for an example of targeted gene disruption in plants see Terada *et al.*, *Nat. Biotechnol.* 20: 1030-1034, 2002);

25 post-transcriptional gene silencing (PTGS) or RNAi of a *CslH* nucleic acid in a cell (for review of PTGS and RNAi see Sharp, *Genes Dev.* 15(5): 485-490, 2001; and Hannon, *Nature* 418: 244-51, 2002);

transformation of a cell with an antisense construct directed against a *CslH* nucleic acid (for examples of antisense suppression in plants see van der Krol *et al.*,
30 *Nature* 333: 866-869; van der Krol *et al.*, *BioTechniques* 6: 958-967; and van der Krol *et al.*,

Gen. Genet. 220: 204-212);

transformation of a cell with a co-suppression construct directed against a *CslH* nucleic acid (for an example of co-suppression in plants see van der Krol *et al.*, *Plant Cell* 2(4): 291-299);

5 transformation of a cell with a construct encoding a double stranded RNA directed against a *CslH* nucleic acid (for an example of dsRNA mediated gene silencing see Waterhouse *et al.*, *Proc. Natl. Acad. Sci. USA* 95: 13959-13964, 1998);

transformation of a cell with a construct encoding an siRNA or hairpin RNA directed against a *CslH* nucleic acid (for an example of siRNA or hairpin RNA mediated gene silencing in plants see Lu *et al.*, *Nucl. Acids Res.* 32(21): e171; doi:10.1093/nar/gnh170, 2004); and/or

insertion of a miRNA target sequence such that it is in operable connection with *CslH* nucleic acid (for an example of miRNA mediated gene silencing see Brown *et al.*, *Blood* 110(13): 4144-4152, 2007).

15

The present invention also facilitates the downregulation of a *CslH* nucleic acid in a cell *via* the use of synthetic oligonucleotides such as siRNAs or microRNAs directed against a *CslH* nucleic acid which are administered to a cell (for examples of synthetic siRNA mediated silencing see Caplen *et al.*, *Proc. Natl. Acad. Sci. USA* 98: 9742-9747, 2001; Elbashir *et al.*, *Genes Dev.* 15: 188-200, 2001; Elbashir *et al.*, *Nature* 411: 494-498, 2001; Elbashir *et al.*, *EMBO J.* 20: 6877-6888, 2001; and Elbashir *et al.*, *Methods* 26: 199-213, 2002).

20

In addition to the examples above, the introduced nucleic acid may also comprise a nucleotide sequence which is not directly related to a *CslH* nucleic acid but, nonetheless, may directly or indirectly modulate the expression of *CslH* nucleic acid in a cell. Examples include nucleic acid molecules that encode transcription factors or other proteins which promote or suppress the expression of an endogenous *CslH* nucleic acid molecule in a cell; and other non-translated RNAs which directly or indirectly promote or suppress endogenous *CslH*-encoded (1,3;1,4)- β -D-glucan

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synthase expression and the like.

In order to effect expression of an introduced nucleic acid in a genetically modified cell, where appropriate, the introduced nucleic acid may be operably connected to one or
5 more control sequences. The term "control sequences" should be understood to include any nucleotide sequences which are necessary or advantageous for the transcription, translation and or post-translational modification of the operably connected nucleic acid or the transcript or protein encoded thereby. Each control sequence may be native or foreign to the operably connected nucleic acid. The control sequences may include,
10 but are not limited to, a leader, polyadenylation sequence, propeptide encoding sequence, promoter, enhancer or upstream activating sequence, signal peptide encoding sequence, and transcription terminator. Typically, a control sequence at least includes a promoter.

15 The term "promoter" as used herein, describes any nucleic acid which confers, activates or enhances expression of a nucleic acid molecule in a cell. Promoters are generally positioned 5' (upstream) to the genes that they control. In the construction of heterologous promoter/gene combinations, it may be desirable to position the promoter at a distance from the gene transcription start site that is approximately the
20 same as the distance between that promoter and the gene it controls in its natural setting, ie. the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function.

A promoter may regulate the expression of an operably connected nucleotide sequence
25 constitutively, or differentially with respect to the cell, tissue, organ or developmental stage at which expression occurs, in response to external stimuli such as physiological stresses, pathogens, or metal ions, amongst others, or in response to one or more transcriptional activators. As such, the promoter used in accordance with the methods of the present invention may include a constitutive promoter, an inducible promoter, a
30 tissue-specific promoter or an activatable promoter.

The present invention contemplates the use of any promoter which is active in a cell of interest. As such, a wide array of promoters which are active in any of bacteria, fungi, animal cells or plant cells would be readily ascertained by one of ordinary skill in the art. However, in some embodiments, plant cells are used. In these embodiments, plant-active constitutive, inducible, tissue-specific or activatable promoters are typically used.

Plant constitutive promoters typically direct expression in nearly all tissues of a plant and are largely independent of environmental and developmental factors. Examples of constitutive promoters that may be used in accordance with the present invention include plant viral derived promoters such as the Cauliflower Mosaic Virus 35S and 19S (CaMV 35S and CaMV 19S) promoters; bacterial plant pathogen derived promoters such as opine promoters derived from *Agrobacterium* spp., eg. the *Agrobacterium*-derived nopaline synthase (*nos*) promoter; and plant-derived promoters such as the rubisco small subunit gene (*rbcS*) promoter, the plant ubiquitin promoter (*Pubi*), the rice actin promoter (*Pact*) and the oat globulin promoter.

“Inducible” promoters include, but are not limited to, chemically inducible promoters and physically inducible promoters. Chemically inducible promoters include promoters which have activity that is regulated by chemical compounds such as alcohols, antibiotics, steroids, metal ions or other compounds. Examples of chemically inducible promoters include: alcohol regulated promoters (eg. see European Patent 637 339); tetracycline regulated promoters (eg. see US Patent 5,851,796 and US Patent 5,464,758); steroid responsive promoters such as glucocorticoid receptor promoters (eg. see US Patent 5,512,483), estrogen receptor promoters (eg. see European Patent Application 1 232 273), ecdysone receptor promoters (eg. see US Patent 6,379,945) and the like; metal-responsive promoters such as metallothionein promoters (eg. see US Patent 4,940,661, US Patent 4,579,821 and US 4,601,978); and pathogenesis related promoters such as chitinase or lysozyme promoters (eg. see US Patent 5,654,414) or PR

protein promoters (eg. see US Patent 5,689,044, US Patent 5,789,214, Australian Patent 708850, US Patent 6,429,362).

5 The inducible promoter may also be a physically regulated promoter which is regulated by non-chemical environmental factors such as temperature (both heat and cold), light and the like. Examples of physically regulated promoters include heat shock promoters (eg. see US Patent 5,447,858, Australian Patent 732,872, Canadian Patent Application 132,4097); cold inducible promoters (eg. see US Patent 6,479,260, US Patent 6,084,08, US Patent 6,184,443 and US Patent 5,847,102); light inducible promoters
10 (eg. see US Patent 5,750,385 and Canadian Patent 132 1563); light repressible promoters (eg. see New Zealand Patent 508103 and US Patent 5,639,952).

“Tissue specific promoters” include promoters which are preferentially or specifically expressed in one or more specific cells, tissues or organs in an organism and/or one or
15 more developmental stages of the organism. It should be understood that a tissue specific promoter may, in some cases, also be inducible.

Examples of plant tissue specific promoters include: root specific promoters such as those described in US Patent Application 2001047525; fruit specific promoters
20 including ovary specific and receptacle tissue specific promoters such as those described in European Patent 316 441, US Patent 5,753,475 and European Patent Application 973 922; and seed specific promoters such as those described in Australian Patent 612326 and European Patent application 0 781 849 and Australian Patent 746032.

25 In some embodiments, the tissue specific promoter is a seed and/or grain specific promoter. Exemplary seed or grain specific promoters include *puroindoline-b* gene promoters (for example see Digeon *et al.*, *Plant Mol. Biol.* 39: 1101-1112, 1999); *Pbf* gene promoters (for example see Mena *et al.*, *Plant J.* 16: 53-62, 1998); *GS1-2* gene promoters (for example see Muhitch *et al.*, *Plant Sci.* 163: 865-872, 2002); glutelin or *Gt1* gene
30 promoters (for example see Okita *et al.*, *J. Biol. Chem.* 264: 12573-12581, 1989; Zheng *et*

al., *Plant J.* 4: 357-366, 1993; Sindhu *et al.*, *Plant Sci.* 130: 189-196, 1997; Nandi *et al.*, *Plant Sci.* 163: 713-722, 2002); *Hor2-4* gene promoters (for example see Knudsen and Müller, *Planta* 195: 330-336, 1991; Patel *et al.*, *Mol. Breeding* 6: 113-123, 2000; Wong *et al.*, *Proc. Natl. Acad. Sci. USA* 99: 16325-16330, 2002); *lipoxygenase 1* gene promoters (for example
 5 see Rouster *et al.*, *Plant J.* 15: 435-440, 1998); *Chi26* gene promoters (for example see Leah *et al.*, *Plant J.* 6: 579-589, 1994); *Glu-D1-1* gene promoters (for example see Lamacchia *et al.*, *J. Exp. Bot.* 52: 243-250, 2001; Zhang *et al.*, *Theor. Appl. Genet.* 106: 1139-1146, 2003); *Hor3-1* gene promoters (for example see Sörensen *et al.*, *Mol. Gen. Genet.* 250: 750-760, 1996; Horvath *et al.*, *Proc. Natl. Acad. Sci. USA* 97: 1914-1919, 2000) and
 10 *Waxy (Wx)* gene promoters (for example see Yao *et al.*, *Acta Phytophysiol. Sin.* 22: 431-436, 1996; Terada *et al.*, *Plant Cell Physiol.* 41: 881-888, 2000; Liu *et al.*, *Transgenic Res.* 12: 71-82, 2003). In one specific embodiment, the seed specific promoter is an endosperm specific promoter.

15 The promoter may also be a promoter that is activatable by one or more defined transcriptional activators, referred to herein as an “activatable promoter”. For example, the activatable promoter may comprise a minimal promoter operably connected to an Upstream Activating Sequence (UAS), which comprises, *inter alia*, a DNA binding site for one or more transcriptional activators.

20

As referred to herein the term “minimal promoter” should be understood to include any promoter that incorporates at least an RNA polymerase binding site and, preferably a TATA box and transcription initiation site and/or one or more CAAT boxes. When the cell is a plant cell, the minimal promoter may be derived from, for
 25 example, the Cauliflower Mosaic Virus 35S (CaMV 35S) promoter. The CaMV 35S derived minimal promoter may comprise, for example, a sequence that corresponds to positions -90 to +1 (the transcription initiation site) of the CaMV 35S promoter (also referred to as a -90 CaMV 35S minimal promoter), -60 to +1 of the CaMV 35S promoter (also referred to as a -60 CaMV 35S minimal promoter) or -45 to +1 of the CaMV 35S
 30 promoter (also referred to as a -45 CaMV 35S minimal promoter).

As set out above, the activatable promoter may comprise a minimal promoter fused to an Upstream Activating Sequence (UAS). The UAS may be any sequence that can bind a transcriptional activator to activate the minimal promoter. Exemplary transcriptional

5 activators include, for example: yeast derived transcription activators such as Gal4, Pdr1, Gcn4 and Ace1; the viral derived transcription activator, VP16; Hap1 (Hach *et al.*, *J Biol Chem* 278: 248-254, 2000); Gaf1 (Hoe *et al.*, *Gene* 215(2): 319-328, 1998); E2F (Albani *et al.*, *J Biol Chem* 275: 19258-19267, 2000); HAND2 (Dai and Cserjesi, *J Biol Chem* 277: 12604-12612, 2002); NRF-1 and EWG (Herzig *et al.*, *J Cell Sci* 113: 4263-4273, 2000);

10 P/CAF (Itoh *et al.*, *Nucl Acids Res* 28: 4291 - 4298, 2000); MafA (Kataoka *et al.*, *J Biol Chem* 277: 49903-49910, 2002); human activating transcription factor 4 (Liang and Hai, *J Biol Chem* 272: 24088 - 24095, 1997); Bcl10 (Liu *et al.*, *Biochem Biophys Res Comm* 320(1): 1-6, 2004); CREB-H (Omori *et al.*, *Nucl Acids Res* 29: 2154 - 2162, 2001); ARR1 and ARR2 (Sakai *et al.*, *Plant J* 24(6): 703-711, 2000); Fos (Szuts and Bienz, *Proc Natl Acad Sci USA*

15 97: 5351-5356, 2000); HSF4 (Tanabe *et al.*, *J Biol Chem* 274: 27845 - 27856, 1999); MAML1 (Wu *et al.*, *Nat Genet* 26: 484-489, 2000).

In some embodiments, the UAS comprises a nucleotide sequence that is able to bind to at least the DNA-binding domain of the GAL4 transcriptional activator. UAS

20 sequences, which can bind transcriptional activators that comprise at least the GAL4 DNA binding domain, are referred to herein as UAS_G. In a particular embodiment, the UAS_G comprises the sequence 5'-CGGAGTACTGTCCTCCGAG-3' or a functional homolog thereof.

25 As referred to herein, a "functional homolog" of the UAS_G sequence should be understood to refer to any nucleotide sequence which can bind at least the GAL4 DNA binding domain and which may comprise a nucleotide sequence having at least 50% identity, at least 65% identity, at least 80% identity or at least 90% identity with the UAS_G nucleotide sequence.

The UAS sequence in the activatable promoter may comprise a plurality of tandem repeats of a DNA binding domain target sequence. For example, in its native state, UAS_G comprises four tandem repeats of the DNA binding domain target sequence. As such, the term “plurality” as used herein with regard to the number of tandem repeats
5 of a DNA binding domain target sequence should be understood to include at least 2, at least 3 or at least 4 tandem repeats.

As mentioned above, the control sequences may also include a terminator. The term “terminator” refers to a DNA sequence at the end of a transcriptional unit which
10 signals termination of transcription. Terminators are 3'-non-translated DNA sequences generally containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. As with promoter sequences, the terminator may be any terminator sequence which is operable in the cells, tissues or organs in which it is intended to be used. Examples of suitable
15 terminator sequences which may be useful in plant cells include: the nopaline synthase (*nos*) terminator, the CaMV 35S terminator, the octopine synthase (*ocs*) terminator, potato proteinase inhibitor gene (*pin*) terminators, such as the *pinII* and *pinIII* terminators and the like.

20 Modulating the level of (1,3;1,4)- β -D-glucan in a cell, by modulating the level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in the cell, has several industrial applications.

For example, (1,3;1,4)- β -D-glucans are known to form viscous solutions. The viscosity-
25 generating properties of soluble cereal (1,3;1,4)- β -D-glucans are critical determinants in many aspects of cereal processing. For example, incompletely degraded (1,3;1,4)- β -D-glucans from malted barley and cereal adjuncts can contribute to wort and beer viscosity and are associated with problems in wort separation and beer filtration (eg. see Bamforth, *Brew. Dig.* 69 (5): 12-16, 1994) Therefore, for example, in some
30 embodiments, the present invention may be applied to reduce the level of (1,3;1,4)- β -D-

glucan in barley grain, by reducing the level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase in one or more cells of the barley grain, to increase its suitability for beer production.

5 Soluble cereal (1,3;1,4)- β -D-glucans are also considered to have antinutritive effects in monogastric animals such as pigs and poultry. The “antinutritive” effects have been attributed to the increased viscosity of gut contents, which slows both the diffusion of digestive enzymes and the absorption of degradative products of enzyme action. This, in turn, leads to slower growth rates. Moreover, in dietary formulations for poultry,
10 high (1,3;1,4)- β -D-glucan concentrations are associated with ‘sticky’ faeces, which are indicative of the poor digestibility of the (1,3;1,4)- β -D-glucans and which may present major handling and hygiene problems for producers. Therefore, in another embodiment, the present invention may be applied to reducing the level of (1,3;1,4)- β -D-glucan in one or more cells of a plant used for animal feed, to improve the suitability
15 of the plant as animal feed.

However, cereal (1,3;1,4)- β -D-glucans are important components of dietary fibre in human and animal diets. As used herein, the term “dietary fibre” should be understood to include the edible parts of plants or analogous carbohydrates that are
20 resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. “Dietary fibre” includes polysaccharides (specifically including (1,3;1,4)- β -D-glucans), oligosaccharides, lignin and associated plant substances. In at least human diets, dietary fibres promote beneficial physiological effects including general bowel health, laxation, blood cholesterol
25 attenuation, and/or blood glucose attenuation.

Humans and monogastric animals produce no enzymes that degrade (1,3;1,4)- β -D-glucans, although there are indications that some depolymerization occurs in the stomach and small intestine, presumably due to the activity of commensal
30 microorganisms. By comparison, the soluble (1,3;1,4)- β -D-glucans and other non-

starchy polysaccharides are readily fermented by colonic micro-organisms and make a small contribution to digestible energy. In contrast to their antinutritive effects in monogastric animals, oat and barley (1,3;1,4)- β -D-glucans at high concentrations in humans have beneficial effects, especially for non-insulin-dependent diabetics, by
5 flattening glucose and insulin responses that follow a meal. High concentrations of (1,3;1,4)- β -D-glucans (eg. 20% w/v) in food have also been implicated in the reduction of serum cholesterol concentrations, by lowering the uptake of dietary cholesterol or resorption of bile acids from the intestine.

10 Therefore, in another embodiment, the present invention may be applied to increasing the dietary fibre content of an edible plant or edible plant part, by increasing the level of (1,3;1,4)- β -D-glucan in the plant, or part thereof. In some embodiments, the edible plant or edible part of a plant is a cereal crop plant or part thereof.

15 (1,3;1,4)- β -D-glucans, in common with a number of other polysaccharides, in particular (1 $\rightarrow$ 3)- β -D-glucans, are also thought to modify immunological responses in humans by a process that is mediated through binding to receptors on cells of the reticuloendothelial system (leucocytes and macrophages). In addition, they may have the capacity to activate the proteins of the human complement pathway, a system that
20 is invoked as a first line of defence before circulating antibodies are produced.

The present invention also facilitates the production of (1,3;1,4)- β -D-glucan in a recombinant expression system. For example, a (1,3;1,4)- β -D-glucan may be recombinantly produced by introducing a *CslH* nucleic acid under the control of a
25 promoter, into a cell, wherein the cell subsequently expresses a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase and produces (1,3;1,4)- β -D-glucan.

A vast array of recombinant expression systems that may be used to express a *CslH* nucleic acid are known in the art. Exemplary recombinant expression systems include:
30 bacterial expression systems such as *E. coli* expression systems (reviewed in Baneyx,

Curr. Opin. Biotechnol. 10: 411-421, 1999; eg. see also *Gene expression in recombinant microorganisms*, Smith (Ed.), Marcel Dekker, Inc. New York, 1994; and *Protein Expression Technologies: Current Status and Future Trends*, Baneyx (Ed.), Chapters 2 and 3, Horizon Bioscience, Norwich, UK, 2004), *Bacillus* spp. expression systems (eg. see *Protein Expression Technologies: Current Status and Future Trends*, *supra*, chapter 4) and *Streptomyces* spp. expression systems (eg. see *Practical Streptomyces Genetics*, Kieser *et al.*, (Eds.), Chapter 17, John Innes Foundation, Norwich, UK, 2000); fungal expression systems including yeast expression systems such as *Saccharomyces* spp., *Schizosaccharomyces pombe*, *Hansenula polymorpha* and *Pichia* spp. expression systems and filamentous fungi expression systems (eg. see *Protein Expression Technologies: Current Status and Future Trends*, *supra*, chapters 5, 6 and 7; Buckholz and Gleeson, *Bio/Technology* 9(11): 1067-1072, 1991; Cregg *et al.*, *Mol. Biotechnol.* 16(1): 23-52, 2000; Cereghino and Cregg, *FEMS Microbiology Reviews* 24: 45-66, 2000; Cregg *et al.*, *Bio/Technology* 11: 905 – 910, 1993); mammalian cell expression systems including Chinese Hamster Ovary (CHO) cell based expression systems (eg. see *Protein Expression Technologies: Current Status and Future Trends*, *supra*, chapter 9); insect cell cultures including baculovirus expression systems (eg. see *Protein Expression Technologies: Current Status and Future Trends*, *supra*, chapter 8; Kost and Condreay, *Curr. Opin. Biotechnol.* 10: 428-433, 1999; *Baculovirus Expression Vectors: A Laboratory Manual* WH Freeman & Co., New York, 1992; and *The Baculovirus Expression System: A Laboratory Manual*, Chapman & Hall, London, 1992); Plant cell expression systems such as tobacco, soybean, rice and tomato cell expression systems (eg. see review of Hellwig *et al.*, *Nat Biotechnol* 22: 1415-1422, 2004); and the like.

Therefore, in a third aspect, the present invention provides a method for producing (1,3;1,4)- β -D-glucan, the method comprising transforming a cell with an isolated *CslH* nucleic acid and allowing the cell to express the isolated *CslH* nucleic acid.

In some embodiments, the cell is a cell from a recombinant expression system as hereinbefore defined.

In a fourth aspect, the present invention also provides (1,3;1,4)- β -D-glucan produced according to the method of the third aspect of the invention.

5 In a fifth aspect, the present invention also provides a cell comprising:

a modulated level and/or activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase relative to a wild type cell of the same taxon; and/or

modulated expression of a *CslH* nucleic acid relative to a wild type cell of the same taxon.

10

In some embodiments, the cell further comprises a modulated level of (1,3;1,4)- β -D-glucan relative to a wild type cell of the same taxon.

In some embodiments, the cell of the fifth aspect of the invention is produced
15 according to the methods of the first or second aspects of the present invention as described herein. In further embodiments, the cell is a plant cell, a monocot plant cell, a Poales plant cell and/or a cereal crop plant cell.

Furthermore, in a sixth aspect, the present invention provides a multicellular structure
20 comprising one or more cells according to the fifth aspect of the invention.

As referred to herein, a "multicellular structure" includes any aggregation of one or more cells. As such, the term "multicellular structure" specifically encompasses tissues, organs, whole organisms and parts thereof. Furthermore, a multicellular structure
25 should also be understood to encompass multicellular aggregations of cultured cells such as colonies, plant calli, suspension cultures and the like.

As mentioned above, in some embodiments of the invention, the cell is a plant cell and as such, the present invention includes a whole plant, plant tissue, plant organ, plant
30 part, plant reproductive material or cultured plant tissue, comprising one or more

plant cells according to the sixth aspect of the invention.

In another embodiment, the present invention provides a cereal crop plant comprising one or more cells according to the fifth aspect of the invention.

5

In a particular embodiment, the present invention provides cereal grain comprising one or more cells according to the fifth aspect of the invention.

Therefore, in a seventh aspect, the present invention provides a cereal grain comprising
10 a modulated level of (1,3;1,4)- β -D-glucan, wherein the grain comprises one or more cells comprising a modulated level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase and/or modulated expression of a *CslH* nucleic acid.

In some embodiments, the grain may have an increased level of (1,3;1,4)- β -D-glucan
15 compared to wild type grain from the same species. In alternate embodiments, the grain may have a decreased level of (1,3;1,4)- β -D-glucan compared to wild type grain from the same species.

In some embodiments wherein the grain is a wheat grain, the wheat grain comprises
20 level of (1,3;1,4)- β -D-glucan of at least 1%, at least 1.1%, at least 1.2%, at least 1.3%, at least 1.4%, at least 1.5%, at least 1.6%, at least 1.7%, at least 1.8% or 1.9% on a fresh weight basis of air dried whole grain.

In an eighth aspect, the present invention also provides flour comprising:

25 flour produced by the milling of the grain of the seventh aspect of the invention; and

optionally, flour produced by the milling of one or more other grains.

As such, the flour produced by the milling of the grain of the seventh aspect of the
30 invention may comprise, for example approximately 10%, 20%, 30%, 40%, 50%, 60%,

70%, 80%, 90% or 100% by weight of the flour of the ninth aspect of the invention.

As referred to herein “milling” contemplates any method known in the art for milling grain, such as those described by Brennan *et al.* (*Manual of Flour and Husk Milling*,
5 Brennan *et al.* (Eds.), AgriMedia, ISBN: 3-86037-277-7).

In some embodiments, the flour produced by the milling of the grain of the seventh aspect of the invention used in the flour comprises an increased level of (1,3;1,4)- β -D-glucan compared to wild type flour.

10

The “flour produced by the milling of one or more other grains” may be flour produced by milling grain derived from any cereal plant, as hereinbefore defined. This component of the flour of the eighth aspect of the invention may, for example, comprise 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% by weight.

15

In some embodiments, the flour produced by the milling of one or more other grains is wheat flour and, therefore, the flour of the eighth aspect of the invention may be particularly suitable for producing bread, cakes, biscuits and the like.

20 As set out above, the present invention is predicated, in part, on the identification and isolation of *CslH* nucleotide sequences and *CslH* amino acid sequences that encode (1,3;1,4)- β -D-glucan synthases.

Therefore, in a ninth aspect, the present invention provides an isolated *CslH* nucleic
25 acid as hereinbefore defined, or a complement, reverse complement or fragment thereof.

In the present invention, “isolated” refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is
30 altered “by the hand of man” from its natural state. For example, an isolated

polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be isolated because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. An “isolated” nucleic acid molecule should also be understood to include a synthetic nucleic acid molecule, including those produced by chemical synthesis using known methods in the art or by *in-vitro* amplification (eg. polymerase chain reaction and the like).

The isolated nucleic acid molecules of the present invention may comprise any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, the isolated nucleic acid molecules of the invention may comprise single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and/or double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the isolated nucleic acid molecules may comprise of triple-stranded regions comprising RNA or DNA or both RNA and DNA. The isolated nucleic acid molecules may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. “Modified” bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, “polynucleotide” embraces chemically, enzymatically, or metabolically modified forms.

As set out above, the present invention also provides fragments of a nucleotide sequence. “Fragments” of a nucleotide sequence should be at least 15, 20, 30, 40, 50, 100, 150, 200, 250, 300, 325, 350, 375, 400, 450, 500, 550, or 600 nucleotides (nt) in length. These fragments have numerous uses that include, but are not limited to, diagnostic probes and primers. Of course, larger fragments, such as those of 601-3000 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of a *CsIH* nucleic acid.

In some embodiments, the fragment may comprise a functional fragment of a *CslH* nucleic acid. That is, the polynucleotide fragments of the invention may encode a polypeptide having (1,3;1,4)- β -D-glucan synthase functional activity as defined herein.

5

In a tenth aspect, the present invention provides a genetic construct or vector comprising an isolated nucleic acid molecule of the ninth aspect of the invention.

The vector or construct may further comprise one or more of: an origin of replication
10 for one or more hosts; a selectable marker gene which is active in one or more hosts; or one or more control sequences which enable transcription of the isolated nucleic acid molecule in a cell.

“Selectable marker genes” include any nucleotide sequences which, when expressed by
15 a cell, confer a phenotype on the cell that facilitates the identification and/or selection of these transformed cells. A range of nucleotide sequences encoding suitable selectable markers are known in the art. Exemplary nucleotide sequences that encode selectable markers include: antibiotic resistance genes such as ampicillin-resistance genes, tetracycline-resistance genes, kanamycin-resistance genes, the AURI-C gene
20 which confers resistance to the antibiotic aureobasidin A, neomycin phosphotransferase genes (eg. *nptI* and *nptII*) and hygromycin phosphotransferase genes (eg. *hpt*); herbicide resistance genes including glufosinate, phosphinothricin or bialaphos resistance genes such as phosphinothricin acetyl transferase encoding genes (eg. *bar*), glyphosate resistance genes including 3-enoyl pyruvyl shikimate 5-phosphate
25 synthase encoding genes (eg. *aroA*), bromoxnil resistance genes including bromoxnil nitrilase encoding genes, sulfonamide resistance genes including dihydropterate synthase encoding genes (eg. *sul*) and sulfonylurea resistance genes including acetolactate synthase encoding genes; enzyme-encoding reporter genes such as GUS and chloramphenicolacetyltransferase (CAT) encoding genes; fluorescent reporter
30 genes such as the green fluorescent protein-encoding gene; and luminescence-based

reporter genes such as the luciferase gene, amongst others.

Furthermore, it should be noted that the selectable marker gene may be a distinct open reading frame in the construct or may be expressed as a fusion protein with the *CslH*-
5 encoded (1,3;1,4)- β -D-glucan synthase polypeptide.

The tenth aspect of the invention extends to all genetic constructs essentially as described herein, which include further nucleotide sequences intended for the maintenance and/or replication of the genetic construct in prokaryotes or eukaryotes
10 and/or the integration of the genetic construct or a part thereof into the genome of a eukaryotic or prokaryotic cell.

In some embodiments, the vector or construct is adapted to be at least partially transferred into a plant cell *via Agrobacterium*-mediated transformation. Accordingly,
15 the vector or construct may comprise left and/or right T-DNA border sequences.

Suitable T-DNA border sequences would be readily ascertained by one of skill in the art. However, the term "T-DNA border sequences" may include substantially homologous and substantially directly repeated nucleotide sequences that delimit a
20 nucleic acid molecule that is transferred from an *Agrobacterium* sp. cell into a plant cell susceptible to *Agrobacterium*-mediated transformation. By way of example, reference is made to the paper of Peralta and Ream (*Proc. Natl. Acad. Sci. USA*, 82(15): 5112-5116, 1985) and the review of Gelvin (*Microbiology and Molecular Biology Reviews*, 67(1): 16-37, 2003).

25

Although in some embodiments, the vector or construct is adapted to be transferred into a plant *via Agrobacterium*-mediated transformation, the present invention also contemplates any suitable modifications to the genetic construct which facilitate bacterial mediated insertion into a plant cell *via* bacteria other than *Agrobacterium* sp.,
30 for example, as described in Broothaerts *et al.* (*Nature* 433: 629-633, 2005).

Those skilled in the art will be aware of how to produce the constructs described herein and of the requirements for obtaining the expression thereof, when so desired, in a specific cell or cell-type under the conditions desired. In particular, it will be known to
5 those skilled in the art that the genetic manipulations required to perform the present invention may require the propagation of a genetic construct described herein or a derivative thereof in a prokaryotic cell such as an *E. coli* cell or a plant cell or an animal cell. Exemplary methods for cloning nucleic acid molecules are described in Sambrook *et al.* (2000, *supra*)

10

In an eleventh aspect, the present invention provides a cell comprising the isolated nucleic acid molecule of the ninth aspect of the invention or genetic construct of the tenth aspect of the invention.

15 The isolated nucleic acid molecule of the tenth or eleventh aspects of the invention or genetic construct of the twelfth aspect of the invention may be introduced into a cell *via* any means known in the art.

The isolated nucleic acid molecule or construct referred to above may be maintained in
20 the cell as a DNA molecule, as part of an episome (eg. a plasmid, cosmid, artificial chromosome or the like) or it may be integrated into the genomic DNA of the cell.

As used herein, the term “genomic DNA” should be understood in its broadest context to include any and all DNA that makes up the genetic complement of a cell. As such,
25 the genomic DNA of a cell should be understood to include chromosomes, mitochondrial DNA, plastid DNA, chloroplast DNA, endogenous plasmid DNA and the like. As such, the term “genomically integrated” contemplates chromosomal integration, mitochondrial DNA integration, plastid DNA integration, chloroplast DNA integration, endogenous plasmid integration, and the like.

30

The isolated nucleic acid molecule may be operably connected to, *inter alia*, a control sequence and/or a promoter such that the cell may express the isolated nucleic acid molecule.

5 The cell may be any prokaryotic or eukaryotic cell. As such, the cell may be a prokaryotic cell such as a bacterial cell including an *E. coli* cell or an *Agrobacterium* spp. cell, or an archaea cell. The cell may also be a eukaryotic cell including a fungal cell such as a yeast cell or mycelial fungus cell; an animal cell such as a mammalian cell or an insect cell; or a plant cell. In a particular embodiment, the cell is a plant cell. In some
10 embodiments, the plant cell is a monocot plant cell, a Poales plant cell, or a cereal crop plant cell.

In a twelfth aspect, the present invention provides a multicellular structure, as hereinbefore defined, comprising one or more of the cells of the eleventh aspect of the
15 invention.

As mentioned above, in some embodiments, the cell is a plant cell and as such, the present invention should be understood to specifically include a whole plant, plant tissue, plant organ, plant part, plant reproductive material, or cultured plant tissue,
20 comprising one or more cells of the eleventh aspect of the invention.

In a further embodiment, the present invention provides a monocot plant, a Poales plant or a cereal crop plant or part thereof, comprising one or more cells of the eleventh aspect of the invention.

25

In some embodiments, the present invention provides cereal grain comprising one or more cells of the eleventh aspect of the invention.

As set out above, the present invention also provides amino acid sequences for *CslH*-
30 encoded (1,3;1,4)- β -D-glucan synthases. Accordingly, in a thirteenth aspect, the present

invention provides an isolated *CslH*-encoded (1,3;1,4)- β -D-glucan synthase as hereinbefore defined, or a fragment thereof.

5 The isolated polypeptides may comprise of amino acids joined to each other by peptide bonds or modified peptide bonds, ie., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The isolated polypeptides of the present invention may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art.

10 Modifications can occur anywhere in the isolated polypeptide, including the peptide backbone, the amino acid side-chains and/or the termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given isolated polypeptide. Also, an isolated polypeptide of the present invention may contain many types of modifications.

15

The polypeptides may be branched, for example, as a result of ubiquitination, and/or they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods.

20

Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, PEGylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance,

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Proteins--Structure And Molecular Properties 2nd Ed., Creighton (ed.), W. H. Freeman and Company, New York, 1993); *Posttranslational Covalent Modification Of Proteins*, Johnson (Ed.), Academic Press, New York, 1983; Seifter *et al.*, *Meth Enzymol* 182: 626-646, 1990); Rattan *et al.*, *Ann NY Acad Sci* 663: 48-62, 1992.).

5

As set out above, the present invention also provides fragments of isolated polypeptides. Polypeptide fragments may be "free-standing" or comprised within a larger polypeptide of which the fragment forms a part or region.

10 The polypeptide fragments can be at least 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length.

In some embodiments, the fragment is a functional fragment and thus comprises (1,3;1,4)- β -D-glucan synthase functional activity. However, even if the fragment does
15 not retain one or more biological functions of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase, other functional activities may still be retained. For example, the fragments may lack *CslH*-encoded (1,3;1,4)- β -D-glucan synthase functional activity but retain the ability to induce and/or bind to antibodies which recognize the complete or mature forms of an isolated *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide. A
20 peptide, polypeptide or protein fragment which has the ability to induce and/or bind to antibodies which recognize the complete or mature forms of the isolated *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide is referred to herein as a "*CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitope".

25 A *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitope may comprise as few as three or four amino acid residues. In some embodiments the epitope may comprise, for example, at least 5, at least 10, at least 20, at least 50, at least 100 or at least 200 amino acid residues. Whether a particular epitope of an isolated *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide retains such immunologic activities can readily be
30 determined by methods known in the art. As such, one *CslH*-encoded (1,3;1,4)- β -D-

glucan synthase polypeptide fragment is a polypeptide comprising one or more *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitopes.

5 A polypeptide comprising one or more *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitopes may be produced by any conventional means for making polypeptides including synthetic and recombinant methods known in the art. In some embodiments, *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitope-bearing polypeptides may be synthesized using known methods of chemical synthesis. For instance, Houghten has described a simple method for the synthesis of large numbers of peptides (Houghten,
10 *Proc. Natl. Acad. Sci. USA* 82: 5131-5135, 1985).

The isolated *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptides and *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitope-bearing polypeptides are useful, for example, in the generation of antibodies that bind to the isolated *CslH*-encoded
15 (1,3;1,4)- β -D-glucan synthase polypeptides of the invention.

Such antibodies are useful, *inter alia*, in the detection and localization of (1,3;1,4)- β -D-glucan synthase polypeptides and in affinity purification of (1,3;1,4)- β -D-glucan synthase polypeptides. The antibodies may also routinely be used in a variety of
20 qualitative or quantitative immunoassays using methods known in the art. For example see Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press 2nd Ed., 1988).

Accordingly, in a fourteenth aspect, the present invention provides an antibody or an
25 epitope binding fragment thereof, raised against an isolated *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide as hereinbefore defined or an epitope thereof.

The antibodies of the present invention include, but are not limited to, polyclonal, monoclonal, multispecific, chimeric antibodies, single chain antibodies, Fab fragments,
30 F(ab') fragments, fragments produced by a Fab expression library and epitope-binding

fragments of any of the above.

The term “antibody”, as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that
5 contain an antigen-binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

10 The antibodies of the present invention may be monospecific, bispecific, trispecific, or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. For example, see PCT publications
15 WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt *et al.*, *J. Immunol.* 147: 60-69, 1991; US Patents 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; and Kostelny *et al.* *J. Immunol.* 148: 1547-1553, 1992).

In some embodiments, the antibodies of the present invention may act as agonists or
20 antagonists of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase. In further embodiments, the antibodies of the present invention may be used, for example, to purify, detect, and target the polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of *CslH*-encoded
25 (1,3;1,4)- β -D-glucan synthase in biological samples. See, e.g., Harlow *et al.*, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2nd ed. 1988).

The term “antibody”, as used herein, should be understood to encompass derivatives that are modified, eg. by the covalent attachment of any type of molecule to the
30 antibody such that covalent attachment does not prevent the antibody from binding to

a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase or an epitope thereof. For example, the antibody derivatives include antibodies that have been modified, eg., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Furthermore, any of numerous chemical modifications may also be made using known techniques. These include specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

10 Antibodies may be generated using methods known in the art.

For example, if *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For example, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde.

20 Animals such as rabbits, rats and mice may be immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 micrograms of peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for example, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

Polyclonal antibodies to a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide or a polypeptide comprising one or more *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitopes can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, for example, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art.

As another example, monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed., 1988) and Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* (Elsevier, NY, 1981). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

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Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. For example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The

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splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Antibody fragments which recognize one or more *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitopes may also be generated by known techniques. For example, Fab and F(ab')₂ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

The antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labelled antigen or antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein.

Examples of phage display methods include those disclosed by Brinkman *et al.* (*J. Immunol. Methods* 182: 41-50, 1995), Ames *et al.* (*J. Immunol. Methods* 184: 177-186, 1995), Kettleborough *et al.* (*Eur. J. Immunol.* 24: 952-958, 1994), Persic *et al.* (*Gene* 187: 9-18,

1997), Burton *et al.* (*Advances in Immunology* 57: 191-280, 1994); PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patents 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

5

After phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax *et al.* (*BioTechniques* 12(6): 864-869, 1992); and Sawai *et al.* (*AJRI* 34:26-34, 1995); and Better *et al.* (*Science* 240: 1041-1043, 1988).

15 Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston *et al.* (*Methods in Enzymology* 203: 46-88, 1991); Shu *et al.* (*Proc. Natl. Acad. Sci. USA* 90: 7995-7999, 1993); and Skerra *et al.* (*Science* 240: 1038-1040, 1988).

20 The present invention is further described by the following non-limiting examples:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows (A) a schematic of the T-DNA of the *HvCslH1::pGBW15* construct used in gain-of-function experiments in *Arabidopsis*. After Gateway cloning, the 3×HA tag was attached at the NH₂-terminal end of the full-length *HvCslH1* ORF. (B) Transcript levels in the leaves of mature *HvCslH1* transgenic T1 plants as determined by Northern blot analysis. Upper panel, X-ray film exposure; lower panel, corresponding ethidium bromide-stained gel. The observed 2.5 kb transcript size corresponds to the expected size of the tagged *HvCslH1* mRNA. (C) 3×HA-tagged *HvCslH1* protein levels in 3-

week-old pooled *HvCslH1* transgenic T₂ lines as determined by Western blot analysis. Thirty micrograms of mixed microsomal membrane protein was loaded per lane and blots probed with the anti-HA antibody. B and C; Numbers refer to transgenic lines, Col-0, wild-type untransformed line. Col-0, lines 8 and 14 from the same blot, all other
 5 lines are from different blots.

Figure 2 shows transmission electron micrographs showing detection of β -glucan in walls of *HvCslH1*-expressing lines with a β -glucan-specific monoclonal antibody (Meikle *et al.*, *Plant J* 5: 1-9, 1994). (A-C) line 8, 16, 11; (D) wild-type Col-0 control; (E)
 10 line 6. A and D show cells of the vascular bundle; B and C, mesophyll cells; E, epidermal cells. Scale bar = 0.5 μ m (A-C, E), 1 μ m (D).

Figure 3 shows HPAEC profiles of oligosaccharides released upon (1,3;1,4)- β -D-glucan endo-hydrolase digestion of alcohol insoluble residue (AIR) prepared from 145 day-old
 15 *Arabidopsis* line 16-1 rosette leaf tissue (—). 16-1 pre-enzyme treatment buffer wash (-----). Barley mature leaf (entire sheath) AIR was used as the positive control sample (.....). G4G3G_R (3-O- β -cellobiosyl D-glucose, DP3) and G4G4G3G_R (3-O- β -cellotriosyl D-glucose, DP4) peaks are indicated.

20 Figure 4 shows transmission electron micrographs showing the detection of the 3 $\times$ HA-tagged *HvCslH1* protein by a gold-labelled anti-HA antibody in sections of high pressure-frozen leaves of *Arabidopsis* transgenic line 11. (A and B) mesophyll cells. G, Golgi body, cw, cell wall, v, vacuole, er, endoplasmic reticulum. Scale bar = 0.5 μ m (A), 0.2 μ m (B). Arrows indicate Golgi-associated vesicle labeling.

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Figure 5 shows *HvCslH1* expression in barley as determined by QPCR and *in situ* PCR analyses. (A) Normalised levels of *HvCslH1* transcript (copies/microlitre cDNA) in a range of barley tissues. Control genes for normalisation were GAPDH, cyclophilin and α -tubulin. (B) Normalised levels of *HvCslH1* transcript in developing endosperm 0-24
 30 days post-pollination. Control genes were GAPDH, α -tubulin and elongation factor-

1a. (C) Normalised levels of *HvCslH1* transcript in 10 day-old first leaf. Control genes were GAPDH, cyclophilin and HSP70. Error bars on QPCR plots indicate standard deviation. (D-F) *In situ* PCR images of the maturing zone of a 7 day-old first leaf using probes for 18S RNA (positive control, D), *HvCslH1* (F) and a negative control (E). Scale
5 bar = 100 μ m.

Figure 6 shows structural features of *HvCslH1*. (A) Exon-intron structure of *HvCslH1*. Black bars indicate exons, the thin black line introns and 5' and 3' UTRs. Numbers above boxes show size of exons, numbers below the line show intron size. Italicised
10 numbers refer to the size of 5' and 3' UTRs, bold-underline, the length of known sequence upstream of the start codon. Numbers are in base pairs. Thick black bars indicate the six consensus trans-membrane domains as predicted by ARAMEMNON (<http://aramemnon.botanik.uni-koeln.de/>). (B) Kyte-Doolittle hydrophobicity plot (Kyte and Doolittle, *J Mol Biol* 157: 105-132, 1982) of *HvCslH1*. A 19 amino acid
15 window with a +1.6 cutoff was used. The six predicted transmembrane domains are indicated by black bars. Numbers refer to amino acids. (C) Predicted membrane topology of *HvCslH1*. NH₂, amino terminal; COOH, carboxy terminal; lumen, interior of ER, Golgi body or vesicle; cyt, cytoplasm, mem, membrane, D,D,D,QXXRW, signature motif of CAZy GT2 family. Sequence of the QXXRW motif in *HvCslH1* is
20 QFKRW.

Figure 7 shows a phylogenetic tree of full-length barley (*Hordeum vulgare*) and rice (*Oryza sativa*) CSLH sequences. *A. thaliana* and poplar (*Populus trichocarpa*) CSLB protein sequences are included because the CSLB family is the most closely related of
25 the CSL families to the CSLH family. The alignment was generated using ClustalX and the in-built distance algorithm with neighbour joining used. The number of bootstrap replicates (from a total of 1,000) supporting each clade is indicated below the internode for that clade. Accession numbers are: *HvCslH1* (FJ459581), *OsCSLH1* (Os10g20090, AC119148), *OsCSLH2* (Os04g35020, AL606632), *OsCSLH3* (Os04g35030, AL606632),
30 *PtCSLB1* (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html; ID no. 572982),

PtCSLB2 (ID no. 684214), AtCSLB1 (At2G32610, NM_128820), AtCSLB2 (At2G32620, NM_128821), AtCSLB3 (At2G32530, NM_179859), AtCSLB4 (At2G32540, NM_128813), AtCSLB5 (At4G15290, NM_117617, AtCSLB6 (At4G15320, NM_117620).

5 Figure 8 shows a partial genomic map of the short arm of chromosome 2H where *HvCslH1* is located. *HvCslH1* and a cluster of four *HvCSLF* genes were mapped to an interval corresponding to 69.2-71.5 Mb on the Steptoe × Morex bin map close to the centromere (indicated by a black circle). *HvCslH1* was placed in bin 8, co-segregating with the wg996 marker. On the Steptoe × Morex reference map, wg996 co-segregates
10 with abc162 and is 2.3 cM south of abc468, the marker that co-segregates with the four *HvCSLF* genes (Burton *et al.*, *Plant Physiol* 146: 1821-1833, 2008). Key markers are shown on the left, their distances from the top of the chromosome in centimorgans (cM) and the LOD (logarithm of the odds to the base 10) score in the malt β-glucan QTL analysis of Han *et al.* (*Theor Appl Genet* 91: 921-927, 1995) are indicated on the right.

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Figure 9 shows (A) HPAEC profiles of oligosaccharides released upon (1,3;1,4)-β-D-glucan endo-hydrolase digestion of AIR prepared from 145 day-old combined leaf and stem material from *Arabidopsis* line 16-2 (—). 16-2 pre-enzyme treatment buffer wash (—). Barley mature leaf (entire sheath) AIR was used as the positive control sample
20 (—). Laminaribiose standard (—). Retention times for maltose (Gα4G) and cellobiose (Gβ4G) are also marked by arrows. (B) MALDI-TOF MS chromatogram of enzyme-digested AIR of sample in A. DP2 (laminaribiose), DP3 (3-O-β-cellobiosyl D-glucose) and DP4 (3-O-β-cellotriosyl D-glucose) peaks are indicated.

25 Figure 10 shows the nucleotide sequence identity, protein sequence identity and protein sequence similarity between CslH sequences derived from Barley (*Hordeum vulgare*) and Rice (*Oryza sativa*).

Figure 11 shows a ClustalW multiple sequence alignment of CslH amino acid
30 sequences derived from Barley (*Hordeum vulgare*) and Rice (*Oryza sativa*).

Figure 12 is a phylogenetic tree showing the relationship of complete CslB, F and H amino acid sequences derived from Barley (*Hordeum vulgare*), Rice (*Oryza sativa*), *Arabidopsis thaliana* and poplar (*Populus trichocarpa*).

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Figure 13 shows transmission electron micrographs illustrating the detection of (1,3;1,4)- β -D-glucan with a (1,3;1,4)- β -D-glucan-specific monoclonal antibody in epidermal cell walls of four transgenic *Arabidopsis* plant lines used as parents in OsCSLF2 x HvCslH1 transgenic plant crosses. HvCslH1 line individuals 15-8-3 and 15-11-7 are shown in panels A and B, respectively, and OsCSLF2 line individuals H37-5 and H17-4-4 in panels C and D, respectively.

Figure 14 shows transmission electron micrographs illustrating the detection of (1,3;1,4)- β -D-glucan with a (1,3;1,4)- β -D-glucan-specific monoclonal antibody in cell walls of progeny resulting from OsCSLF2 x HvCslH1 transgenic plant crosses. An individual from a cross of 15-8-3 x H37-5 is shown in (A), a sib of 15-8-3 x H37-5 (B), 15-8-3 x H37-7 (C), a sib of 15-8-3 x H37-7 (D), 15-8-15 x H37-16 (E and F), 15-11-13 x H37-11 (G) and 15-11-7 x H17-4 (H). Panels A-E, G-H show epidermal cells, panel F, mesophyll cells.

20

Figure 15 shows a vector map of the pGWB15 vector used to express the *CslH* gene in *Arabidopsis*.

Figure 16 shows the DNA sequence and translated amino acid sequence of the *CslH1* gene cDNA from barley cv. Himalaya. The DNA sequence is shown numbered every ten bases and the translated amino acid sequence of the single large open reading frame is shown beneath in single letter form.

Figure 17 shows a comparison of the barley *CslH1* gene cDNA and genomic sequences against the genomic sequences of the three wheat *CslH1* gene homeologs (*TaCslH1-1*, 1-

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2 and 1-3). The DNA sequences of the barley cDNA (top, *HvCslH1* from *cv.* Schooner (SEQ ID NO: 1) and *HvCslH1Him* from *cv.* Himalaya, (SEQ ID NO: 69) and genomic clones (*HvCslH1g* from *cv.* Morex (SEQ ID NO: 9) and *HvCslH1gHim* from *cv.* Himalaya (SEQ ID NO: 71) were aligned with the three wheat sequences (*TaCslH1-1*, 1-2 and 1-3, (SEQ ID NO: 78, 80 and 81, respectively) in BioEdit using the Muscle comparison programme. The alignment position is numbered above the sequences and dashes indicate gaps introduced to optimise the alignment. Nucleotides identical to the wheat genomic sequence (*TaCslH1-1*) are indicated by dots. The exon/intron boundaries are shown in bold in the wheat genomic sequence (*TaCslH1-1*). For reference, the ATG initiation codon of the *CslH* coding region starts at alignment position 98 and the stop codon TAA starts at position 3320, both are underlined.

Figure 18 shows a comparison of the amino acid sequences of the barley *cv.* Himalaya and wheat *CslH1* proteins. The translated amino acid sequences of the barley gene (top, *HvCslH1(Him)*) were aligned with the three wheat sequences (indicated as *TaCslH1-1pro*, 1-2pro and 1-3pro) in BioEdit using the Muscle comparison programme. The alignment position is numbered above the sequences and there is a single dash (indicating a gap) in the barley sequence introduced to optimise the alignment. Amino acids are shown in their single letter form and those identical to the barley sequence (*HvCslH1(Him)*) are indicated by dots.

Figure 19 shows the results of semi quantitative RT-PCR and Q-PCR expression analysis of the barley *cv.* Himalaya *CslH1* gene during coleoptile development. Panel A shows semi quantitative RT-PCR showing the expression pattern of the barley *CslH1* gene during growth of the coleoptile and in young leaf(L), root (R) and mid stage endosperm(E). A constitutively expressed gene (alpha tubulin) is shown as a control. Panel B shows normalized expression levels (Q-PCR) for *HvCslH1* in developing coleoptiles at various times (days) after the initiation of germination.

Figure 20 shows the results of semi quantitative RT-PCR expression analysis of the

barley *CslF* and *CslH1* genes during leaf development. Semi quantitative RT-PCR showing the expression pattern of the barley *CslH1* gene compared to other barley *CslF* genes. A constitutively expressed gene (alpha tubulin) is shown as a control.

5 Figure 21 shows the results of semi quantitative RT-PCR expression analysis of the barley cv. Himalaya and wheat *CslH1* genes during endosperm development. Semi quantitative RT-PCR showing the difference in expression pattern of the *CslH1* gene in the developing endosperm of barley cv. Himalaya gene (upper panel) compared to wheat cv. Westonia (lower panel). DPA = days post anthesis.

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Figure 22 shows the results of quantitative RT-PCR expression analysis of the barley cv. Himalaya and wheat *CslH1* genes during endosperm development. Quantitative real time RT-PCR showing the difference in expression pattern of the barley *CslH1* gene compared to wheat *CslH1* gene in developing endosperm. The Ta0 dpa sample
15 has been set to one and the other expression levels are relative to this.

Figure 23 shows a plasmid map of the plant transformation vector used to express the barley cv. Himalaya *CslH1* genomic sequence under control of the Bx17 promoter. A schematic representation of the plant transformation vector designated
20 pZLBx17HvgH1. The boxes inside the circular plasmid represent various genetic elements: Bx17prom = Bx17 promoter driving expression of the barley *HvCslH1* genomic sequence; Hvg9185_1 = *HvCslH1* genomic clone number 1 isolated with primer pair SJ91 and SJ85; Nos3' = nopaline synthase polyadenylation sequence; NPTII = bacterial kanamycin resistance gene. The position of selected restriction sites is
25 indicated outside of the plasmid map.

Figure 24 shows a plasmid map of the plant selectable marker plasmid conferring kanamycin resistance. A schematic representation of the plant transformation vector designated pCMSTLSneo. The boxes inside the circular plasmid represent various
30 genetic elements: 35Sprom = CaMV 35S promoter driving expression of the plant

selectable marker gene; NPTII = plant kanamycin resistance gene; STLS intron = *Solanum tuberosum* large subunit intron; 35S polyA = CaMV 35S polyadenylation sequence; Amp res = bacterial ampicillin resistance gene. The position of selected restriction sites is indicated outside of the plasmid map.

5

Figure 25 shows the beta glucan contents of single wheat grains from T0 plant line 10 expressing the barley cv. Himalaya *CslH1* gene. Graph showing beta glucan content of individual wheat grains from a T0 line number 10. Beta glucan is given as a percentage of flour weight.

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Figure 26 shows a quantitative RT-PCR expression analysis of *CslH1* genes in empty vector control (208) and transgenic (236) barley. Expression is shown in leaf and developing grain at 7 days after pollination (7D) and 14 days after pollination (14D).

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Figure 27 shows a comparison of the DNA coding sequence and amino acid sequence identity/similarity for barley and wheat *CslH* sequences. HvCslH1 = DNA coding sequence from barley cv. Schooner (SEQ ID NO: 1) and corresponding amino acid sequence (SEQ ID NO: 2); HvCslH1 (Him) DNA coding sequence from barley cv. Himalaya (SEQ ID NO: 69) and corresponding amino acid sequence (SEQ ID NO: 70);

20

TaCslH1-1 = DNA coding sequence from wheat cv. Chinese Spring (SEQ ID NO: 72) and corresponding amino acid sequence (SEQ ID NO: 75); TaCslH1-2 = DNA coding sequence from wheat cv. Chinese Spring (SEQ ID NO: 73) and corresponding amino acid sequence (SEQ ID NO: 76); TaCslH1-3 = DNA coding sequence from wheat cv. Chinese Spring (SEQ ID NO: 74) and corresponding amino acid sequence (SEQ ID NO:

25

77).

EXAMPLE 1

Barley has only one *CSLH* gene

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Candidate *CSLH* genes in barley were initially identified by querying online EST

databases, such as the discontinued Stanford cell wall website, NCBI, HarvEST, GrainGenes, Barley Gene Index and BarleyBase, with rice *CSLH* sequences. All *CSLH*-related ESTs from barley could be aligned into a single contiguous sequence of ~1,500 bp that included the entire 3' untranslated region (UTR) and a region encoding the

5 COOH-terminal 488 (of an expected ~750) amino acid residues of the protein (Table 2). This gene was designated *HvCslH1*. Screening of a barley BAC library with *HvCslH1*-derived probes identified several genomic clones all containing *HvCslH1*, from which the missing 5' end was obtained (data not shown). A 2,430 bp *HvCslH1* cDNA fragment was PCR-amplified, contains a single 2,256 bp ORF, and encodes a protein

10 with a predicted MW of 82.6 kDa and a pI of 7.0 (Figure 6A). Analysis of the conceptual translation of this sequence with ARAMEMNON found between five and nine transmembrane domains (TMDs), with the consensus among the different programs being two NH₂-terminal and four COOH-terminal TMDs (Figure 6B) and both termini of the mature protein predicted to be cytoplasmic. This topology also

15 places the large, central domain containing the D,D,D,QFKRW motif within the cytoplasm (Figure 6C). At the nucleotide level, *HvCslH1* shares 68-74% identity (62-69% amino acid identity) (see Example 6) with the three rice *CSLH* genes (Hazen et al., *Plant Physiol* 128: 336-340, 2002). A phylogenetic reconstruction shows *HvCslH1* to be the likely barley ortholog of *OsCSLH1* (Figure 7). Genetic mapping of *HvCslH1* using a

20 Sloop × Halcyon doubled haploid population (Read et al., *Aust J Agr Res* 54: 1145-1153, 2003) showed that *HvCslH1* is on the short arm of chromosome 2H, approximately 1.5 cM from a cluster of four *HvCSLF* genes (*HvCSLF3*, 4, 8, 10) that Burton et al. (*Plant Physiol* 146: 1821-1833, 2008) reported was within a major QTL controlling β-glucan content in ungerminated barley grain (Han et al., *Theor Appl Genet* 91: 921-927, 1995;

25 Figure 8).

Supporting Information

A BAC library screening was employed to obtain a complete set of full-length *HvCslH* family members. BAC filters containing 6.5 equivalents of the barley genome (cv.

30 Morex) were screened and three clearly positive clones identified (data not shown).

When a blot of BAC DNA from these clones digested with *Hind III* was probed, the same three clones, 3-5-10, 3-7-3 and 3-7-8, were verified as being positive. The digestion pattern of BACs 3-5-10 and 3-7-8 appeared identical and many bands were common to BAC 3-7-3, indicating that all 3 BACs cover identical or very similar regions of the barley genome. When a genomic DNA blot was hybridised with the same probe, single bands were observed in lanes digested with *Hind III*, *Eco RI* or *Eco RV*, corroborating the BAC digestion results. As all *HvCslH* ESTs are also derived from a single gene (Table 2), these data strongly suggest that there is only one *CSLH* gene in the barley genome.

10

An adaptor primer PCR method (Siebert et al., *Nucl Acids Res* 23: 1087-1088, 1995) was used to identify the 5' end of *HvCslH1*. DNA was isolated from BACs 3-5-10 and 3-7-3, digested with a range of restriction enzymes producing blunt-ended DNA fragments to which adaptors were ligated. Nested PCR was then performed with adaptor- and *HvCslH1*-specific primers (Table 3) in order to amplify fragments containing the 5' end of the gene. Amplification of BAC 3-7-3 DNA digested with *Nru I* using primers AP2 and H1R6 successfully amplified a 1.3 kbp fragment that contained all but ~20 amino acids of the N-terminal sequence. Direct sequencing of BAC 3-7-3 DNA with the H1R10 primer, an antisense primer designed to anneal near the 5' end of the 1.3 kb fragment, enabled the remainder of the open reading frame plus 748 bp of upstream sequence to be identified. As predicted from earlier results, the sequence obtained from BAC 3-5-10 was identical to BAC 3-7-3, confirming that there is only one *CSLH* gene within the barley genome.

20

TABLE 2 – List of ESTs derived from *HvCslH1*. ESTs are listed in order of alignment 5' to 3'.

Accession no. (5' to 3')	Cultivar	Source tissue
CA013594	Barke	early endosperm, 0-16 hours after imbibition
BJ470984	Haruna Nijo adult	top three leaves at heading stage

25

BJ471865	Haruna Nijo adult	top three leaves at heading stage
BJ473288	Haruna Nijo adult	top three leaves at heading stage
BJ452043	Akashinriki	vegetative stage leaves
BJ471909	Haruna Nijo adult	top three leaves at heading stage
AV932844	Haruna Nijo adult	top three leaves at heading stage
BJ469514	Haruna Nijo adult	top three leaves at heading stage
AV933503	Haruna Nijo adult	top three leaves at heading stage
AV933012	Haruna Nijo adult	top three leaves at heading stage
AV932649	Haruna Nijo adult	top three leaves at heading stage
AV932549	Haruna Nijo adult	top three leaves at heading stage
BJ475824	Haruna Nijo adult	top three leaves at heading stage
BJ476822	Haruna Nijo adult	top three leaves at heading stage
AV934650	Haruna Nijo adult	top three leaves at heading stage
BJ477472	Haruna Nijo adult	top three leaves at heading stage
AV935479	Haruna Nijo adult	top three leaves at heading stage
AV935951	Haruna Nijo adult	top three leaves at heading stage
AV832539	Akashinriki	vegetative stage leaves
AV936586	Haruna Nijo adult	top three leaves at heading stage
CB881459	Barke	male inflorescences (approx. 2 mm in size), green anther stage
AV934667	Haruna Nijo adult	top three leaves at heading stage
BJ475744	Haruna Nijo adult	top three leaves at heading stage
BJ459600	Akashinriki	vegetative stage leaves
AV832391	Akashinriki	vegetative stage leaves

TABLE 3 – Primers used in cloning and amplifying *HvCslH1* and *in situ* PCR analysis

Gene	Primer name	Primer sequence (5' to 3')	Technique
<i>HvCslH1</i>	H1F1	TTGACCGGACAACGGATCC (SEQ ID NO: 13)	DNA blot analysis, BAC screening, gene mapping, <i>in situ</i> PCR
<i>HvCslH1</i>	H1F2	CTGGAGATACTCATCAGC (SEQ ID NO: 14)	Northern blotting,

			transgenic plant genomic DNA PCR screening
<i>HvCslH1</i>	HvCslH1cF1	TCGAGCGGTTGTTGCTTGTG (SEQ ID NO: 15)	HvCslH1 cDNA amplification
<i>HvCslH1</i>	HvH1TOPOf	CACCATGGCGGGCGGCAAGAAGCTG (SEQ ID NO: 16)	Binary vector construction
<i>HvCslH1</i>	H1R1	CGTCACCGGGATGAAAAC (SEQ ID NO: 17)	DNA blot analysis, BAC screening, genome walking PCR, <i>in situ</i> PCR
<i>HvCslH1</i>	H1R2	TGACGCTCCACGGCATTTC (SEQ ID NO: 18)	<i>In situ</i> PCR (priming cDNA synthesis)
<i>HvCslH1</i>	H1R5	GGCTGGCCATCGAAATATTG (SEQ ID NO: 19)	BAC screening, genome walking PCR, gene mapping, <i>in situ</i> PCR
<i>HvCslH1</i>	H1R6	GAGCGTTGGTCATCACGG (SEQ ID NO: 20)	Genome walking PCR
<i>HvCslH1</i>	H1R7	CACATCGCGTGTAGGGC (SEQ ID NO: 21)	Genome walking PCR
<i>HvCslH1</i>	H1R10	CACTTGCCGTTTCATGTTG (SEQ ID NO: 22)	Adaptor ligation PCR, BAC sequencing
<i>HvCslH1</i>	HvCslH1cR1	CCTGCTTGAGTCTTCGTTACATGTTC (SEQ ID NO: 23)	HvCslH1 cDNA amplification
<i>HvCslH1</i>	HvH1TOPOr	CGCTTCCAATATTTGATG (SEQ ID NO: 24)	Binary vector construction, Northern blotting, transgenic plant genomic DNA PCR screening

Generic	Adaptor 1	CTAATACGACTCACTATAGGGCTCGAG CGGCCGCCCCGGGCAGGT (SEQ ID NO: 25)	Adaptor ligation PCR
Generic	Adaptor 2	P-ACCTGCCC-NH ₂ (SEQ ID NO: 26)	Adaptor ligation PCR
Generic	AP1	GGATCCTAATACGACTCACTATAGGGC (SEQ ID NO: 27)	Adaptor ligation PCR
Generic	AP2	AATAGGGCTCGAGCGGC (SEQ ID NO: 28)	Adaptor ligation PCR
18S rRNA	Hv18SRT _r	GTTTCAGCCTTGCGACCATACT (SEQ ID NO: 29)	<i>In situ</i> PCR (priming cDNA synthesis)
18S rRNA	Hv18S _f	GGTAATTCCAGCTCCAAT (SEQ ID NO: 30)	<i>In situ</i> PCR
18S rRNA	Hv18S _r	GTTTATGGTTGAGACTAG (SEQ ID NO: 31)	<i>In situ</i> PCR

EXAMPLE 2

Expression of *HvCslH1* in *Arabidopsis* results in deposition of (1,3;1,4)-β-D-glucan

- 5 For heterologous expression in *Arabidopsis*, the *HvCslH1* ORF was cloned into the Gateway-enabled binary vector pGWB15 (Nakagawa *et al.*, *J Biosci Bioeng* 104: 34-41, 2007; Figure 15), which placed *HvCslH1* under the control of the CaMV 35S promoter and added a 3×HA epitope tag to the encoded protein's NH₂-terminal end (Figure 1A). Initial selection of transformed *Arabidopsis* seeds identified a number of putative
- 10 transgenic seedlings which PCR confirmed contained *HvCslH1*. RNA blot analysis of these T₁ plants showed that approximately 90% accumulated *HvCslH1* transcripts in rosette leaves (Figure 1B). Western blotting using an anti-HA tag antibody was used to detect HvCslH1 protein in these lines (Figure 1C). A mixed microsomal membrane fraction (50,000-100,000 ×g pellet) was prepared from pooled three-week old
- 15 kanamycin-resistant T₂ seedlings. Western blotting with the anti-HA antibody showed that only four of the 28 lines containing *HvCslH1* transcripts accumulated a polypeptide of the expected size (~90 kDa) (Figure 1C). Occasionally proteins of higher

and lower molecular mass were also detected (e.g. lane 11). The 90 kDa-protein was not observed in total protein extracts (data not shown) or in mixed-membrane fractions prepared from untransformed *Arabidopsis* plants (Figure 1C, Col-0 lane). It is not known why HA-tagged HvCslH1 accumulated in only some of the plant lines that expressed *HvCslH1* mRNA or why no correlation was apparent between HvCslH1 protein levels and either *HvCslH1* transcript levels (compare Figs. 1B and C) or with the number of *HvCslH1* transgenes present in a plant (data not shown), although this has been previously observed (Burton *et al.*, *Science* 311: 1940-1942, 2006) Lines 8, 11, 16 and 24, which expressed the HA-tagged HvCslH1, and line 6, which did not express detectable levels of the protein (control), were selected for subsequent experimental work.

Immuno-EM was used to determine whether the walls of the transgenic *Arabidopsis* plants accumulated detectable levels of β -glucan. Thin sections of mature leaf pieces from self-pollinated progeny of lines 8, 11, 16, 24 and 6 (T_2 generation) were probed with a monoclonal antibody specific for β -glucan (Meikle *et al.*, *Plant J* 5: 1-9, 1994), followed by detection using a secondary antibody conjugated to 18 nm gold particles. Gold particles were clearly evident in walls of the HA-tagged HvCslH1 positive lines 8, 11 and 16 (Figure 2A, C, B, respectively) but not in the walls of either line 24, which also expressed HvCslH1 (data not shown), or line 6 (Figure 2E) which had no detectable HvCslH1 protein. Each positive transgenic line showed a different pattern of tissue labeling. In line 8, patchy labeling was observed in the walls of epidermal cells and occasionally in xylem walls (Figure 2A) whereas in line 11, epidermal and vascular tissue walls were only lightly labelled, but heavier (albeit more patchy) labeling was observed in mesophyll walls (Figure 2C). Broadly distributed, light labeling was present in all walls of the mature leaf of line 16 (Figure 2B). Irregular and inconsistent patterns of ectopic polysaccharide production by transgenic *Arabidopsis* lines expressing genes driven by the “constitutively”-expressed 35S promoter have been observed previously (Burton *et al.*, 2006, *supra*). No labeling was seen in leaf sections of untransformed *Arabidopsis* (Figure 2D). Reduced levels of labeling were

seen in leaf sections of transgenic plants that had been pre-incubated with a *Bacillus subtilis* endo-hydrolase which specifically hydrolyses this β -glucan (Burton *et al.*, 2006, *supra*; data not shown).

5 To provide biochemical confirmation of the presence of β -glucan in transgenic *Arabidopsis* walls and to examine the fine structure of the nascent β -glucan, leaf and/or stem material was pooled from the self-pollinated T₃ and T₄ progeny of lines derived from plants 8, 11 and 16. These lines were homozygous for the *HvCslH1* transgene. Because β -glucan was found to accumulate with plant age, samples were taken when
10 plants were in senescence. Walls were prepared and digested with a (1,3;1,4)- β -glucan-specific endo-hydrolase and the released oligosaccharides profiled by HPAEC and MALDI-TOF MS. (1,3;1,4)- β -D-Glucan endo-hydrolase specifically hydrolyses (1,4)- β -glucosidic linkages when these linkages are on the reducing-end side of a (1,3)- β -D-glucosyl residue. The action of this enzyme yields a series of oligosaccharides with
15 different degrees of polymerization (DP). The diagnostic oligosaccharides in this series are the trisaccharide G₄G₃G_R and the tetrasaccharide G₄G₄G₃G_R (where G is β -D-glucopyranose, 3 and 4 indicate (1,3) and (1,4) linkages, respectively, and G_R refers to the reducing terminal residue). Variable quantities of G₄G₃G_R and G₄G₄G₃G_R were released when walls prepared from leaf or leaf and stem from lines 8 and 11 and two
20 independent lines derived from plant 16 (lines 16-1 and 16-2) were treated with (1,3;1,4)- β -D-glucan endo-hydrolase (Figures 3, 9A). These oligosaccharides were not detected in the no-enzyme treatment control. The ratio of G₄G₃G_R to G₄G₄G₃G_R (DP₃:DP₄) was estimated to be 3.5 in line 16-1, which is similar to the DP₃:DP₄ ratio of 3.6 obtained for β -glucan from the barley leaf sample. A peak that co-eluted with
25 laminaribiose, a (1,3)- β -linked disaccharide of glucose (G₃G_R), was also observed in lines 8, 11 and 16-2 at varying levels across samples (Figure 9A, data not shown). This product was absent from the barley and no-enzyme treatment control samples (Figure 9A), verifying its appearance is not due to a contaminating enzyme in the (1,3;1,4)- β -D-glucan endo-hydrolase preparation or to endogenous disaccharide or enzyme activity
30 within *Arabidopsis*. The identities of oligosaccharides in this profile were further

confirmed by MALDI-TOF MS analysis, which showed the presence of Hex₂, Hex₃ and Hex₄ in ratios similar to those observed in the HPAEC profile (Figure 9B). The amounts of β -glucan in lines 16-1 and 16-2, as estimated from the areas of the G4G3G_R peaks, were 0.005% and 0.003% (w/w) of total wall, respectively.

5

EXAMPLE 3

HvCslH1 is located in ER- and Golgi-associated vesicles but not the plasma membrane of transgenic *Arabidopsis* plants expressing *HvCslH1*

10 Sections of high pressure-frozen leaves from line 11 were incubated with the gold-labelled anti-HA antibody to determine the sub-cellular location of HvCslH1. Labelling was seen in the endoplasmic reticulum and in Golgi-derived vesicles but not in the plasma membrane (Figure 4A, B). Similar results were observed in labelled sections of roots and seedlings (data not shown).

15

EXAMPLE 4

HvCslH1 is transcribed in barley at low levels in developing grain, floral tissues and cells of the leaf undergoing secondary cell wall thickening

20 The levels of *HvCslH1* transcripts in various barley tissues were determined using quantitative RT-PCR (QPCR). The gene-specific primers are presented in Table 4.

TABLE 4 – List of primers used in Q-PCR analysis. PCR primers and PCR product sizes are given in base pairs, together with optimal acquisition temperatures for genes
25 analysed. Hv, *Hordeum vulgare*.

Gene	Forward Primer (5' - 3')	PCR product (bp)	Acquisition Temp. (°C)
	Reverse Primer (5' - 3')		
<i>Hv GAPDH</i>	GTGAGGCTGGTGCTGATTA (SEQ ID NO: 32)	198	80

	CGTGGTGCAGCTAGCATTTGAGAC (SEQ ID NO: 33)		
<i>Hv Cyclophilin</i>	CCTGTCGTGTCGTCTGCTCTAAA (SEQ ID NO: 34)	122	79
	ACGCAGATCCAGCAGCCTAAAG (SEQ ID NO: 35)		
<i>Hv α-Tubulin</i>	AGTGTCTGTCCACCCACTC (SEQ ID NO: 36)	248	80
	AGCATGAAGTGGATCCTTGG (SEQ ID NO: 37)		
<i>Hv HSP70</i>	CGACCAGGGCAACCGCACCAC (SEQ ID NO: 38)	108	83
	ACGGTGTTGATGGGGTTCATG (SEQ ID NO: 39)		
<i>Hv EL1a</i>	GGTACCTCCCAGGCTGACTGT (SEQ ID NO: 40)	164	80
	GTGGTGGCGTCCATCTTGTTA (SEQ ID NO: 41)		
<i>HvCslH1</i>	TGCTGTGGCTGGATGGTGTT (SEQ ID NO: 42)	295	82
	GCTTTATTATTGAGAGAGATTGGGAGA (SEQ ID NO: 43)		

Figure 5 (A-C) shows that across a set of barley vegetative and floral tissue cDNAs, *HvCslH1* transcripts were accumulated to levels that were routinely less than 1,000s copies/ μ l cDNA. This value is lower than some of the other barley *CESAs* and *CSLs* we

5 have studied where values are typically in the range of 10,000s and 100,000s copies/ μ l

cDNA. Levels of *HvCslH1* transcripts were relatively low in tissues comprising rapidly elongating cells, including coleoptile and leaf base, which are those that are actively synthesising β -glucan.

5 The highest levels of *HvCslH1* transcripts were in leaf tip, where cells are no longer actively growing and less β -glucan accumulates (Figure 5C; 2, 4). *HvCslH1* transcription in leaf was characterised further using RNAs isolated from six zones within the ~13 cm-long leaves of 10 day-old seedlings, starting from the leaf tip. These zones comprises fully mature cells (zone A), to the leaf base comprising dividing cells
10 (zone F). *In situ* PCR (see Example 5) was used to identify those cells in the leaf tip that contained the *HvCslH1* mRNA. In this technique, cells in which gene transcripts are detected stain purple to dark brown (Figure 5D, 18S RNA positive control). Cells where no transcription is detected stain light brown, as in the negative control (Figure 5E). *HvCslH1* was mostly transcribed in cells that are undergoing secondary wall
15 thickening, such as interfascicular sclerenchymal fibre and xylem cells (Figure 5F). Immuno-EM using sections taken from barley leaf and probed with the β -glucan antibody identified β -glucan in the walls of these cells.

HvCslH1 transcript levels were also investigated in more detail in a 24-day developing
20 endosperm series (Figure 5B). *HvCslH1* expression was low throughout the starchy endosperm during development. Maximum transcript levels were reached at 4 DPA, approximately 1 day before β -glucan is first detected in endosperm walls. This transcription pattern is similar to that of several barley *CSLF* genes (*HvCSLF3*, 4, 7, 8 and 10) that are also expressed in developing grain, although *HvCSLF9* and 6 show
25 much higher transcript levels.

EXAMPLE 5

Discussion

30 The data presented here indicate that the product of *HvCslH1*, a member of the grass-

specific *CSLH* gene family, mediates β -glucan biosynthesis in *Arabidopsis*. Barley appears to have only a single *CSLH* gene based on EST database analyses, genomic DNA blot analysis and BAC library screening. EST analyses of other grasses such as bread wheat, *Lolium multiflorum*, *Festuca arundinaceae* and *Brachypodium distachon* (all subfamily Pooideae) have one identified *CSLH* gene, similar to barley, whereas maize, sorghum and sugar cane (all subfamily Panicoideae), like rice (subfamily Ehrhartoideae), appear to have multiple *CSLH* genes. When an epitope-tagged version of the *HvCslH1* cDNA was heterologously expressed in *Arabidopsis*, three of four plant lines in which protein was detected accumulated a polysaccharide in their walls that was recognized by a β -glucan-specific monoclonal antibody. When isolated walls of the transgenic lines were digested with a specific (1,3;1,4)- β -D-glucan endo-hydrolase, the characteristic trisaccharide (G4G3G_R) and tetrasaccharide (G4G4G3G_R) were detected at ratios similar to those found in β -glucans from barley endosperm, demonstrating that the walls from the transgenic *Arabidopsis* lines contained β -glucan. Furthermore, epitope-tagged *HvCslH1* was found in the endoplasmic reticulum and in Golgi-derived vesicles in cells of transgenic plants. The morphological phenotype of the transgenic *Arabidopsis* lines that expressed *HvCslH1* appeared identical to wild-type plants.

Although the overall proportion of (1,3)- and (1,4)- β -glucosyl linkages and the ratios of the G4G3G_R and G4G4G3G_R products from (1,3;1,4)- β -D-glucan endo-hydrolase digestion of walls derived from plant line 16-1 was similar to those observed in β -glucans isolated from barley tissues, one unusual feature that was observed was that the major oligosaccharide released by (1,3;1,4)- β -D-glucan endo-hydrolase from the walls of line 16-2 was laminaribiose (G3G_R; Figure 9A). The presence of G3G_R in variable levels was also associated with increased levels of trisaccharide relative to the tetrasaccharide and, thus increased DP3:DP4 ratios. The presence of G3G_R in wall digests of the majority of plant lines indicates a polysaccharide containing sections of alternating (1,3)- β - and (1,4)- β -linked glucosyl residues (-G3G4G3G4-). It is not known if these reside in a separate polysaccharide or constitute a portion of a β -glucan chain

that also has the usual fine structural features. Alternating (1,3)- β -D-glucosyl and (1,4)
 β -D-glucosyl residues are not common in barley and other cereal β -glucans, but do
represent a significant component of the β -glucan from the non-flowering plant
Equisetum and are also detected in β -glucans from a number of fungi, including
5 basidiomycetes and ascomycetes. It is possible that G3G_R arises through misregulation
of the β -glucan synthase in transgenic *Arabidopsis*, possibly because its membrane
micro-environment is different or because an unknown factor that in barley suppresses
(1,3)- β glucosidic linkage formation (or alternatively promotes (1,4)- β glucosidic
linkage formation) is present at suboptimal levels in *Arabidopsis*. Minor variations in
10 the level of this factor among the lines derived from plant 16 would account for the
different structures that were obtained. Another possible explanation for the structural
variability in the β -glucan may relate to subtle differences in post-assembly processing
(see also Supporting Information below).

15 In barley, *HvCslH1* was most highly transcribed in leaf tips, a tissue comprising fully
mature cells. There is no evidence to indicate coordinate transcription of *HvCslH1* and
any of the barley *CSLFs*, suggesting that their encoded products are not components of
a protein complex. *HvCslH1* transcription, for example, was not high in elongating
cells such as the coleoptile or developing endosperm, which in barley are the tissues
20 where β -glucan is characteristically accumulated. Although usually found in primary
cell walls of vegetative tissues where it is implicated in the control of cell expansion
and possibly as a temporary store of glucose that can be mobilized as an energy source
in the dark, β -glucan has also been found in the lignified cell walls of xylem tracheary
elements and sclerenchyma fibres, where immuno-EM using the antibody to β -glucan
25 shows labeling in both the middle lamella region (primary wall) and secondary wall of
sclerenchyma cells. Because *in situ* PCR showing transcription of the *HvCslH1* gene in
the leaf was restricted to cells such as interfascicular sclerenchymal fibre and xylem
cells, we suggest that a major role of this gene is in β -glucan synthesis during
secondary wall development, although we cannot exclude a role in primary wall β -
30 glucan synthesis elsewhere in the plant.

Regardless of how the fine structures of β -glucans are generated, it is clear that the CSLHs can mediate the synthesis of β -glucan in *Arabidopsis*, a finding that has implications for our understanding of how this polysaccharide is synthesised. Any mechanism(s) being considered for the assembly of β -glucan must account for the synthesis of the predominant celotriosyl and celotetraosyl units, the random linking of these (1,4)- β -units together by single (1,3)- β -linkages and the means by which the molar ratio of tri- to tetra-saccharide units is regulated. At least two glycosyltransferase activities might act in concert: one that processively adds (1,4)- β -linked glucose residues to assemble the tri- and tetra-saccharides and the other that adds single (1,3)- β -linkages. Based on our current knowledge of polysaccharide synthases several mechanisms are hypothetically possible. The simplest explanation is that the one polypeptide is responsible for the synthesis of both glucosidic linkage types. Our transgenic experiments indicate that CSLH proteins are independently able to make a β -glucan and could therefore conceivably make both types of β -linkages. The CSLH family is classified by the Carbohydrate Active Enzymes (CAZy) database as members of glycosyltransferase family 2 (GT2) (<http://www.cazy.org>; Coutinho *et al.*, *J Mol Biol* 328: 307-317, 2003), a family that includes enzymes capable of independently catalyzing the synthesis of either (1,3)- β - or (1,4)- β -linkages but also examples of bifunctional enzymes, i.e. enzymes that can synthesize two types of glycosidic linkages. For example, hyaluronan synthases (HAS) synthesize a repeating disaccharide of (1,4)- β -glucuronic acid-(1,3)- β -N-acetylglucosamine units and both transferase activities reside in the one polypeptide. In mouse HAS1, the region that includes the D,D,D,QXXRW motif is responsible for both these activities. The active site of the CSLHs, also containing the D,D,D,QXXRW motif, might be similarly bifunctional. Another possibility is that the CSLHs synthesise only one type of glucosidic linkage with another glycosyltransferase, common to monocots and dicots, responsible for synthesis of a second linkage.

EXAMPLE 6

Materials and Methods*Binary vector construction and plant transformation*

5 The *HvCslH1* ORF was amplified from barley cv. Schooner mature leaf tip cDNA with Herculase[®] (Stratagene) using primers HvH1TOPOf and HvH1TOPOr (Table 3) and the PCR product cloned into pENTR/D-TOPO (Invitrogen). Using the manufacturer's protocol (Invitrogen), an LR reaction was used to clone the cDNA into the destination vector pGWB15 containing an NH₂-terminal 3× HA tag (Nakagawa *et al.*, *J Biosci Bioeng*
10 104: 34-41, 2007) and the predicted sequence confirmed by DNA sequencing. The *HvCslH1::pGWB15* construct was transferred from *Escherichia coli* into *Agrobacterium tumefaciens* strain AGL1 *via* triparental mating using the helper plasmid pRK2013. *Arabidopsis thaliana* Col-0 plants were transformed using the floral dip method (Clough and Bent, *Plant J* 16: 735-743, 1998).

15

RNA blot analysis

Samples of ~10 µg total RNA extracted from mature rosette leaves of T1 plants using TRIzol[®] (Invitrogen) were prepared and separated on a 1% w/v agarose-formaldehyde gel (Farrell, *RNA methodologies: A laboratory guide for isolation and characterization*,
20 Academic Press, Inc., San Diego, 1993). RNA was transferred to Hybond[™] N⁺ membranes, pre-hybridised and hybridised according to the method outlined in the Gene Images CDP-Star detection module (Amersham-Biosciences). A *HvCslH1* fragment amplified with primers H1F2 and HvH1TOPOr (Table 3) was labeled using the Gene Images Random Prime labeling module (Amersham) following the
25 manufacturer's protocol and used as the probe.

Quantitative PCR analysis

RNA extractions, cDNA syntheses and QPCR were carried out as described in Burton *et al.* (*Science* 311, 1940-1942, 2006; *Plant Physiol* 134, 224-236, 2004) with the
30 modifications listed in Burton *et al.* (*Plant Physiol* 146, 1821-1833, 2008). The primer

sequences of the barley control genes are listed in Table 4.

In situ PCR

In situ PCRs were conducted according to the method of Koltai & Bird (*Plant Physiol*
 5 123: 1203-1212, 2000) with the following modifications. After tissue sectioning, genomic
 DNA was removed by treatment for 6 h at 37°C in 1× DNase buffer and 4 U RNase-free
 DNase (Promega). cDNA synthesis was carried out using Thermoscript™ RT
 (Invitrogen) except that the RNase H step was omitted and a gene-specific primer (1
 µg, Table 3) used for reverse transcription. PCRs were carried out in a final volume of
 10 50 µL containing 1× PCR buffer, 200 µM dNTPs (Promega), 0.2 nmol digoxigenin-11-
 dUTP (Roche), 2 mM MgCl₂, 200 ng of each primer and 2 U Taq DNA polymerase
 (Invitrogen). Cycling parameters were as follows: initial denaturation at 96°C for 2 min,
 then 40 cycles of 94°C for 30 sec, 59°C for 30 sec, 72°C for 1 min. Sections were then
 washed, incubated with 1.5 U alkaline phosphatase-conjugated anti-digoxigenin Fab
 15 fragments (Roche) and developed for 10-20 min as outlined by Koltai & Bird (2000,
supra). For negative control sections, reverse transcriptase was omitted and all the Hv
 18S rRNA primers included to check whether there was any amplification from
 genomic DNA.

20 *Preparation of mixed microsomal membranes*

T1 seed of *HvCslH1* transgenic plants was collected and ~100 seeds sown onto 1× MS
 agar media containing 50 mg/L kanamycin (Sigma). After 3 weeks, kanamycin-
 resistant seedlings were pooled, frozen in liquid N₂ and ground at 4 °C in a mortar and
 pestle containing homogenising buffer (50 mM NaPO₄ buffer, pH 7.5, 0.5 M sucrose, 20
 25 mM KCl, 10 mM DTT, 0.2 mM PMSF, 83 µL plant protease inhibitor cocktail (Sigma,
 P9599)). Homogenate was filtered through a 50 µM mesh and the S/N centrifuged at
 6,000 ×g for 10 min at 4°C. The S/N was decanted and centrifuged at 50,000 ×g for 30
 min at 4°C in 4.5 ml ultracentrifuge tubes (Beckmann). The 50,000 ×g S/N was decanted
 and the pellet resuspended in 10 mM Tris-MES buffer, pH 7.5 using a glass-teflon
 30 homogenizer. The resuspended pellet was diluted to 4.5 mL with Tri-MES buffer and

centrifuged at 100,000 ×g for 1 h at 4°C. The pellet was resuspended in 0.25 M sucrose, 10 mM Tris-MES buffer, pH 7.5, as outlined above. Protein concentration was measured using Bradford assay reagent (BioRad) using bovine serum albumin as the standard.

5

Western blotting

Samples of membrane protein (30 µg) were incubated at 60°C for 20-60 min in 200 mM dithiothreitol and sample buffer (37.5 mM Tris-HCl, pH 7.0, 10% glycerol, 3% sodium dodecylsulphate (SDS), 0.025% bromophenol blue) to give an SDS:protein ratio of 1.5
10 mg SDS to 30 µg protein before loading onto an 8% SDS-PAGE gel. After electrophoresis, gels were blotted onto nitrocellulose (OSMONIC™ Nitropure 22 µm) in Towbin buffer (25 mM Tris base, 192 mM glycine, 20% methanol) containing 0.05% SDS at 100 V for 90 min at 4 °C. Membranes were then blocked overnight in Tris-buffered saline (TBS; 20 mM Tris base, 150 mM NaCl) containing 3% w/v milk powder
15 before incubation for 1 h at RT in rat anti-HA polyclonal antibody (Roche) diluted 1:1000 in TBS containing 1% BSA. Membranes were washed 3× in TBS containing 0.05% SDS (TBST), then incubated in anti-rat IgG HRP-conjugated antibody (Dako) diluted 1:1000 in TBS containing 3% w/v nonfat milk powder. Membranes were washed 3× in TBST before signal was detected with the SuperSignal® West Pico chemiluminescent
20 substrate (Pierce).

Immuno-Electron Microscopy

Arabidopsis tissues were fixed and labeled with anti-(1,3;1,4)-β-D-glucan specific antibody (Meikle *et al.*, *Plant J* 5: 1-9, 1994) according to Burton *et al.* (*Science* 311, 1940-
25 1942, 2006). For labeling with anti-HA antibody, plant tissue was placed between two copper planchets and rapidly frozen in a Leica EM high pressure freezer (set at 2.7×10^5 kPa and at an approximate rate of $-10,000^\circ\text{C s}^{-1}$). The planchets were transferred into 100% ethanol in a Leica automated freeze-substitution unit set at -50°C for 72 h. Samples were brought to room temperature (RT) overnight, removed and infiltrated
30 with LR White resin and embedded in gelatin capsules as detailed in Burton *et al.*

(2006, *supra*). Thin sections of embedded leaf tissue were collected on formvar-coated gold grids and incubated in a 1:200 dilution of the rat anti-HA polyclonal antibody in phosphate buffered saline (PBS; 137 mM NaCl, 10 mM NaPO₄, 2.7 mM KCl, pH 7.4) containing 1% w/v BSA for 1 h at RT and then overnight at 4 °C. Grids were washed
5 several times in PBS, then incubated in a 1:20 dilution of anti-rat secondary antibody conjugated to 18 nm gold (Jackson ImmunoResearch) in PBS containing 1% w/v BSA for 1 h at RT. The grids were then washed, post stained and viewed under the TEM as described by Burton *et al.* (2006, *supra*)

10 *Preparation of cell wall material*

Alcohol insoluble residue (AIR) was prepared by grinding plant material in liquid N₂ using a mortar and pestle. Five volumes of 80% ethanol was added to the homogenate prior to mixing by rotation for 1 h at 4 °C. After centrifugation at 3,400 ×g for 5 min, the supernatant was removed and the residue was refluxed twice at RT in 80% ethanol for
15 1 h, followed by refluxing in 50% ethanol twice for 1 h. The ethanol-soluble fraction was removed and the AIR was washed once in 100 % ethanol prior to drying at 40°C under vacuum.

(1,3;1,4)-β-D-glucan specific endo-hydrolase digestion

20 AIR (100 mg, prepared as described above) was incubated in 5 mL 20 mM NaPO₄ buffer, pH 6.5 for 2 h at 50°C with continuous mixing in an incubator with shaking at 200 rpm. After 2 h, the suspension was centrifuged (3,400 ×g, 5 min) and the supernatant (S/N) removed. Another 5 mL of buffer was added and the incubation and centrifugation repeated. The S/N from this second incubation was used as the no
25 enzyme negative control. The pelleted AIR was resuspended in 5 mL NaPO₄ buffer to which 100 µl (1,3;1,4)-β-D-glucan endo-hydrolase (McCleary *et al.*, *J Inst Brew* 91: 285-295, 1985) was added. The mixture was incubated for 2 h at 50°C with continuous mixing after which the S/N was collected as the (1,3;1,4)-β-D-glucan endo-hydrolase-released oligosaccharides. The negative control and (1,3;1,4)-β-D-glucan endo-
30 hydrolase-treated S/Ns were desalted on a graphitised carbon cartridge as described by

Packer *et al.* (*Glycoconj J* 15: 737-747, 1998) and dried.

HPAEC analysis

The dried (1,3;1,4)- β -D-glucan endo-hydrolase -released oligosaccharides were
 5 dissolved in 100 μ L Milli H₂O and 20 μ L injected onto a CarboPac PA1 column
 (Dionex) equilibrated with 50 mM NaOAc in 0.2 M NaOH using a Dionex BioLC ICS
 300 system (Dionex) equipped with a pulsed amperometric detector (PAD) and
 autosampler. Oligosaccharides were eluted at 1 mL/min with a linear gradient of
 NaOAc from 50 mM in 0.2M NaOH to 350 mM in 0.2 M NaOH over 15 min.
 10 Laminaribiose (Seigaku), maltose and cellobiose (both from Sigma) were run as
 standards.

MALDI-TOF MS analysis

Aliquots (30 μ L) of the remaining (1,3;1,4)- β -D-glucan endo-hydrolase-released
 15 oligosaccharides were lyophilised, dissolved in DMSO and methylated using the
 NaOH method (Ciucanu and Kerek, *Carb Research* 131: 209–217, 1984). Methylated
 oligosaccharides were partitioned into dichloromethane (DCM) and the DCM phase
 washed 3 $\times$ with MilliQ water. The DCM phase was dried under a N₂ stream before re-
 dissolving in 10 μ L 50% acetonitrile. A 1 μ L aliquot was mixed with 1 μ L 2,5-
 20 dihydroxy benzoic acid matrix (10 mg/mL dissolved in 50% acetonitrile) and 1 μ L of
 the mix was spotted onto a MALDI plate for analysis in a MALDI TOF mass
 spectrometer (Voyager DSTR, Applied Biosystems).

EST analyses, contig assembly and bioinformatics

25 CSLH ESTs were obtained by querying public databases including the now
 discontinued Stanford Cell Wall website, NCBI (<http://www.ncbi.nlm.nih.gov/>),
 HarvEST (<http://harvest.ucr.edu/>), GrainGenes
 (<http://wheat.pw.usda.gov/GG2/index.shtml>), Barley Gene Index
 (<http://compbio.dfci.harvard.edu/tgi/plant.html>) and BarleyBase (www.barleybase.org)
 30 using the BLAST search tool (Altschul *et al.*, *Nucl Acids Res* 25: 3389-3402, 1997).

Sequences were assembled into contigs using either Sequencer™ 3.0 (GeneCodes) or ContigExpress, a module of Vector NTI® Advance 9.1.0 (Invitrogen). DNA or protein sequences were aligned using ClustalX (Thompson *et al.*, *Nucl Acids Res* 24: 4876-4882, 1997). Phylogenetic analysis was carried out using the in-built neighbour joining
5 algorithm and tree robustness assessed using 1000 bootstrapped replicates. Sequence similarities were calculated using MatGat 2.02 (<http://bitincka.com/ledion/matgat/>) (Campanella *et al.*, *BMC Bioinformatics* 4: 29, 2003). Transmembrane domains were predicted using the suite of programs described in ARAMEMNON (<http://aramemnon.botanik.uni-koeln.de>) (Schwacke *et al.*, *Plant Physiol* 131: 16-26,
10 2003). Motifs predicting post-translational modifications were identified using the programs listed in ExPasy under the Tools menu (<http://www.expasy.org/tools/#pattern>). Protein parameters were calculated using ProtParam at ExPasy (<http://www.expasy.org/cgi-bin/protparam>).

15 *Barley BAC screening*

BAC filters containing 6.5 equivalents of the barley genome from the non-Yd2 cv. Morex (Clemson University Genomics Institute, CUGI) were blocked for 6 h at 65°C in prehybridisation solution (0.53 M NaPO₄ buffer pH 7.2, 7.5% w/v SDS, 1 mM EDTA, 11 µg/ml salmon sperm DNA). The radiolabeled cDNA and gDNA fragment amplified
20 with primers H1F1 and H1R1 or H1R5 (Table 3) was added and incubated for 24 h at 65°C. Filters were washed 3× with 2× SSC, 0.1% SDS at RT. Final washes were done with 1× SSC, 0.1% SDS. Filters were exposed to X-ray film for 2 d. Positive BAC clones were identified and ordered as directed on the CUGI website (<http://www.genome.clemson.edu>). Clones were streaked onto LB agar containing 25
25 µg/ml chloramphenicol and grown overnight at 37°C. Colonies for each clone were picked, placed on gridded nylon membranes resting on LB agar containing 25 µg/ml chloramphenicol and incubated overnight at 37°C. DNA was fixed to the membrane and denatured by placing on filter paper soaked in 0.4 M NaOH for 20 min, then neutralized by placing on filter paper soaked in neutralizing solution (1.5 M NaCl, 0.5
30 M Tris-HCl pH 7.2, 1 mM EDTA). Membranes were then washed 3× in 2× SSC, 0.1%

SDS and hybridized using standard conditions (Sambrook *et al.*, *Molecular cloning: a laboratory manual*, Cold Spring Harbour Laboratory Press, New York, 1989).

BAC DNA isolation

5 Positive clones were cultured overnight in LB broth containing 25 µg/ml chloramphenicol at 37°C. Cells were pelleted by centrifugation (12,000 ×g, 3 min) and the pellet resuspended in 90 µL TES buffer (25 mM Tris-HCl pH 8.0, 10 mM EDTA, 15% w/v sucrose). An aliquot (180 µL) of lysis solution (0.2 M NaOH, 1% SDS) was added and mixed gently, followed by 135 µL 3 M NaOAc pH 4.6. The chromosomal
10 DNA was pelleted by centrifugation (12,000 ×g, 15 min). The S/N was collected and 2 µL RNase A (10 mg/mL) added and incubated for 1 h at 37°C. A 400 µL aliquot of Tris-saturated phenol-chloroform (1:1 ratio) was added and the samples centrifuged again (12,000 ×g, 5 min). The S/N was collected and BAC DNA precipitated using 2-3 volumes chilled 95% ethanol for 10 min at RT. The BAC DNA was pelleted by
15 centrifugation (15,000 ×g, 15 min), washed in 70% ethanol, resuspended in 20-50 µL TE and stored at 4 °C.

Genome walking

The adaptor ligation method of Siebert *et al.* (*Nucl Acids Res* 23: 1087-1088, 1995) was
20 used to amplify fragments of genomic DNA upstream of known *CSLH* EST sequence. Restriction enzymes used to digest barley genomic DNA were *Eco RV*, *Nru I*, *Pvu II*, *Sca I* or *Ssp I*. Primary PCR reactions were conducted in 25 µL volumes containing 2 µL ligated DNA (1:10 dilution), 1× PCR buffer, 2 mM MgCl₂, 100 ng each of adaptor primer AP1 and antisense primer H1R7 (Table 3), 0.4 mM dNTPs and 1 unit Taq
25 polymerase (Invitrogen). Cycle parameters were as follows: 96°C for 2 min then 40 cycles of 94°C for 30 sec, 59°C for 30 sec, 72°C for 1 min, and a final step at 72°C for 7 min. A secondary PCR reaction was conducted with 1 µL of the primary PCR using 100 ng each of adaptor primer AP2 and the nested primer H1R6. Reaction composition and cycle parameters were the same as above except that an annealing temperature of 61°C
30 was used.

BAC sequencing

For sequencing, between 0.5 and 1 µg of isolated BAC DNA was combined with 5 pmol primer and 1× Big Dye Terminator v 3.1 (BDT) mix (Applied Biosystems, USA) in a final volume of 20 µL. Cycle parameters were as follows: 96°C for 15 min, then 65 cycles of 96°C for 10 sec, 55°C for 10 sec and 60°C for 4 min. DNA was precipitated with 0.1 vol 3M NaOAc pH 5.2 and 2.5 vol 95% ethanol on ice for 10 min, then pelleted by spinning at 12,000× g for 30 min. The pellet was rinsed with 70% ethanol, dried and sent to AGRF (Brisbane, Australia) for sequencing.

10

Mapping of HvCslH1

Genetic mapping was done using a Sloop × Halcyon doubled haploid (DH) mapping population of 60 lines (Read *et al.*, *Aust J Agric Res* 54: 1145-1153, 2003). Using standard methods of DNA blot hybridization (Sambrook *et al.*, 1989, *supra*) a *HvCslH1* probe PCR-amplified using primers H1F1 and H1R5 (Table 3) was hybridized to membranes containing parental line genomic DNA digested with one of six restriction enzymes (*Bam* HI, *Dra* I, *Eco* RI, *Eco* RV, *Hind* III, *Xba* I). The dihybrid population was then digested with enzymes that gave a clear polymorphism (*Dra* I). Polymorphisms were scored and *HvCslH1* map location determined using the 'find best location' function of MapManager QT version 0.30 (Manly *et al.*, *Mamm Genome* 12: 930-932, 2001). Map locations were correlated with QTL data using resources available at <http://www.barleyworld.org/>.

20

Arabidopsis growth conditions

Arabidopsis seeds were surface-sterilized in a sterilization solution (sodium hypochlorite (2% available chlorine), drop of Tween-20) for 15 min then rinsed 4× with sterile MilliQ water. Surface-sterilized seed was spread onto 85 × 25 mm Petri dishes containing 50 mL of sterile 1× MS medium (4.33 g/L Murashige and Skoog basal salts (Phytotechnology Laboratories), 2 % w/v sucrose, 1% w/v bactoagar). For selection of transformants, 50 mg/L kanamycin (Sigma) was added to the medium. Plates were

30

placed in a cold room for 3-5 days at 4°C to synchronize germination. Cold-stratified plates were then transferred into controlled environment growth cabinets (Thermoline L+M model TPG 1260 TO-5×400, Smithfield, NSW, Australia) with day and night temperatures of 23°C and 17°C, respectively. The average light intensity at rosette leaf level was ~70 $\mu\text{E m}^{-2} \text{ sec}^{-1}$ supplied by 3-foot fluorescent tubes (Sylvania Standard F30W/133-T8 Cool White) during a 16 h light cycle. After 3 weeks on MS plates, individual plantlets were transferred into hydrated 42 mm diameter Jiffy pellets. Nine rows of six pellets were arranged in trays with three trays being housed on each 2 × 3.5-foot wire rack shelf. Relative humidity was measured to be between 60 and 70%. Plants were watered with tap water supplemented with Peter's Professional™ General Purpose plant fertilizer (Scotts Australia) by sub-irrigation every 2-3 days.

Genomic DNA extraction and PCR analysis of Arabidopsis transgenics

DNA was extracted from a single *Arabidopsis* leaf according to the method described in Edwards *et al.* (*Nucl Acids Res* 19: 1349, 1991). A 1 μL aliquot of genomic DNA was used as template in PCR screens of transgenic plants using primers H1F2 and HvCslH1TOPOr (Table 3) with the following cycling regime: 94°C for 2 min followed by 35 cycles of 94°C for 20 sec, 57°C for 30 sec, 72°C for 30 sec.

EXAMPLE 7

Alignment of CslH DNA and Amino Acid Sequences from Rice and Barley

An alignment of the DNA and amino acid sequences for the *CslH* sequences in both rice and barley was performed to calculate the percent identity and similarity between the sequences, the results of which are shown in Figure 10. The DNA and protein sequences were aligned and compared using the default parameters in MatGAT version 2.02 downloaded from <http://bitincka.com/ledion/matgat/>.

Multiple sequence alignments and phylogenetic tree generation was performed using the ClustalX program as described by Thompson *et al.* (*Nucl Acids Res* 25: 4876-4882,

1997). The protein alignment and resultant phylogenetic tree are shown in Figures 11 and 12, respectively.

EXAMPLE 8

5 Cross of *HvCslH1* and *OsCSLF2* Transgenic *Arabidopsis* Lines

Two transgenic *Arabidopsis* lines, 15-8 and 15-11, in which the tagged *HvCslH1* protein was detected using an anti-HA antibody, were chosen to genetically cross with two other transgenic *Arabidopsis* lines containing *OsCslF2*, H37 and H17-4, as
10 described by Burton *et al.* (*Science* 311: 1940-1942, 2006). It was thought that by expressing the *HvCslH1* and *OsCSLF2* proteins in the same cell types, higher levels of (1,3;1,4)- β -D-glucan above those observed in single gene (*CSLH* or *CSLF* only) transgenic *Arabidopsis* plants, could potentially be deposited into cell walls. In addition, this would aid in detecting (1,3;1,4)- β -D-glucan in immuno-electron
15 microscopy studies as well as in chemical cell wall analyses.

All four of the parental lines were confirmed to contain (1,3;1,4)- β -D-glucan in their cell walls by immuno-electron microscopy (Figure 13). Individuals from each of the four populations were used as male and female parents. Flowers of the female parent (e.g.
20 individual H37-5) were emasculated prior to anther dehiscence and pollinated using dehisced anthers from the male parent (e.g. individual 15-8-3). Each crossed flower was labelled and the resulting seed pods collected upon dehydration.

The progeny of each cross were sown in soil and their genotypes determined by PCR
25 using leaf genomic DNA as template and *HvCslH1*- specific primers and, in a separate reaction, *OsCslF2*-specific primers. Mature leaves were fixed, embedded, sectioned and labeled with (1,3;1,4)- β -D-glucan monoclonal antibody. A number of the progeny were found to have greater levels of labelling than the parental lines, as observed in Figure 14. For example, the labelling in the epidermal cells of the individual shown in Panel D
30 is much heavier than compared to its 15-8-3 x H37-7 parents (Figure 13). A sib with the

same genotype (Figure 14, panel C) showed consistent yet lower levels of epidermal cell wall labeling.

EXAMPLE 9

5 Cloning of *CslH* cDNA and genomic sequences from barley cultivar Himalaya and
wheat

A full length cDNA sequence of the *CslH1* gene was isolated from barley cultivar Himalaya using a combination of barley EST sequences, PCR from cDNA using
10 primers based on the rice *CslH1* gene sequence (LOC_Os10g20090) and 5'RACE.

The 2333 bp consensus sequence designated *HvCslH1(Him)* (SEQ ID NO: 69) is shown in Figure 16. There is a single long open reading frame of 751 amino acids (SEQ ID NO: 70).

15

Oligonucleotide primers SJ91 and SJ85 were designed from the 5' and 3' ends of the consensus sequence and used to amplify a 3203 bp DNA fragment from genomic DNA designated *HvCslH1gHim* (SEQ ID NO: 71) in Figure 17.

20 Alignment of the barley cDNA sequence and genomic sequences indicated that the CslH gene has eight small (approximately 100 bp) introns each flanked by the consensus GT..AG splice donor/acceptor sites (Figure 17).

A wheat homolog of *CslH1* was identified in the TIGR database as TC255929. Three
 25 classes of sequences made up this tentative consensus as exemplified by ESTs
 CJ614392, CJ609729 and CJ721204. PCR primers were designed from the barley
 sequence surrounding the ATG initiation codon (SJ163) and from the consensus
 sequence of all three EST types at the 3' end (SJ164) and used to amplify a full length
 genomic fragment from wheat cultivar Chinese Spring. Two sequence types were
 30 identified and designated *TaCslH1-1* (SEQ ID NO: 78) and *TaCslH1-2* (SEQ ID NO: 79).

The third homeolog designated *TaCslH1-3* (SEQ ID NO: 80) was isolated using primers SJ204 and SJ164 as described in more detail in materials and methods.

Comparison with the barley sequences indicated that the intron-exon junctions were conserved in all three genes (Figure 17). The three wheat genes are 94.8 - 96.1% identical.

The predicted coding region sequences of the three wheat *CslH1* genes (SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74) each encode a polypeptide of 752 amino acids (SEQ ID NO: 75, SEQ ID NO: 76 and SEQ ID NO: 77).

The DNA coding sequences and amino acids sequences of the barley and wheat CSLH1 genes were aligned using the muscle alignment program and the percentage identity and similarity was calculated using a PAM250 matrix. A table showing the percentage identity and similarity is show in Figure 27.

As shown in Figure 27, the wheat proteins are about 94-95.0% identical to each other and about 92.6-93.1% identical to the barley proteins.

20

EXAMPLE 10

CslH gene expression in barley and wheat

Expression of the *CslH1* gene was examined by semi quantitative (RT-PCR and gel electrophoresis) and quantitative (real time PCR) methods.

25

The coleoptile is a good tissue to examine expression of genes related to beta glucan biosynthesis since the levels of beta glucan increase as the coleoptile grows and then decline after growth has stopped. The *CslH1* gene shows maximum expression only after growth has ceased and is high in the oldest tissues (6-8 days old, as shown in Figures 19A/B).

30

Other tissues were also examined. In developing leaf, the *CslH1* gene shows differential and maximum expression in the oldest tissue at the tip of the leaf (Figure 20). It appears from these results that the *CslH1* gene is preferentially (although not
5 exclusively) expressed in cells that have stopped dividing and elongating and are thus differentiating. Cells in the maturing endosperm would be in a similar phase of development, ie. cell division would have stopped, cell enlargement would be slowing with the cells differentiating into specialised starch storage parenchyma.

10 In barley endosperm tissue, *CslH1* gene expression peaked around 4 days post anthesis and then increased during later stages to reach a maximum at 28 days (Figure 21).

There was a large difference in *CslH1* gene expression in wheat where expression peaked at 4 days post anthesis after which expression was very low. These results were
15 confirmed by real time PCR which showed that at 28 days post anthesis, the *CslH* gene was expressed about 10 fold higher levels in barley than in wheat (Figure 22).

EXAMPLE 11

Overexpression of the barley *CslH* gene in wheat grain

20

Transgenic wheat plants were generated by biolistics transformation with the full length genomic *HvCslH1* (cv. Himalaya) gene under control of the glutenin promoter such that expression should only occur in endosperm tissues (Figure 23). Lines were screened for the presence or absence of the transgene by PCR of young leaf material.
25 Twelve PCR positive lines and three PCR negative lines (H1-2, -7 and -11) were grown to maturity in the glasshouse. RNA was isolated from developing grain at approximately 15 days post anthesis and cDNA was made using Superscript III. Expression of the barley transgene was then analysed by real time PCR. Table 5 shows the relative expression levels compared to the endogenous wheat *CslH* gene as the
30 primers used amplify both the wheat and barley genes.

TABLE 5 – Relative expression of *CslH* gene in wheat endosperm

Line	Run 1	Run 2	Run 3
H1-1	468	225	
H1-2		2	
H1-3	206		
H1-4	620	497	243
H1-5	299	411	
H1-6	952		
H1-7	63		
H1-8	140		230
H1-9		1771	
H1-10		4396	4052
H1-11	26		6
H1-12	10	1103	
H1-14	1013	352	
H1-15	10		
H1-13	1	1	

Most of the lines expressed the barley *CslH* gene at several hundred fold higher levels

5

than the controls with line 9, 10, 12 and 14 showing the highest expression (greater than one thousand fold higher).

At maturity, single grains from were analysed for beta glucan content and a summary of the results are shown in Table 6:

10

TABLE 6 – Beta glucan content of transgenic wheat flour

Transgenic line	Average Beta glucan	std dev	Max beta glucan
H1-1	0.81	0.08	.8
H1-2	0.68	0.02	.7

H1-3	0.89	0.05	.9
H1-4	0.82	0.05	.8
H1-5	0.83	0.21	1.1
H1-6	0.91	0.09	1.0
H1-7	0.65	0.05	.7
H1-8	0.87	0.15	1.1
H1-9	1.17	0.33	1.9
H1-10	1.12	0.39	1.9
H1-11	0.82	0.14	1.0
H1-12	1.23	0.26	1.7
H1-14	0.99	0.19	1.4
H1-15	0.60	0.26	.8
H1-16	1.00	0.11	1.2
PCR - (2,7,11) average	0.69	0.10	1.0
PCR + (rest) average	0.97	0.11	1.9

The PCR negative lines all had the lowest beta glucan contents averaging 0.69% of grain weight, whereas grain from the PCR positive lines had an increased average beta glucan content of 0.97%. The last column of Table 6 shows the maximum beta glucan content of any single grain from a given line – the highest PCR negative line was 1.0% (and most grains were much lower than this) but several of the PCR positive lines had grains with significantly increased beta glucan levels with line 9 and line 10 (the highest expressers) having grains with up to 1.9% beta glucan. These levels of beta glucan have never been seen before in wheat.

10

The heads from these T0 plants contain T1 seed which are segregating for the transgene. If the DNA is inserted at a single locus a ratio of three transgenic to one wild type seed should be observed. Figure 25 shows the beta glucan levels of individual T1 seeds from the H1 transgenic line 10 from which it can be seen that approximately three quarters (47/61) have higher beta glucan levels than the average of the PCR negative lines (0.7%). From the ratio of the highest beta glucan level (1.9%) to the

15

average PCR negative level (0.7%) the increase in beta glucan content is 2.7 times that normally seen in wild type wheat grains. A further significant observation is that a high proportion of the grains have at least 1.4% beta glucan.

- 5 It is expected that further increases in beta glucan will be seen in these grains when the lines are made homozygous and gene dosage increases.

EXAMPLE 12

Materials and Methods for Examples 9 to 11

10

Plant Material

Barley (*Hordeum vulgare*) cultivar Himalaya and wheat (*Triticum aestivum*) cultivar Chinese Spring, Westonia and Bob White²⁶, were grown under standard glasshouse conditions.

15

Primer Sequences

The primer sequences referred to in Examples 9 to 11 and this example are shown below in Table 7:

20 TABLE 7 – Primer sequences for Examples 9 to 12

Primer	Target gene	Sequence (5'- 3')	Sequence Identifier
SJ27	<i>CslH1</i>	AGGCGTGGTTCGCGTTCG	SEQ ID NO: 44
SJ28	<i>CslH1</i>	CAGCGCGTAGTACGTCAC	SEQ ID NO: 45
SJ72	<i>CslH1</i>	CAGCCGTGATGACCAACG	SEQ ID NO: 46
SJ73	<i>CslH1</i>	GTTGCCAAAGGGATCGTC	SEQ ID NO: 47
SJ79	<i>CslH1</i>	GCGGTCGTGACGAACATGTCCAC	SEQ ID NO: 48
SJ75	<i>CslH1</i>	GACGCTCCACGGGATTC	SEQ ID NO: 49
SJ85	<i>CslH1</i>	GGTTAGTTCCTTGTGCAGAGGT	SEQ ID NO: 50
SJ91	<i>CslH1</i>	GAGCTGTGTTCTGTGGAGCTTAG	SEQ ID NO: 51
SJ163	<i>CslH1</i>	CTGCTCTCGGCCACGGCCAT	SEQ ID NO: 52

SJ164	<i>CslH1</i>	CCGCCGGTTAGTTCCTTGTGCAGA	SEQ ID NO: 53
SJ183	<i>CslH1</i>	GGAGAGTTCGTGTGCTGTGG	SEQ ID NO: 54
SJ204	<i>CslH1</i>	CACCATGAGCCCCGTCCGGTTCGACA	SEQ ID NO: 55
TUB	Alpha tubulin	CAAACCTCAGGGAAGCAGTCA	SEQ ID NO: 56
TUB2F	Alpha tubulin	AGTGTCCTGTCCACCCACTC	SEQ ID NO: 57
SJ107	<i>CslF6</i>	GCATCGTACTGGTGCTGCT	SEQ ID NO: 58
SJ82	<i>CslF6</i>	GCGCTTCTCACGGGACACGTACA	SEQ ID NO: 59
SJ94	<i>CslF4</i>	GATGCGTACAACCTCGAGCAA	SEQ ID NO: 60
SJ95	<i>CslF4</i>	CGTTGCTGAAGTCAAGTGGA	SEQ ID NO: 61
SJ97	<i>CslF9</i>	CGCTGCAAACGAGAAAGAAGG	SEQ ID NO: 62
SJ93	<i>CslF9</i>	GGCGCTGAAATCCAGAGG	SEQ ID NO: 63
SJ44	<i>CslF3</i>	CGGAAATCCATAGGAAAGG	SEQ ID NO: 64
SJ38	<i>CslF3</i>	CGGCGGAACATGCAAC	SEQ ID NO: 65
SJ96	<i>CslF8</i>	GGATTGACCCAGCTGAAAAC	SEQ ID NO: 66
SJ37	<i>CslF8</i>	GAGTTGTTGACGTAGTGGTC	SEQ ID NO: 67
SJ244	Bx17 prom	CGAGCACCCCAATCTACAGA	SEQ ID NO: 68

DNA, RNA isolation and cDNA synthesis

Plant DNA was isolated from fully expanded leaf tissue using a CTAB based method (Murray and Thompson, *Nucleic Acids Res.* 8: 4321–4325, 1980). Total RNA was isolated from leaf and coleoptile tissues using an RNAeasy kit from Qiagen. RNA was isolated from developing endosperm using a phenol SDS method and LiCl precipitation (Clarke *et al.*, *Functional and Integrative Genomics* 8, 211-221, 2007). RNA was treated with DNase using a “DNA-free” kit from Ambion and then cDNA was synthesised using SuperscriptIII reverse transcriptase according to the manufacturer’s instructions (Clontech).

Cloning of CslH genes

The methods for cloning *CslH* genes were similar to those described in the cloning and characterisation of *CslF* genes (Burton *et al.*, *Plant Physiol* 146: 1821-1833, 2008). A 1.8 kb tentative consensus sequence (TC140327) of a barley homolog of the rice Cellulose

synthase like H1 gene (LOC_Os10g20090) was identified in the TIGR database. PCR primer pairs (SJ27-SJ73 and SJ72-SJ75) were designed based on the rice *CslH1* sequence and used to amplify sequences from cDNA. The 5' end of the gene was then amplified by 5'RACE using a SMART cDNA library and nested *CslH1* primers SJ28 and SJ79
5 according to the manufacturer's instructions (Clontech).

A full length genomic clone was isolated by amplification with primers SJ91 and SJ85 and Phusion Taq polymerase (Finnzymes) according to the manufacturers recommend cycling conditions (denature 30 sec at 98°C followed by 35 cycles of 98°C for 5 sec, 63°C
10 for 7 sec and 72°C for 3 min) and cloned into the pCRBluntII TOPO cloning vector (Invitrogen).

Wheat *CslH* genomic clones were isolated by PCR with Phusion polymerase from the cultivar Chinese Spring using primers SJ163 and SJ164 and an annealing temperature
15 of 70°C. A genome walking kit was used according to the manufacturers instructions (Clontech) to obtain sequences extending upstream of the coding region of all three wheat *CslH* homeologs from the variety Bob White (data not shown). A primer (SJ204) was designed that was specific to the third homeolog and used with SJ164 to isolate the third full length genomic clone. It was confirmed that the predicted exon/intron
20 boundaries could be spliced correctly by sequencing cDNA fragments (data not shown).

Expression analysis of CslH gene in wheat and barley by RT-PCR

Total RNA was isolated from sections of the first leaf of a 7 day old plant, from dark
25 grown coleoptiles of different ages, and from developing grain collected at 4 day intervals post anthesis (DPA), DNase treated and reverse transcribed with Superscript III according to the manufacturer's instructions (Invitrogen). PCR reactions were performed using HotStarTaq (Qiagen). The cDNA was diluted and used in PCR reactions at a level equivalent to 1 ng of original RNA per microlitre. For semi-
30 quantitative RT-PCR, *CslH1* primers SJ72 and SJ74, for the *CslF* genes, primer pairs

were as follows; (CslF6; SJ107-SJ82), (CslF4; SJ94-SJ95), (CslF9; SJ97-SJ93), (CslF3; SJ44-SJ38), (CslF8; SJ96- SJ37). An annealing temperature of 59°C was used. Test amplifications were performed to ensure that the amplification was not saturated (approx 32-35 cycles except tubulin 24 cycles) and the products were analysed by
 5 ethidium bromide staining after agarose gel electrophoresis. Real time PCR was performed on triplicate samples on a Rotorgene 6000 machine (Corbett Life Sciences, AU) using HotStarTaq (Qiagen), SybrGreen and primers SJ183 and SJ164 and an annealing temperature of 60°C. Relative expression levels were calculated using the machine software with wheat 0 dpa samples as the comparator (set to one). The Ct
 10 value of this sample was 25.5 cycles. For analysis of transgenic grain at 15 dpa, the relative expression values were normalised against tubulin and compared to the lowest expression line (H1-13).

Expression analysis of CslH gene in barley by Q-PCR

15 *HvCslH1* transcript was measured in developing coleoptile 0.5 to 7 days post germination. *HvCslH1* transcript was shown to accumulate only after the completion of the elongation phase and the emergence of the leaf. Highest levels of expression were seen at 7 days when the coleoptile is senescing (twisting and shrinking) (Gibeaut
et al., *Planta* 221:729-738, 2005).

20

Production of transgenic wheat plants overexpressing the barley CslH gene in endosperm

The full length barley cv. Himalaya genomic *CslH* sequence (SEQ ID NO: 71) was amplified using primers SJ91 and SJ85, was inserted as an *EcoRI* fragment between a 1.9 kb fragment of the high molecular weight glutenin Bx17 promoter and the nopaline
 25 synthase terminator (Figure 23). The Bx17 promoter confers high level expression in developing endosperm (Reddy and Appels, *Theor Appl Genet* 85: 616-624, 1993).

Bob White 26 wheat plants were transformed using the biolistics method (Pellegrineschi *et al.*, *Genome* 45: 421-430, 2002) with 50 mg/L G418 as the selection
 30 agent. The *HvCslH* expression vector (pZLBx17HvgH1 and a second plasmid with the

CaMV 35S promoter driving expression of the NPTII selectable marker (pCMSTLSneo, Figure 24) were mixed in equimolar amounts and co bombarded into immature embryos.

- 5 Transgenic plants were screened for the presence of the transgene using young leaf tissue and the RedExtractnAmp™ kit from Sigma with primers SJ244 and SJ79.

At anthesis (emergence of the anthers and shedding of pollen) heads were tagged to enable grain to be sampled at approximately 15 dpa. Three grains from a head were
10 pooled, RNA extracted and reverse transcribed and levels of transgene expression were analysed by real time PCR using primers SJ183 and SJ85. Expression levels were normalised against alpha tubulin (primers TUB and TUB2F) and finally expressed as a ratio compared to the lowest expresser.

- 15 Flour from mature single grains was analysed for beta glucan content using a scaled down version of the lichenase enzymatic method (AACC Method 32-33, Megazyme assay kit, McCleary and Glennie-Holmes, *J. Inst Brewing* 91: 285-295, 1985). Beta glucan contents are expressed as a percentage (w/w) of the milled whole grain flour.

20

EXAMPLE 13

Overexpression of the barley *CslH* gene in barley cv. Golden Promise

The full-length coding region of the barley *CslH* cDNA (SEQ ID NO: 1) was transferred into two Gateway-enabled barley transformation vectors. The vector pRB474 contains
25 the oat globulin promoter (Vickers *et al.*, *Plant Mol Biol* 62: 195-214, 2006) which provides endosperm specific expression and the vector pMDC32 (Curtis and Grossniklaus, *Plant Physiol.* 133: 462-9, 2003) contains a double 35S promoter which drives constitutive expression in all plant tissues.

30

Barley Transformation

The vectors were transferred into *Agrobacterium tumefaciens* and immature scutella of the barley cultivar Golden Promise were transformed using established protocols to produce two populations of transgenic plants. Insertion of the transgene was confirmed by Southern blotting. Plants 236-1 to 236-18 contain the barley *CslH* gene driven by the oat globulin promoter. Plants 237-1 and -2 contain the barley *CslH* gene driven by the 35S promoter. Plants 208-2,-3,-5 and -7 are control plants and are transgenic for the empty vector pRB474 carrying the oat globulin promoter only.

10 *Transcript Analysis*

Leaf and developing grain samples, from 7 and 14 days after pollination (DAP) were collected from the 236 plants. Total RNA was extracted using TRIzol reagent (Invitrogen) following a standard protocol and cDNA was synthesized according to Burton *et al.*, (*Plant Physiol* 146: 1821-1833, 2008). Quantitative real-time PCR (QPCR) was carried out according to Burton *et al.* (2008, *supra*). The transcript levels of the *CslH* gene were compared in the endosperm of the transgenic grain to wild type endosperm levels which are generally very low.

As shown in Figure 26, the empty vector control lines (208) have typical wild type levels of *CslH* transcript. The transgenic lines (236) show significantly increased *HvCslH1* mRNA levels at 7 days (7D) and further increases at 14 days (14D) after pollination.

Beta-glucan Analysis

25 The T1 seed from the transgenic plants were collected. A sample of the bulked T1 grain from each individual plant was ground to flour and the amount of beta-glucan present was assayed using Megazyme method (described *supra*). The data from each plant are presented as the mean value of two replicates and the amount of beta-glucan as a percentage of grain weight is shown in Table 8, below:

TABLE 8 – (1,3;1,4)- β -D-glucan content of bulked transgenic barley flour

Plant Line	(1,3;1,4)- β -D-glucan as % of grain weight
208-2 (empty vector control)	3.9
208-3 (empty vector control)	4.4
208-5 (empty vector control)	3.0
208-7 (empty vector control)	4.3
236-1	5.0
236-2	5.6
236-3	4.4
236-4	5.2
236-5	5.9
236-6	3.3
236-7	4.5
236-8	4.2
236-9	3.2
236-10	5.3
236-11	5.4
236-13	4.7
236-14	4.7
236-15	3.4
236-16	3.4
236-17	4.4
236-18	3.7
237-1	4.6
237-2	3.0

The empty vector control lines (208) have a (1,3;1,4)- β -D-glucan content around 4% which is typical for wild type Golden Promise grain. Even though the T1 grain is bulked (and therefore contains null-segregant grains) a significant number of the transgenic lines (shaded) show an overall (1,3;1,4)- β -D-glucan content greater than the control, with the highest value at 5.9%.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The
5 invention also includes all of the steps, features, compositions and compounds referred to, or indicated in this specification, individually or collectively, and any and all combinations of any two or more of the steps or features.

Also, it must be noted that, as used herein, the singular forms “a”, “an” and “the”
10 include plural aspects unless the context already dictates otherwise. Thus, for example, reference to “a transgene” includes a single transgene as well as two or more transgenes; “a plant cell” includes a single cell as well as two or more cells; and so forth.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for modulating the level of (1,3;1,4)- β -D-glucan produced by a cell, the method comprising modulating the level and/or activity of a *CslH*-encoded
5 (1,3;1,4)- β -D-glucan synthase in the cell.
2. A method for modulating the level and/or activity of a (1,3;1,4)- β -D-glucan synthase in a cell, the method comprising modulating the expression of *CslH* nucleic acid in the cell.
10
3. A method for producing (1,3;1,4)- β -D-glucan, the method comprising transforming a cell with an isolated *CslH* nucleic acid and allowing the cell to express the isolated *CslH* nucleic acid.
- 15 4. The method of any one of claims 1 to 3 wherein the cell is a plant cell.
5. The method of claim 4 wherein the cell is a monocot plant cell.
6. The method of claim 4 or 5 wherein the cell is a cereal crop plant cell.
- 20 7. (1,3;1,4)- β -D-glucan produced according to the method of claim 3.
8. A cell comprising any one or more of:
a modulated level and/or activity of *CslH*-encoded (1,3;1,4)- β -D-glucan synthase
25 relative to a wild type cell of the same taxon; and/or
modulated expression of a *CslH* nucleic acid relative to a wild type cell of the same taxon.
9. The cell of claim 8 wherein the cell further comprises a modulated level of
30 (1,3;1,4)- β -D-glucan relative to a wild type cell of the same taxon.

10. The cell of claim 8 or 9 wherein the cell is produced according to the method of claim 1 or claim 2.
- 5 11. The cell of any one of claims 8 to 10 wherein the cell is a plant cell.
12. The cell of claim 11 wherein the cell is a monocot plant cell.
13. The cell of claim 11 or 12 wherein the cell is a cereal crop plant cell.
- 10 14. A multicellular structure comprising one or more cells according to any one of claims 8 to 13.
- 15 15. The multicellular structure of claim 14 wherein the multicellular structure is selected from the list consisting of a whole plant, a plant tissue, a plant organ, a plant part, plant reproductive material or cultured plant tissue.
- 20 16. The multicellular structure of claim 14 or 15 wherein the multicellular structure comprises a cereal crop plant or a tissue, organ or part thereof.
- 25 17. The multicellular structure of claim 16 wherein the multicellular structure comprises a cereal grain.
18. A cereal grain comprising a modulated level of (1,3;1,4)- β -D-glucan, wherein the grain comprises one or more cells comprising a modulated level and/or activity of a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase and/or modulated expression of a *CslH* nucleic acid.
- 30 19. Flour comprising:
flour produced by the milling of the grain of claim 17 or 18; and

optionally, flour produced by the milling of one or more other grains.

20. An isolated *CslH* nucleic acid or a complement, reverse complement or fragment thereof.

5

21. A genetic construct or vector comprising an isolated nucleic acid molecule according to claim 20.

10

22. A cell comprising either the isolated nucleic acid molecule of claim 20 or the genetic construct of claim 21.

23. The cell of claim 22 wherein the cell is a plant cell.

15

24. The cell of claim 23 wherein the cell is a monocot plant cell.

25. The cell of claim 23 or 24 wherein the cell is a cereal crop plant cell.

20

26. A multicellular structure which comprises one or more of cells of any one of claims 22 to 25.

27. The multicellular structure of claim 26 wherein the multicellular structure is selected from the list consisting of a whole plant, a plant tissue, a plant organ, a plant part, plant reproductive material or cultured plant tissue.

25

28. The multicellular structure of claim 26 or 27 wherein the multicellular structure comprises a cereal crop plant or a tissue, organ or part thereof.

29. The multicellular structure of claim 28 wherein the multicellular structure comprises a cereal grain.

30

30. An isolated polypeptide comprising an amino acid sequence defining a *CslH*-encoded (1,3;1,4)- β -D-glucan synthase polypeptide or a fragment thereof.
31. An isolated polypeptide of claim 31 wherein the polypeptide or fragment
5 thereof comprises one or more *CslH*-encoded (1,3;1,4)- β -D-glucan synthase epitopes.
32. An antibody or an epitope binding fragment thereof, raised against the isolated polypeptide or fragment thereof of claim 30 or 31.

FIGURE 1

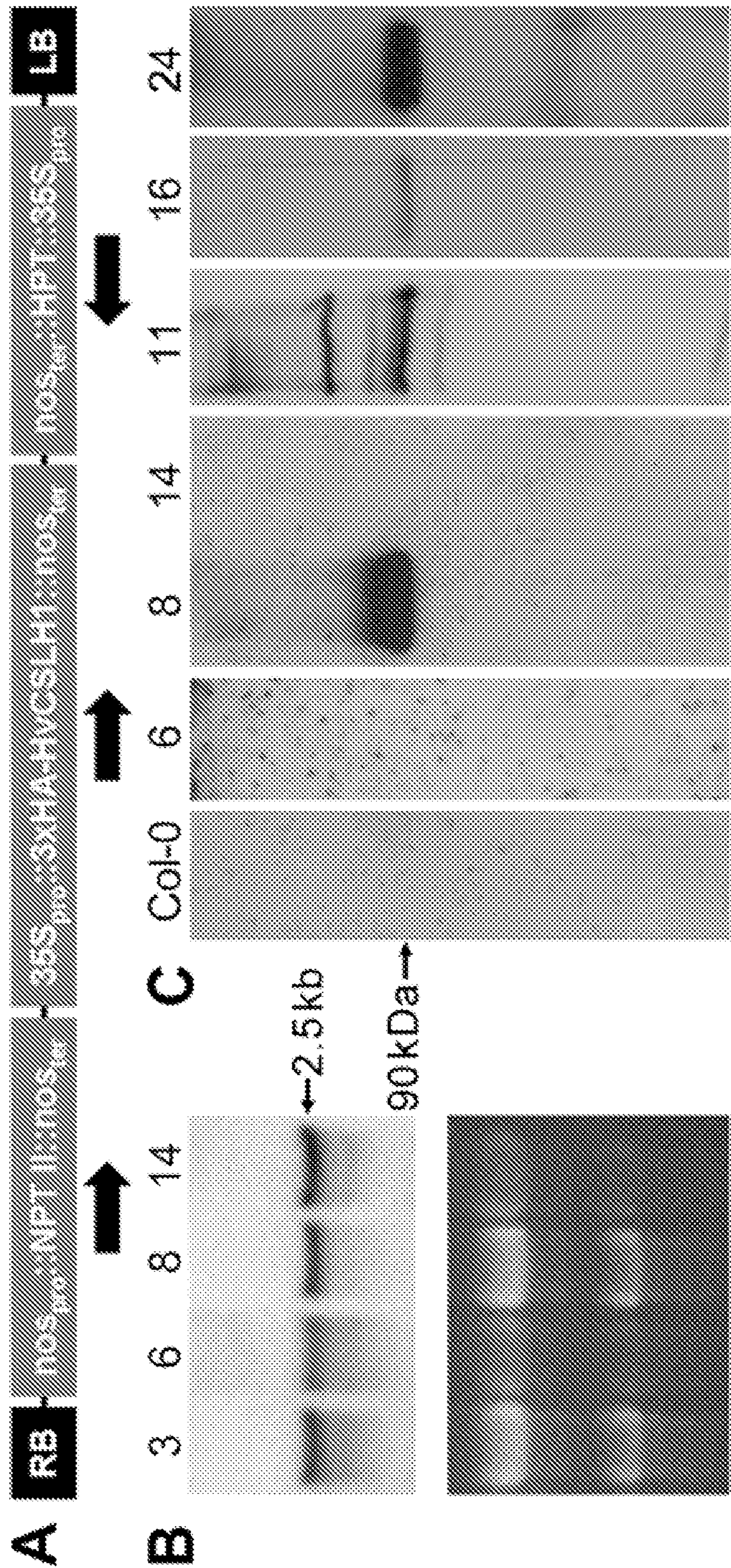


FIGURE 2

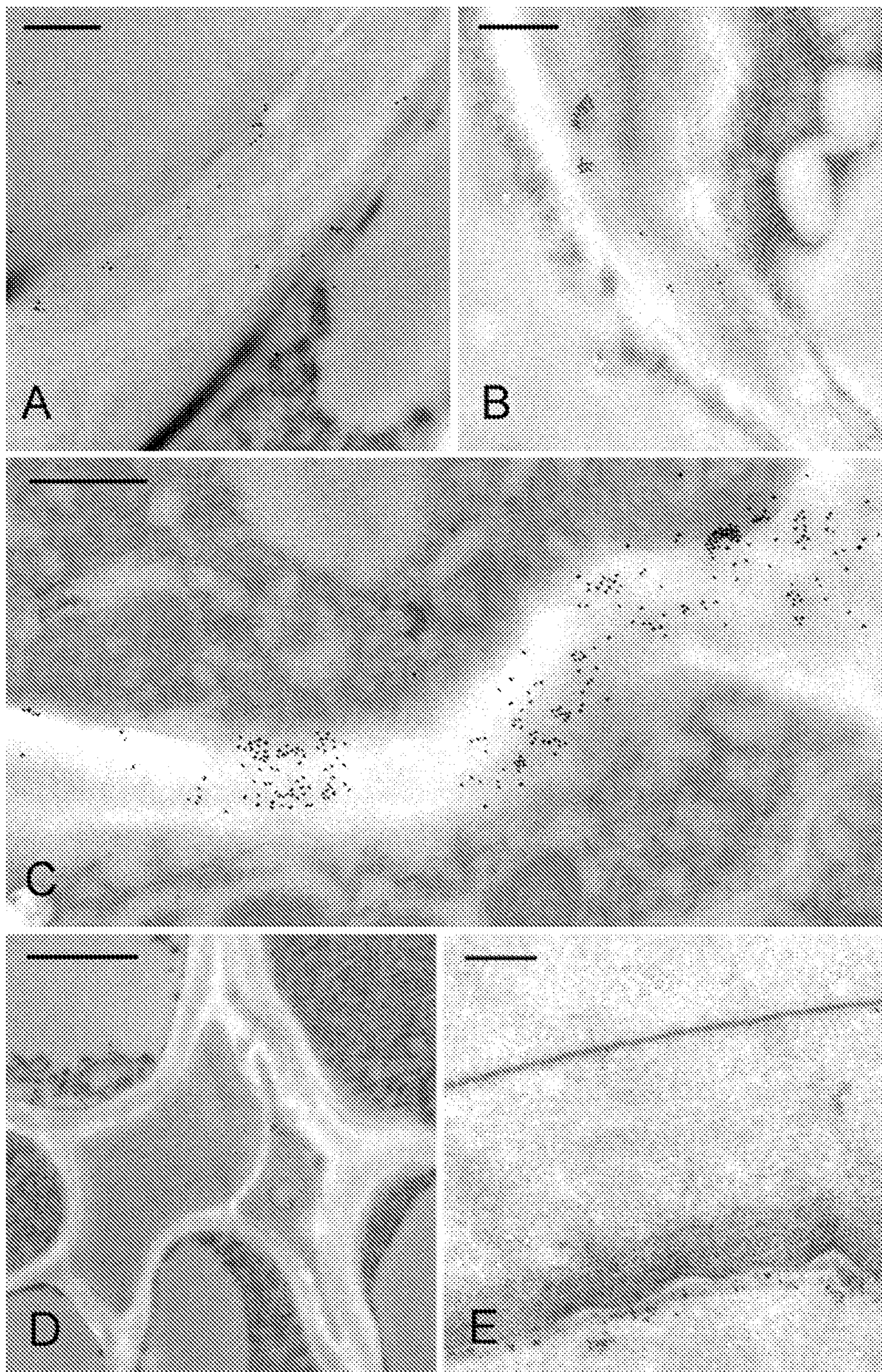


FIGURE 3

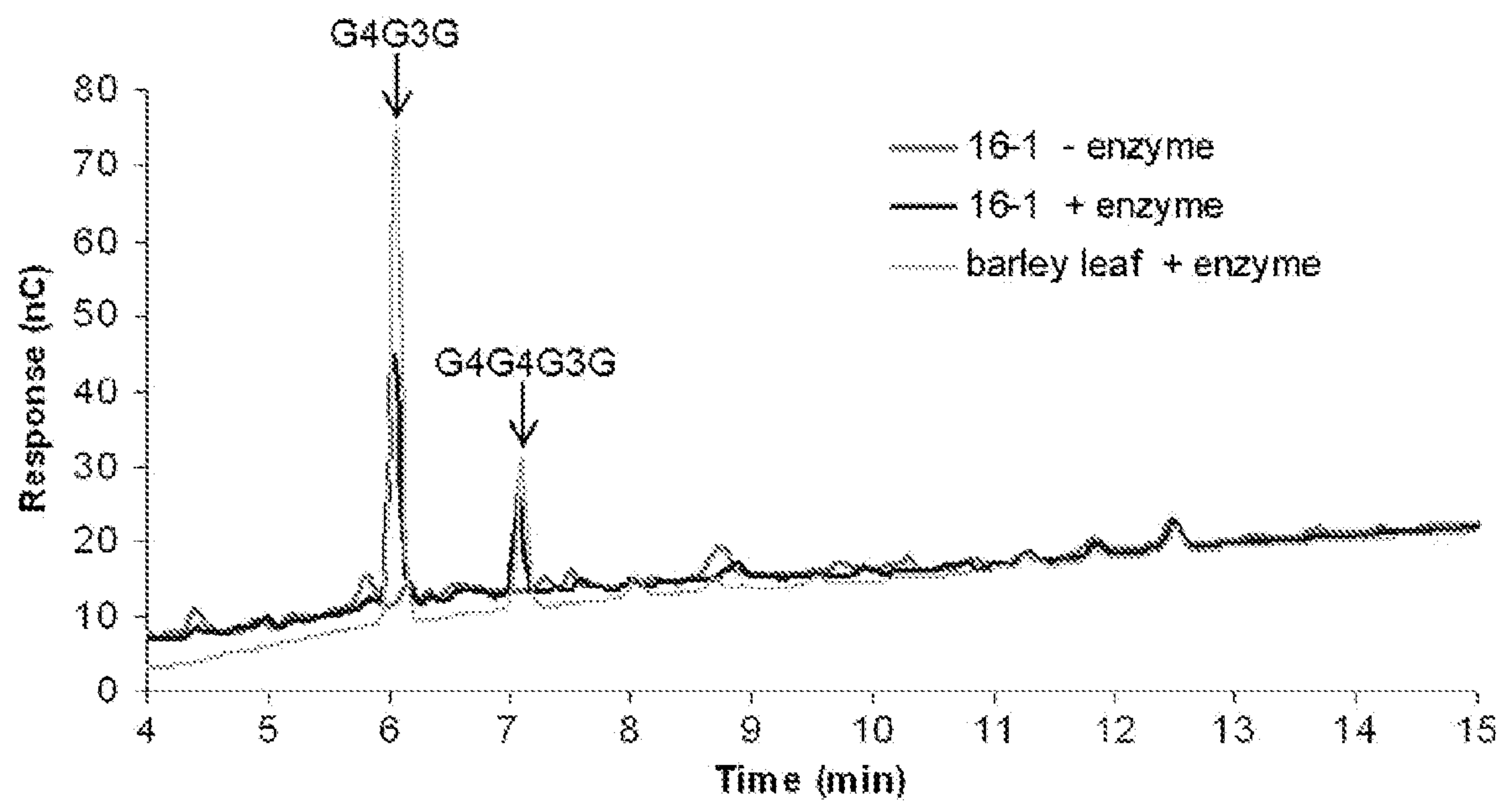


FIGURE 4

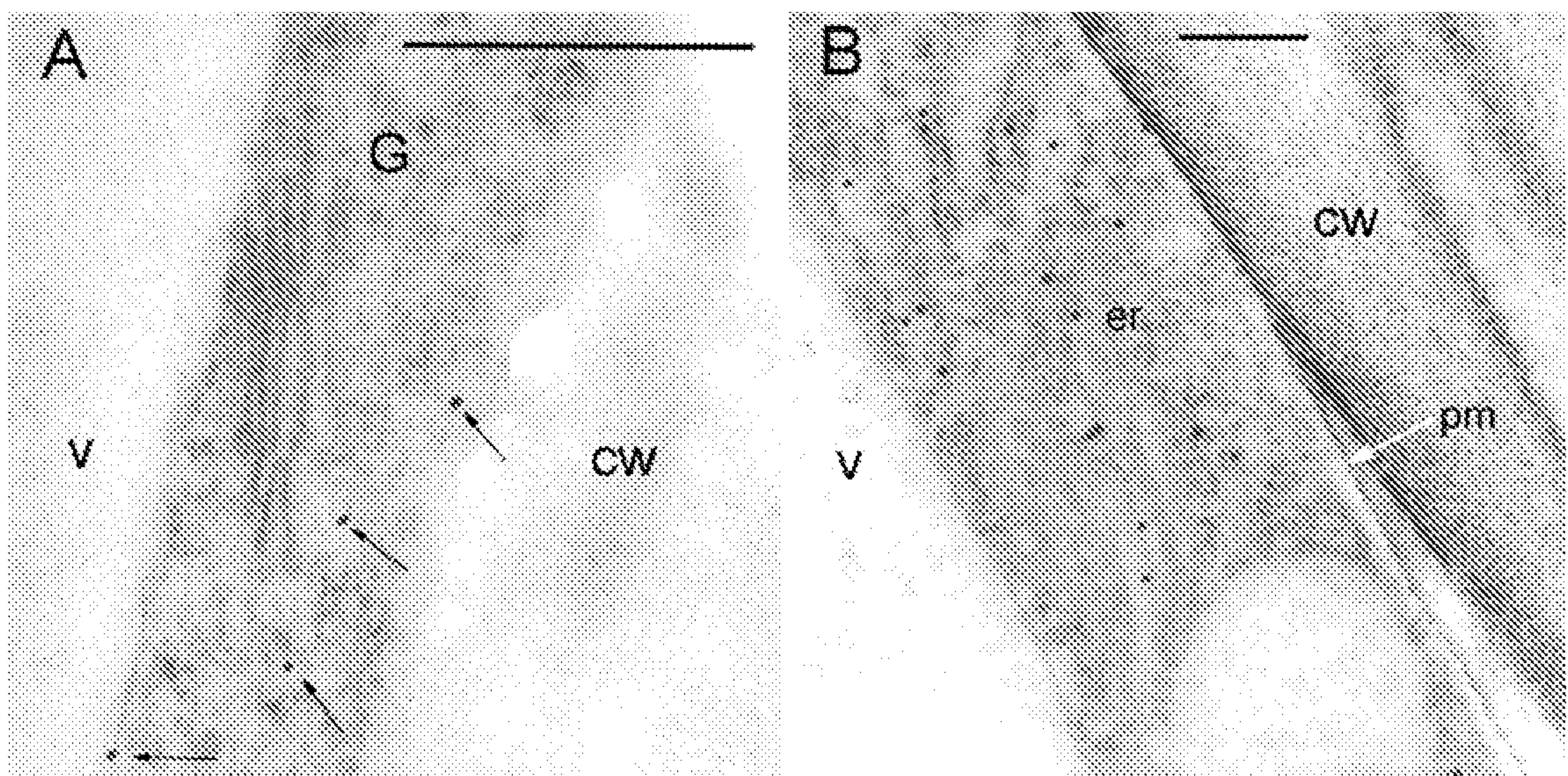


FIGURE 5

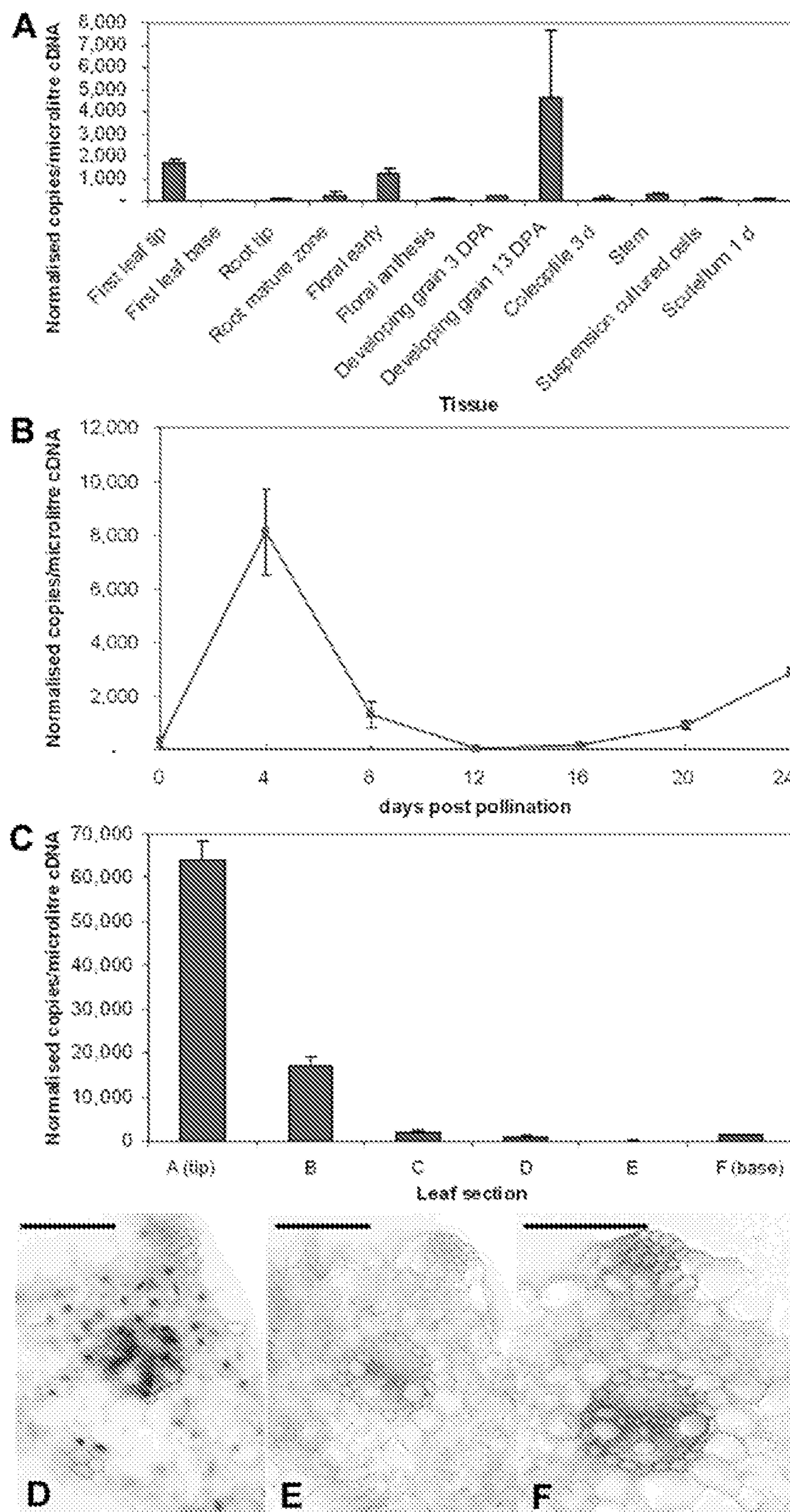


FIGURE 6

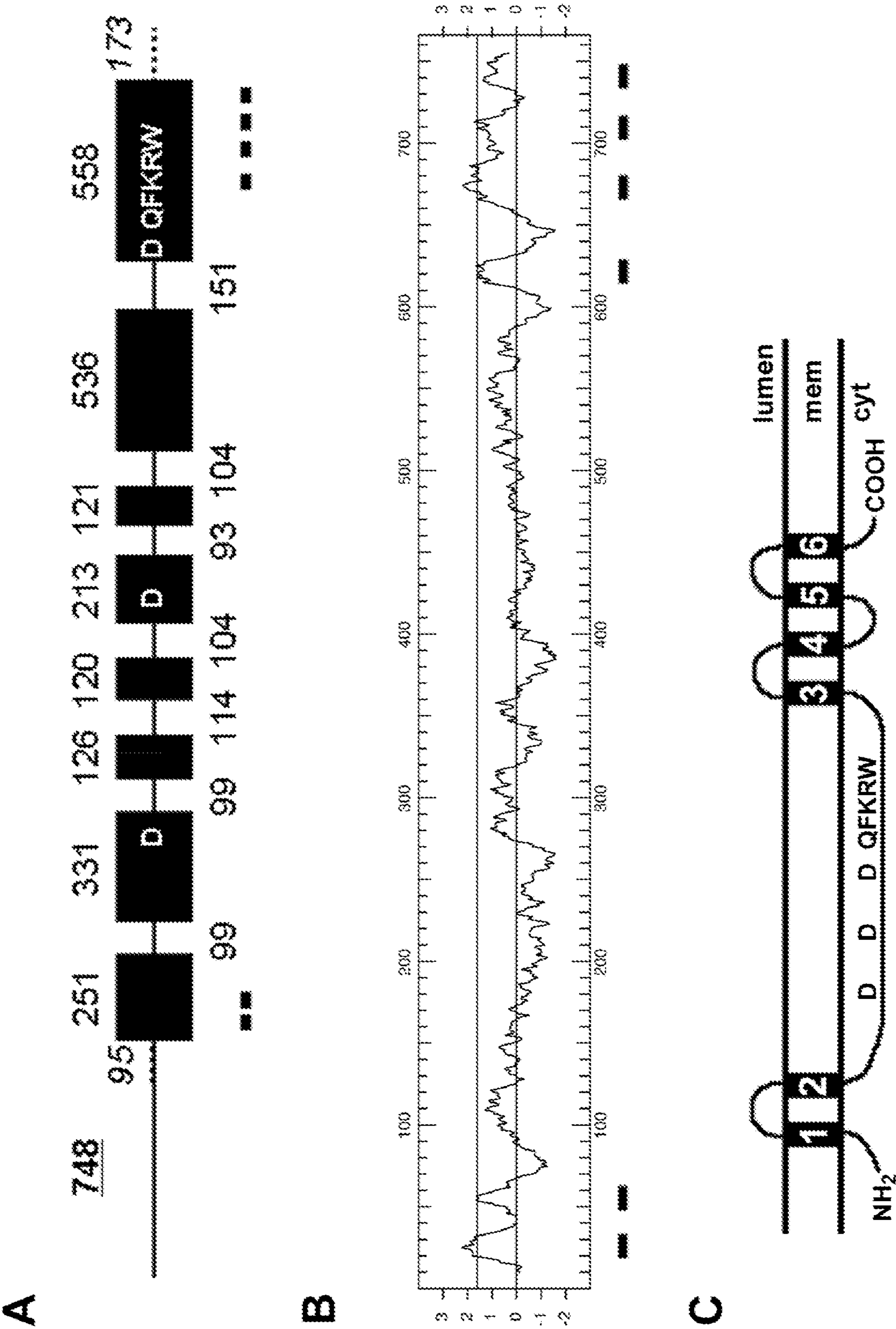


FIGURE 7

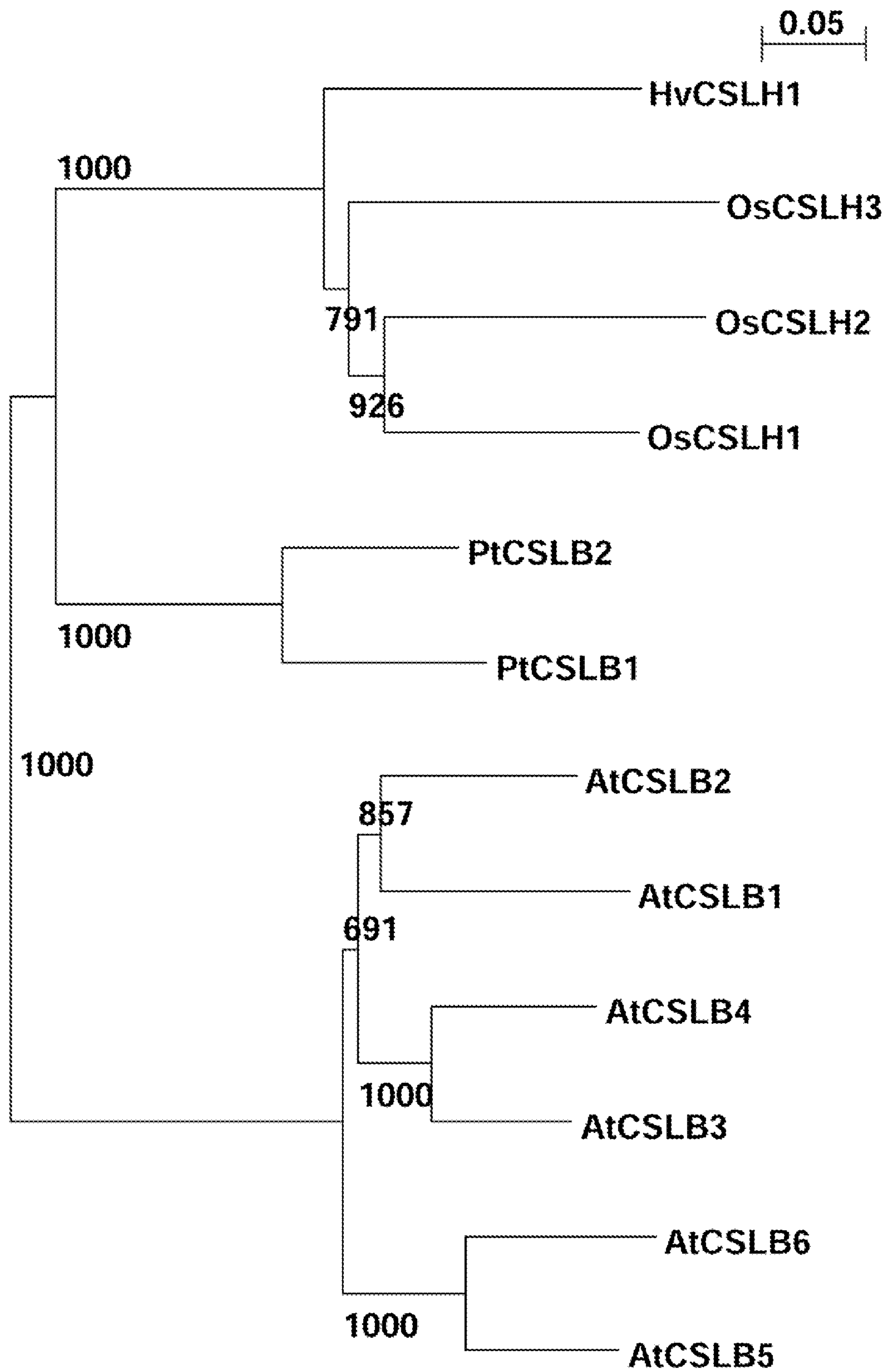


FIGURE 8

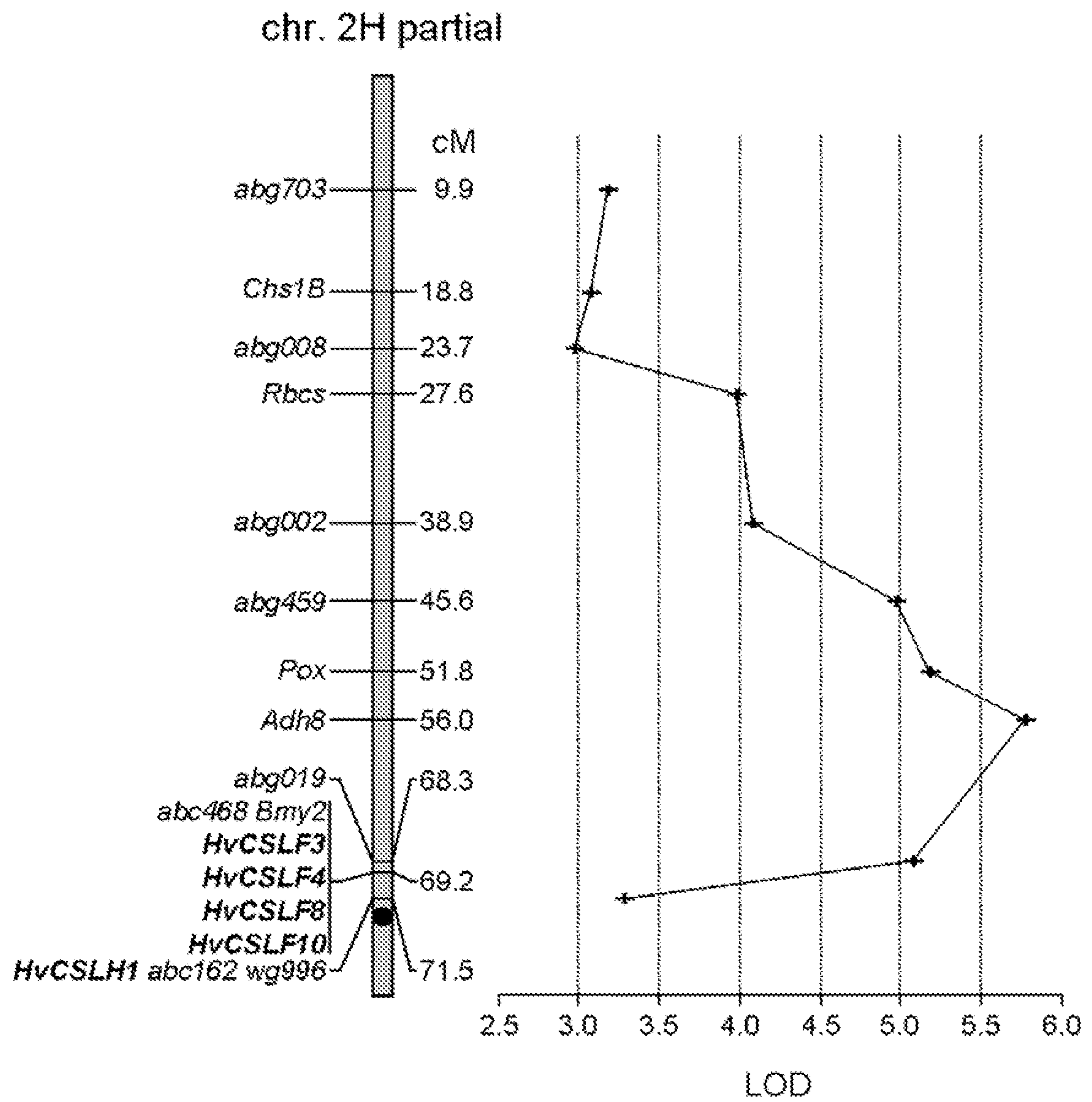


FIGURE 9

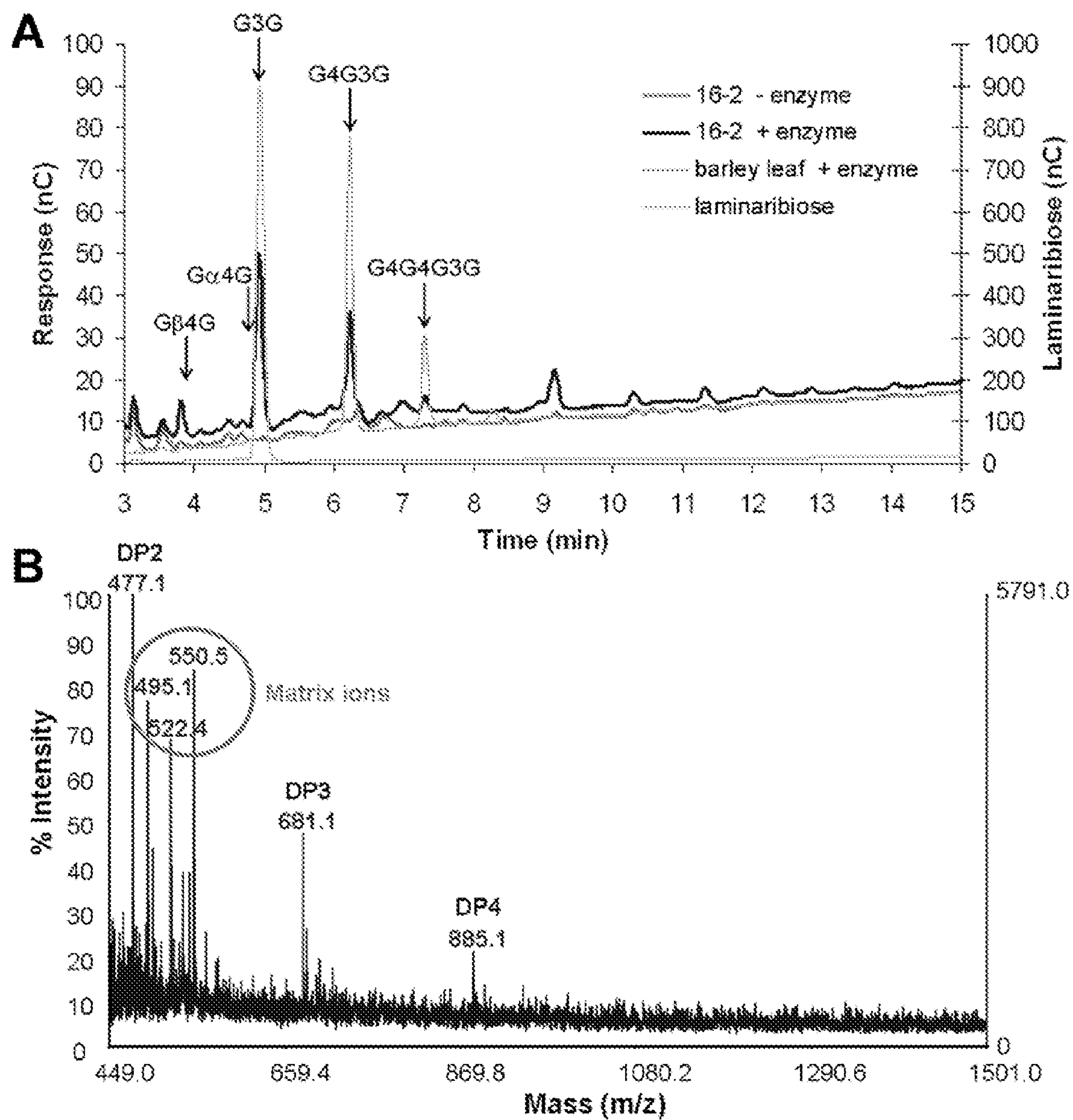


FIGURE 10

DNA id.		HvCslH1	OsCslH1	OsCslH2	OsCslH3			
Prt id.	Prt sim.							
HvCslH1	100%							
OsCslH1	74		100%					
	69	83						
OsCslH2	68		72		100%			
	62	76	65	78				
OsCslH3	71		76		71		100%	
	62	74	64	76	58	70		

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FIGURE 11

HvCs1H1	-----MAGG-----KQLQERVALAR---TAWMLADFAILFL	28
OsCSLH3	-----MAAASGEKEEE-----EKKLQERAPIRR---TAWMLANFVVLFL	36
OsCSLH1	-----MEAAARGN-----KKLQERVPIRR---TAWRLADLAILFL	32
OsCSLH2	MAVVAAAAATGSTTRSGGGGEGTRSGRKKPPPPPLQERVPLGRRAAWARLAGLAVLLL	60
	..*****: * ** **.:.:*:	
HvCs1H1	LLAIVARR--AASLRER---GGTWL-AALVCEAWFAFVWILNMNGKWSPVRFDTYPDNL	82
OsCSLH3	LLALLVRRATAADAEERGVGGAAR-VAFACEAWFAFVWLLNMNAKWSPARFDTYPENL	95
OsCSLH1	LLALLLHR---VLHDS---GAPWRRALACEAWFTFMWLLNVNAKWSPVRFDTFPENL	85
OsCSLH2	LLALLALRLLRHGGAGG-DAGVWR-VALVCEAWFAALCALNVSAKWSPVRFVTRPENLV	118
	:; * .. * .*:.*: : **:..***.* * *:***.	
HvCs1H1	NR-----MEELPAVDMFVTTADPALEPPLITVNTVLSL	116
OsCSLH3	GRCGAAHRPRKSSCISGHLDMRRQCALMQDRRAAGGRHVRDDGGPGARAAGGDGEQGAL	155
OsCSLH1	ER-----IDELPAVDMFVTTADPVLEPPLVTVNTVLSL	119
OsCSLH2	AEG-----RTPSTTAAEYGELPAVDMLVTTADPALEPPLVTVNTVLSL	162
	. : *.. . * . : . *	
HvCs1H1	ALDYPDVG-----KLACYVSDDGCSPVTCYALREAAKFAGLWVPFCRHRDVAVRAP	167
OsCSLH3	AARRRLLPGRRRRRRRRLACYVSDDGCSPTYALREAAGFARTWVPFCRRHGVAVRAP	215
OsCSLH1	ALDYPAAG-----EKLACYVSDDGCSPLTCYALREAAKFARTWVPFCRRHGVAVRAP	171
OsCSLH2	ALDYPRAG-----ERLACYVSDDGCSPLTCHALREAAGFAAAWVPFCRRYGVAVRAP	214
	* :*****:* :***** ** *****:;.******	
HvCs1H1	FMFYSS--TPEVGTGTADHEFLESWALMKSEYERLASRIENADEGSIMRDSGDE-FAEFI	224
OsCSLH3	FRYFAS--APEFGP--ADRKFLLDDWTFMKSEYDKLVRRIEDADETTLLRQGGGE-FAEFM	270
OsCSLH1	FRYFSS--TPEFGP--ADGKFLEDWTFMKSEYDKLVHRIEDADEPSLLRHGGGE-FAEFL	226
OsCSLH2	FRYFSSSSSPESGG-PADRFKLDDWTFMKDEYDKLVRIKNTDERSLLRHGGGEFFAEFL	273
	* **:* :** * ** :*:.*:***.**:*. **:::*** ::*:..*.* *****:	
HvCs1H1	DAERGNHPTIVKVLWDN-SKSKVGE--GFPHLVYLSREKSPRHRHNFQAGAMNVLTRVSA	281
OsCSLH3	DAKRTNHRAIVKVIWDNNSKNRIGEEGGFPHLIYVSREKSPGHHHHYKAGAMNALTRVSA	330
OsCSLH1	DVERGNHPTIIKVLWDN-NRSRTGD--GFPRLIYVSREKSPNLHHHHYKAGAMNALTRVSA	283
OsCSLH2	NVERRNHPTIVK-----TRVSA	290
	:.:* ** :*: *****	
HvCs1H1	VMTNAPIMLNVDCEMFANNPQVALHAMCLLLGFDDEIHSGFVQVPQKFYGGKDDPFGNQ	341
OsCSLH3	VMTNAPIMLNVDCEMFANDPQVVLHAMCLLLGFDDEISSGFVQVPQSFYGDLDKDDPFGNK	390
OsCSLH1	LMTNAPFMLNLDCDMFVNPNRVVLHAMCLLLGFDDEISCAFVQTPQKFYGALKDDPFGNQ	343
OsCSLH2	VMTNAPIMLNMDCEMFVNPNQAVLHAMCLLLGFDDEASSGFVQAPQRFYDALKDDPFGNQ	350
	:*****:***:*****.**:;..***** ***** .*.***.* ** . *****:	
HvCs1H1	MQVITTKIGGGIAGIQGMFYGGTGCFHRRKVIYGMPPPDVTK--HETRGSPSYKELQVRF	399
OsCSLH3	LEVIYK-----GLFYGGTGCFHCRKAIYGIEPDSIVVGREGAAGSPSYKELQFKE	440
OsCSLH1	LEVSLMKVGRGIAGLQGIFYCGTGCFHRRKVIYGMRTG----REGTTGYSSNKEHLSKF	398
OsCSLH2	MECFFKRFISGVQGVQGAIFYAGTGCFHRRKAVYGVPPNFNGAEREDTIGSSSYKELHTRF	410
	:; * ** ***** **:***: . . : * .* *****: ;*	
HvCs1H1	GSSKVLIESSRNIIISGDLARPTVDVSSRIEMAKQVGDCNIEAGTCWGKEIGWVYGSMT	459
OsCSLH3	ESSEELKESARYIIISGDMSGEPIVDISSHIEVAKEVSSCNYESGTHWGLEVGWAYGSMT	500
OsCSLH1	GSSNFKESARDVIYGNLSTEPVDISSCVDVAKEVAACNIEIGTCWGQEVGWVYGSLTE	458
OsCSLH2	GNSEELNESARNIIW-DLSSKPMVDISSRIEVAKAVSACNYDIGTCWGQEVGWVYGSLTE	469
	.*: : **:* :* :; .* **:*** :::*** *. *****: ** ** *:***.***:***	
HvCs1H1	DILTGQRIHAAGWKSALLDTNPPAFLGCAPTGGPASLTQFKRWATGVLEILISRNSPILG	519
OsCSLH3	DILTGQRIHAAGWRSKLETEPPAFLGCAPTGGPACLTQFKRWATGLFEILISQNNPLLL	560
OsCSLH1	DVLTGQRIHAAGWRSTLMEIEPPAFMGCAPNGGPACLTQLKRWASGFLEILISRNNPILT	518
OsCSLH2	DILTGQRIHAMGWRSVLMVTEPPAFMGSAPIGGPACLTQFKRWATGQSEIIISRNNPILA	529
	*:***** **:*. : :*****:.* *****.*****:* **:***:***:*	
HvCs1H1	TIFQRLQLRQCLGYLIVEAWPVRAFPELCYALLGPFCLLTNQSFLLPTASDEGFRIPVALF	579
OsCSLH3	SIFKHLQFRQCLAYLTLYVWAVRGFVELCYELLVPYCLLTNQSFLSKASENCFNITLALF	620
OsCSLH1	TTFKSLQFRQCLAYLHSYVWPVRAFPELCYALLGPYCLLSNQSFLLPKTSEDGFYIALALF	578
OsCSLH2	TMFKRLKFRQCLAYLIVLGWPLRAPFPELCYLLGPYCILTNQSFLLPKASEDGFVPLALF	589
	: * : *:***** ** *: * ***** ** *:*****:***: * :*****	

FIGURE 11 (Cont.)

HvCs1H1	LSYHIYHLMEYKECGLSARAWWNNHRMQRITSASAWLLAFLTVILKTLGLSETVFEVTRK	639
OsCSLH3	LTYNNTYNFVEYMECGLSVRAWWNNHRMQRIISASAWLLAFFTVLLKTIGLSETVFEVTRK	680
OsCSLH1	IAYNTYMFMEFIECGQSARACWNNHRMQRITSASAWLLAFLTVILKTLGFSETVFEVTRK	638
OsCSLH2	ISYNTYNFMEYMACGLSARAWWNNHRMQRIISVSAWTLAFLTVLLKSLGLSETVFEVTGK	649
	:.*: * :*: ** *.** ***** *.**.* ***:**:*:*.**.* ** *	
HvCs1H1	ESSTSDGGAGTDEADPGLFTFDSAPVFIPVTALSVLNIVALAVGAWRAVIGTAAVVHGGP	699
OsCSLH3	EKSTSDGNGQNDEVDPERFTFDASPVFIPVTALTMLNIVAITIGTWRAVFGTTEDVPGGP	740
OsCSLH1	DKSTSDGDSNTDEPEPGRFTFDESTVFIPVTALAMLSVIAIAGAWRVVLVTTEGLPGGP	698
OsCSLH2	DKSMSDDDDNTDGADPGRFTFDSLVPFIPVTALAMLNIVAVTVGACRVAFGTAEGVPCAP	709
	:.* **.. .* :* ***** .*****:*.**:*:*.**.* **..: *: : .*	
HvCs1H1	GIGEFVCCGWMVLCFWPFVRLVS-RGKHGIPWSVKVKAGLIVAAFVHLCTRN	751
OsCSLH3	GISEFMSCGWLLLCLLPFVRGLVG-KGSYGIPWSVKLKASLLVALFLFCSNRN	792
OsCSLH1	GISEFISCGWLVLCFMPLLRGLVG-SGRYGIPWSIKMKACLLVAIFLLFCKRN	750
OsCSLH2	GIGEFMCCGWLVLCFFPFVRGIVWGKGSYGIPWSVKLKASLLVAMFVTFCKRN	762
	.:.**:.**: *:.**: * :*****:*.**.**:** *: **.* *	

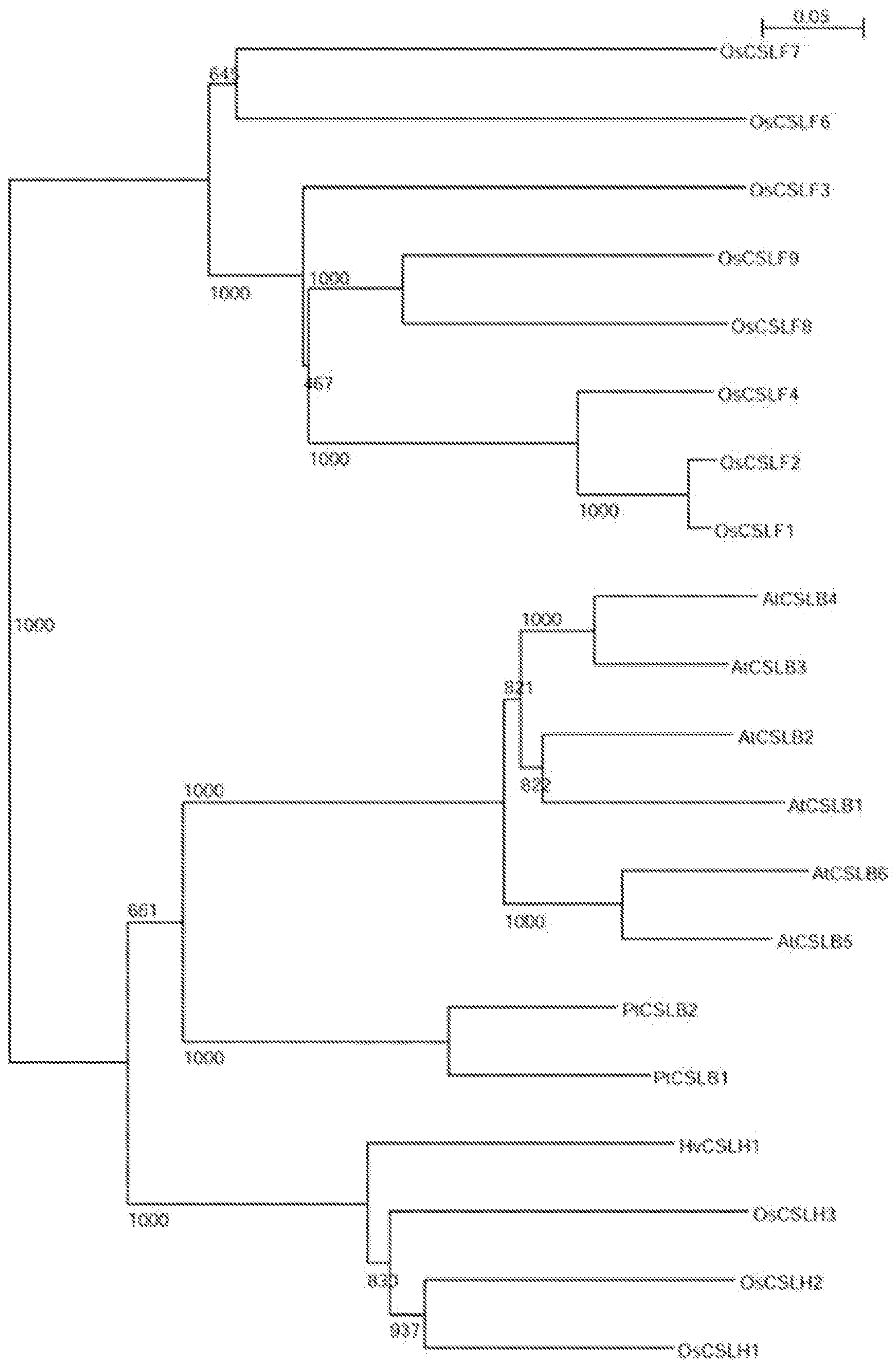


FIGURE 13

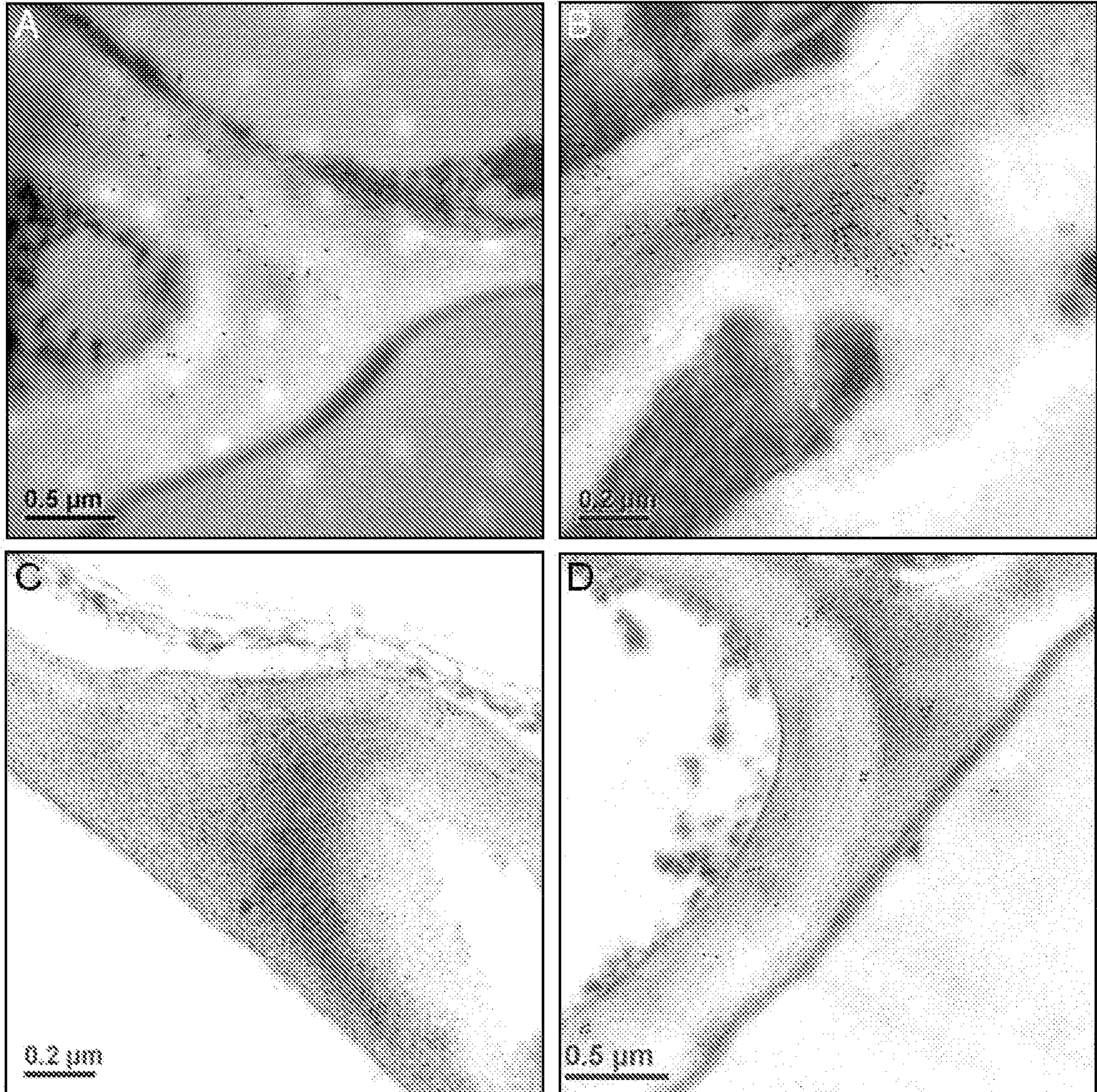


FIGURE 14

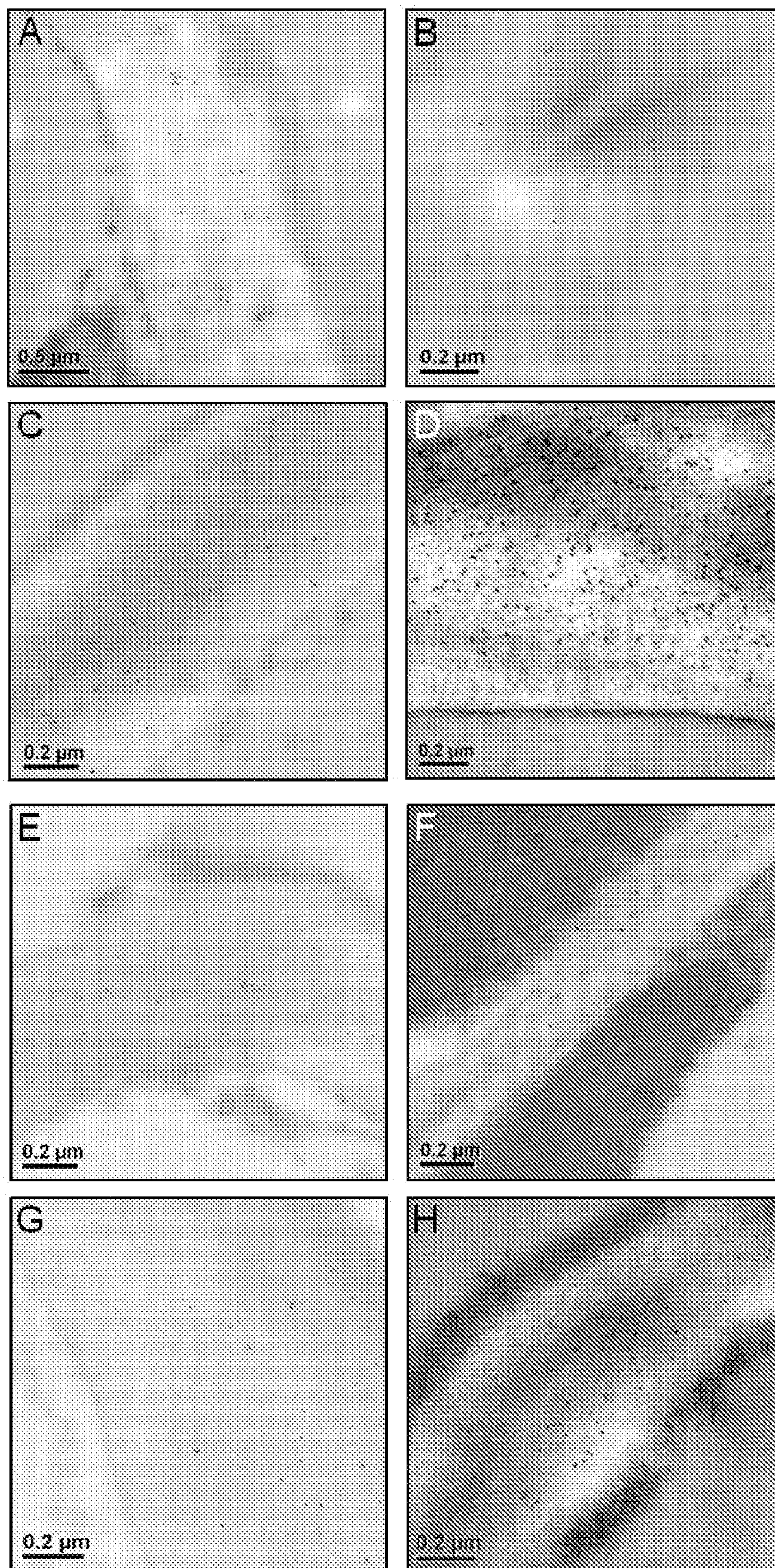


FIGURE 15

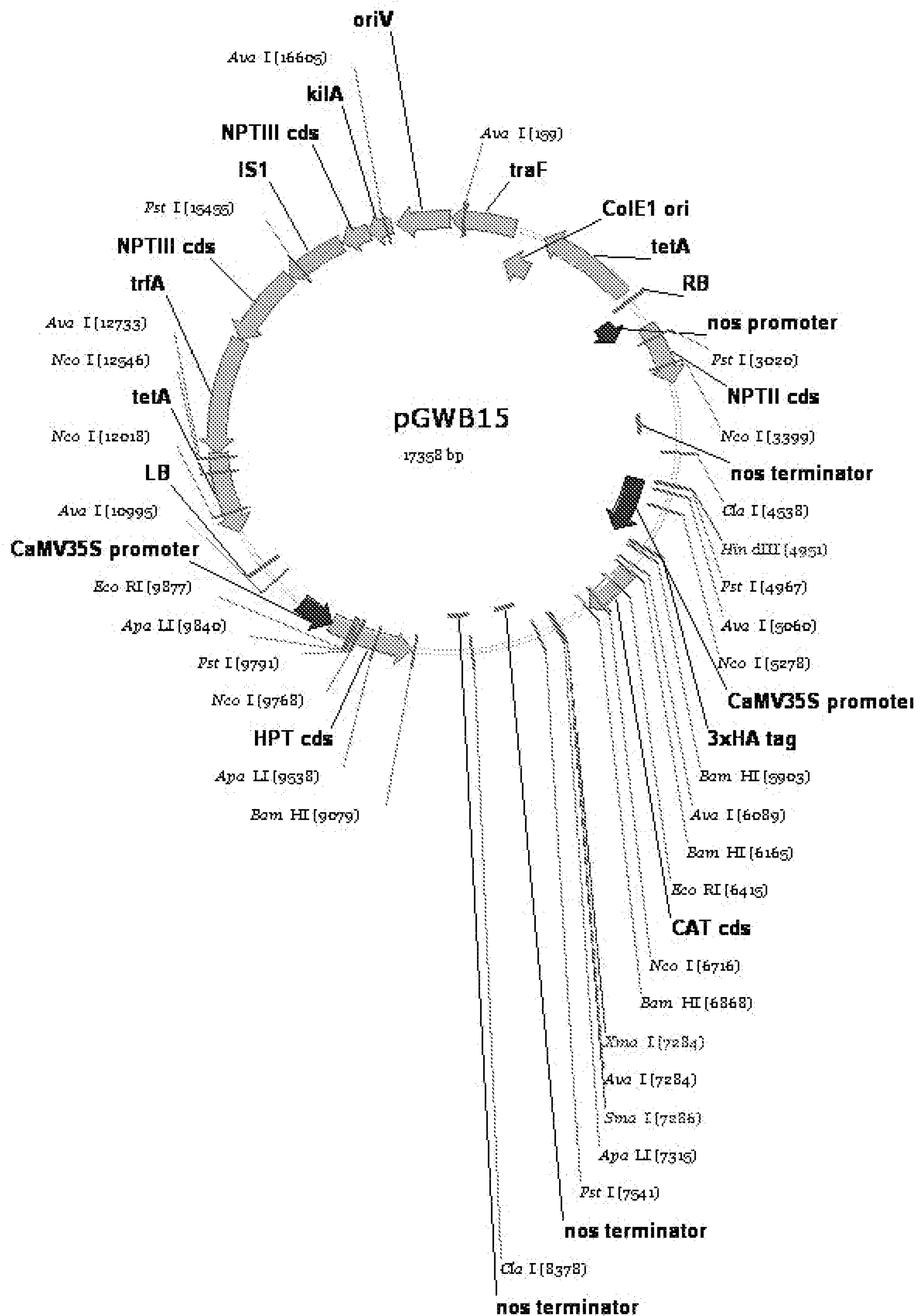


FIGURE 16

[illegible]

FIGURE 16 (Cont.)

HVCs1H11(H1m)	820	830	840	850	860	870	880	890	900
									
	GTGGGGAGGATCCACATCTGGTGTACCTCTCTCGAGAGAAAGCCCGAGACATCGCCACAACTTCAGGCTGGTGCATGAATGTT								
	V G E G F P H L V Y L S R E K S P R H R H N F K A G A M N V								
HVCs1H11(H1m)	910	920	930	940	950	960	970	980	990
									
	CTGACAAAGGGTGTCAAGCGGTGATGACCAAGGCTCCCATCATGCTGAAATGTGGACTGGGAGATGTTGCGCCAAATCCGCAGGTGCCCCTA								
	L T R V S A V M T N A P I M L N V D C D M F A N N P Q V A L								
HVCs1H11(H1m)	1000	1010	1020	1030	1040	1050	1060	1070	1080
									
	CACGCGATGTGCCTCCTATTGGGGTTCGAGACGAGATCCACAGCGGTTGTCCTCAAGTCCACAGAGATTCACGGTGGCCTCAAGGAC								
	H A M C L L L G F D D E I H S G F V Q V P Q K F Y G G L K D								
HVCs1H11(H1m)	1090	1100	1110	1120	1130	1140	1150	1160	1170
									
	GATCCCTTTGGCAACCAGATGCAGGTATTAACCAAGAAATGGAGGTGGAAATCGCGGATCCAAAGCATGTTCTACGGCGGCACGGGC								
	D P F G N Q M Q V I T K K I G G I A G I Q G M F Y G G T G								
HVCs1H11(H1m)	1180	1190	1200	1210	1220	1230	1240	1250	1260
									
	TGTTTTCACCGCAGGAAGTCATTACGGCATGCCGCCACCTGACACCGTCAACACAGAGACAAGGTTACCATCTTACAGGAGCTG								
	C F H R R K V I Y G M P P P D T V K H E T R G S P S Y K E L								
HVCs1H11(H1m)	1270	1280	1290	1300	1310	1320	1330	1340	1350
									
	CAAGTCAGGTTTGGGAGCTCAAGGTGTGATCGAATCATCTAGGAACATCATCTCAGGAGACCTGCTCGCTAGACCAACCGTTGATGTA								
	Q V R F G S S K V L I E S S R N I I S G D L L A R P T V D V								
HVCs1H11(H1m)	1360	1370	1380	1390	1400	1410	1420	1430	1440
									
	TCGAGTCGCATCGAAATGGCAAAACAGTCGGCGGATTGCAACTATGAGGCTGGCACCGTGTGGGCAAGGAGATTGGTTGGTCTATGGA								
	S S R I E M A K Q V G D C N Y E A G T C W G K E I G W V Y G								
HVCs1H11(H1m)	1450	1460	1470	1480	1490	1500	1510	1520	1530
									
	TCAATGACAGAGACATTTTGACCGACACCGGATCCATGGCGGTTTGGAATCGGCCTTGTGGACACCAACCCACCGGCATTCTTG								
	S M T E D I L T G Q R I H A A G W K S A L L D T N P P A F L								

FIGURE 16 (Cont.)

HVCs 1H1(Him)	1540	1550	1560	1570	1580	1590	1600	1610	1620
									
	GGATGTGCTCCGACCGGGGACCGGCTTGACCCAGTTCAAGAGATGGCAACAGGGGTTCTGGAGATACTCATGAGCCGGACAGC								
	G C A P T G G P A S L T Q F K R W A T G V L E I L I S R N S								
HVCs 1H1(Him)	1630	1640	1650	1660	1670	1680	1690	1700	1710
									
	CCTATCCTCGGCACCATCTTCCAAAGCCTCCAACTCCGGCAATGCCTTGGCTATCTCATCTGTCGAGGGTGCCCGTGAGGGCGCCTTTC								
	P I L G T I F Q R L Q L R Q C L G Y L I V E A W P V R A P F								
HVCs 1H1(Him)	1720	1730	1740	1750	1760	1770	1780	1790	1800
									
	GAGCTGTGCTATGCACCTATTGGGACCTTTCTGCCCTTCTCACAACCAAGTCCTTCTTGCCAAAGGATCGGATGAAGTTTTTCGCATCCCA								
	E L C Y A L L G P F C L L T N Q S F L P T A S D E G F R I P								
HVCs 1H1(Him)	1810	1820	1830	1840	1850	1860	1870	1880	1890
									
	GTAGCTCTATTCTTGAGTTACCATATACCACTTGATGGAGTACAGGAGTGCGGCTCTCTGCCCGCCCTGGTGGAAACAACACAGS								
	V A L F L S Y H I Y H L M E Y K E C G L S A R A W N N H R								
HVCs 1H1(Him)	1900	1910	1920	1930	1940	1950	1960	1970	1980
									
	ATGCAACGCATCACCCTGGGCTCCGCTGCTCCCTGCTTCTCCTACCGTGATCCTCAAGACACTAGGGCTCTCTGAGACCGTGTTCGAG								
	M Q R I T S A S A W L L A F L T V I L K T L G L S E T V F E								
HVCs 1H1(Him)	1990	2000	2010	2020	2030	2040	2050	2060	2070
									
	GTCACCGCAAGGAACAGCACGTCCGATGGCGGCGCGCACCGACCGGATCCAGGACTGTTTCACATTCGACTCGCGCGCGGTT								
	V T R K E S S T S D G G A G T D E A D P G L F T F D S A P V								
HVCs 1H1(Him)	2080	2090	2100	2110	2120	2130	2140	2150	2160
									
	TTCATCCCGGTGACGGGCTCTCAGTGTGAACATTGTGGCCCTCCCGTGGGGCATGCCGCGTCAATCGGACTCGCGCGGTGTT								
	F I P V T A L S V L N I V A L A V G A W R A V I G T A A V V								
HVCs 1H1(Him)	2170	2180	2190	2200	2210	2220	2230	2240	2250
									
	CATGGTGGCCCGGCATCGGAGATTCTGTGCTGTGGCTGGATGGTTGTGCTTCTGCGGTGAGAGGGCTTGTGAGCAGGGGA								
	H G G P G I G E F V C C G W M V L C F W P F V R G L V S R G								
HVCs 1H1(Him)	2260	2270	2280	2290	2300	2310	2320	2330	
									
	AAGCATGGAAATCCCGTGGAGGCTCAAGGTGAAGGCTGGTTGATTGGCTGGCTTGGTGCACCTCTGCACAGGAACTAACC								
	K H G I P W S V K V K A G L I V A A F V H L C T R N *								

FIGURE 17

	10	20	30	40	50	60	70	80	90
									
HvCslH1	-----								
HvCslH1Him	--GAGCTGTGTTTCGTGGAGCTTAGCTAGTCTGCTACTGCTACTGCTGGCTAGTGGCTAC...								
HvCslH1g	-----								
HvCslH1gHim	--GAGCTGTGTTTCGTGGAGCTTAGCTAGTCTGCTACTGCTACTGCTGGCTAGTGGCTAC...								
TaCslH1-1	-----					CTG-----			CTCTCGGC
TaCslH1-2	-----								
TaCslH1-3	GGAAATGCACCGAGCAGAGGTTAGTTTATCCGGTCTTTATAAAATGCACTCTGTGGTGTG..					CTAGCTAGCTAGTCTGCTAC.....			
	100	110	120	130	140	150	160	170	180
									
HvCslH1	-----								
HvCslH1Him	.A.....								
HvCslH1g	-----								
HvCslH1gHim	.A.....								
TaCslH1-1	CGCGGCCA								
TaCslH1-2	.A.....								
TaCslH1-3									
	190	200	210	220	230	240	250	260	270
									
HvCslH1	A.....								
HvCslH1Him	A.....								
HvCslH1g	A.....								
HvCslH1gHim	A.....								
TaCslH1-1	CCTCCTCGCCCTCGTGGCCCCGCCGCGCCGCTCGCTCGGGGAGCGCGGGACGTGGCTGGCGGCGCTCGTCTGCGAGGCGTGGTTTCGC								
TaCslH1-2	.G.....								
TaCslH1-3									
	280	290	300	310	320	330	340	350	360
									
HvCslH1	G.....								
HvCslH1Him	G.....								
HvCslH1g	G.....								
HvCslH1gHim	G.....								
TaCslH1-1	CTTCGTCTGGATCCTCAACATGAACGGCAAGTGGAGCCCCGTCCGGTTCGACACCTACCCCGAGAACCTCTCCCACAG GT ACGTACGTTC								
TaCslH1-2	G.....								
TaCslH1-3									
	370	380	390	400	410	420	430	440	450
									
HvCslH1	-----								
HvCslH1Him	-----								
HvCslH1g	AC..A.GT..CC..G..G.C.AG.C-CCAC.A.G.T.ACC.C.A..A....C..AAATT..G.T..AGC.A.....A.....								
HvCslH1gHim	AC..A.GT..CC..G..G.C.AG.C-CCAC.A.G.T.ACC.C.A..A....C..AAATT..G.T..AGC.A.....A.....								
TaCslH1-1	TTGTGCACACTAACTGCAAAATAATGTTGACCTACAGCTTCGTGCAGCTTCTTC----CTTAAACTGTGTCGTGTCTGTGATGATTTTGC								
TaCslH1-2									
TaCslH1-3									
	460	470	480	490	500	510	520	530	540
									
HvCslH1	---.A.....								
HvCslH1Him	---.A.....								
HvCslH1g	A.....								
HvCslH1gHim	A.....								
TaCslH1-1	TAG GCTGGAGGAGCTCCCGCGGTGGACATGTTTCGTCACGACGGCGGACCCGGCGCTGGAGCCGCCGTTGATCACGGTGAACACGGTGCT								
TaCslH1-2	A....A.....								
TaCslH1-3	A.....								
	550	560	570	580	590	600	610	620	630
									
HvCslH1									
HvCslH1Him									
HvCslH1g									
HvCslH1gHim									
TaCslH1-1	CTCGCTGCTCGCCCTGGACTACCCGGACGTCGGCAAGCTGGCGTGCTACGTCTCCGACGACGGCTGCTCCCCGGTGACGTGCTACGCGCT								
TaCslH1-2									
TaCslH1-3									

FIGURE 17 (Cont.)

	640	650	660	670	680	690	700	710	720
									
HvCslH1	...T.....G.....G.C..T.....C.C.....C.....A.....								
HvCslH1Him	...T.....G.....G.C..T.....C.C.....C.....A.....								
HvCslH1g	...T.....G.....G.C..T.....C.C.....C.....A.....								
HvCslH1gHim	...T.....G.....G.C..T.....C.C.....C.....A.....								
TaCslH1-1	GCGCGAGGCCGCCAAGTTCGCCAGCCTCTGGATTCCCTTCTGCAAGAGGTATGACGTTGGTGTGAGGGCCCCCTTTCATGTACTTCTCTTC								
TaCslH1-2	G.....C.C.....								
TaCslH1-3	G.....G.....T.....C.C.....								
	730	740	750	760	770	780	790	800	810
									
HvCslH1	.A.....A..C.....G.....-----								
HvCslH1Him	.A.....A..C.....G.....-----								
HvCslH1g	.A.....A..C.....G.....A.....G...								
HvCslH1gHim	.A.....A..C.....G.....A.....G...								
TaCslH1-1	CGCGCCGAGGTTGGCACCAGGTACAGCCGACCACGAGTTCCCTGGAAAAGCTGGGCACTCATGAAG GT TAGGCGCC-ATGGTGACCATTTC								
TaCslH1-2	A.....T.....C..C.---...A...TG..G...								
TaCslH1-3	A..A...T.....T....-.....T...GC.G								
	820	830	840	850	860	870	880	890	900
									
HvCslH1	-----G.....GA..								
HvCslH1Him	-----G.....GA..								
HvCslH1g	A.AAA.....A.....G...T.....G.....GA..								
HvCslH1gHim	A.AAA.....A.....G...T.....G.....GA..								
TaCslH1-1	GTTTCC---ATAATGTTTGGTCGTCATCGTCGCCATGACCATGCATCTTCCTCGTGTACGTGTGACTTT CAG ACCGAATATGAGAAGCT								
TaCslH1-2	---.....A.....T.....G.....								
TaCslH1-3	-----.....A.....-.....G.C.....								
	910	920	930	940	950	960	970	980	990
									
HvCslH1	A.....A.....T...G.....A.....A.....C.....								
HvCslH1Him	A.....A.....T...G.....A.....A.....C.....								
HvCslH1g	A.....A.....T...G.....A.....A.....C.....								
HvCslH1gHim	A.....A.....T...G.....A.....A.....C.....								
TaCslH1-1	GGCCAGCCGGATCGAGAACGCCGACGAGGTCTCCATTCTGCGTGACGGCGGCGAAGAGTTCGCCGAGTTCATCGACCCGAGCGCGGGAA								
TaCslH1-2	-...-.....-.....C.....								
TaCslH1-3	C.....G.A.A..A.....C.....								
	1000	1010	1020	1030	1040	1050	1060	1070	1080
									
HvCslH1	-----								
HvCslH1Him	-----								
HvCslH1g	TGTCATGCATG.....A.....A.....A.....GT								
HvCslH1gHim	TGTCATGCATG.....A.....A.....A.....GT								
TaCslH1-1	CCATCCTACCATCGTTAAG GT CGCCGCACTGACCA-----TGTCATGTACATCGTGTGATGCCAAACGCGTAGCAAATCC--								
TaCslH1-2	T.....TATC----CACG.....G.....A...G....--								
TaCslH1-3	GT.....TGTT----CATT.....G.....A.....--								
	1090	1100	1110	1120	1130	1140	1150	1160	1170
									
HvCslH1	-----G.....TG.....								
HvCslH1Him	-----G.....TG.....								
HvCslH1g	TT.G.GCC.....G.....TG.....								
HvCslH1gHim	TT.G.GCC.....G.....TG.....								
TaCslH1-1	GTCTCGTGCTAATATCGTCACGGTTAACCT-GTGTGAGTT CAG GTTCTCTGGGATAACAGCAAGAGCAAAGCAGGGGAAGGATTCCCA								
TaCslH1-2	-..C.....A...A...T.....								
TaCslH1-3	T.-..C.....C.....A.....								
	1180	1190	1200	1210	1220	1230	1240	1250	1260
									
HvCslH1	-----C...T.....-----								
HvCslH1Him	-----C...T.....-----								
HvCslH1g	C...T.....TCG								
HvCslH1gHim	T.....TCG								
TaCslH1-1	TCTGGTGTACCTCTCTCGAGAGAAAAAGCCCCAGACATCGCCACAACCTTCAAGGCCGGTGCCATGAATGTTCT GT GAGCACTCTCTTGTA								
TaCslH1-2	GA.....T.....T.....C..								
TaCslH1-3	T.....T.....C..								

FIGURE 17 (Cont.)

	1270	1280	1290	1300	1310	1320	1330	1340	1350
									
HvCslH1	-----								
HvCslH1Him	-----								
HvCslH1g	.T..AC.....G.....C.....--.....G.....-----CCA.AT..								
HvCslH1gHim	.T..AC.....G.....C.....--.....G.....-----CCA.AT..								
TaCslH1-1	CACA--ACAGTGTTCAC	TGGTAATCAGTGTGT	CACACAAACAGCACAATAAGTGGCAGTTGAAAGTTCAGACATGTGTACAATGCGCTT						
TaCslH1-2	.T..AT.....G....AC.....A.--.....A.A....C.....GGT..C..G.CA.AT..								
TaCslH1-3	.T..AT...A....G....AC.....A.--.....A..A.A.....G---.C.TG.CA.TT..								
	1360	1370	1380	1390	1400	1410	1420	1430	1440
									
HvCslH1	-----				A.....C.....				
HvCslH1Him	-----				A.....C.....				
HvCslH1g	T..-----	.C.....	T.A.....	A.....			C.....		
HvCslH1gHim	T..-----	.C.....	T.A.....	A.....			C.....		
TaCslH1-1	GATAATTTGCAAGCAAATAATTAAGCTGAGCGTTTTCGTGGTGC	AG ACAAGGGTGTCGGCCGTGATGACCAACGCTCCAATCATGCTGAAT							
TaCslH1-2	...-----	.C.....	A.....				G.....		
TaCslH1-3	...-----	.C.....	A.....				G.....		
	1450	1460	1470	1480	1490	1500	1510	1520	1530
									
HvCslH1			T.....	A.....	A.....				
HvCslH1Him			T.....	A.....	A.....				
HvCslH1g			T.....	A.....	A.....				
HvCslH1gHim			T.....	A.....	A.....				
TaCslH1-1	GTGGACTGCGACATGTTCGCCAACAACCCGCGAGGTGCGCCCTGCACGCGATGTGCCTCCTGTTGGGGTTCGACGACGAGATCCACAGCGGG								
TaCslH1-2			T.....	A.....					
TaCslH1-3				A.....					
	1540	1550	1560	1570	1580	1590	1600	1610	1620
									
HvCslH1	A.T.....			C..T.....				-----	
HvCslH1Him	A.T.....			C..T.....				-----	
HvCslH1g	A.T.....			C..T.....					
HvCslH1gHim	A.T.....			C..T.....					
TaCslH1-1	TTCTGTCAGGCGCCACAGAAAGTTCTACGGTGGCCTCAAGGATGACCCCTTTGGCAACCAGATGCAGGTTATAACCAAG	GT ACTACATATG							
TaCslH1-2									
TaCslH1-3				C.....				-----	
	1630	1640	1650	1660	1670	1680	1690	1700	1710
									
HvCslH1	-----								
HvCslH1Him	-----								
HvCslH1g				C...C..C...TGTGC.....	-----A.....		T.....G.....		
HvCslH1gHim				C...C..C...TGTGC.....	-----A.....		T.....G.....		
TaCslH1-1	CATGTGCACAAGTGCTGTTGTGGTAGTGC	----ACCACT		-----AGGGTAGTGTTACAGTTGCACTGGTTTTTCTGGCAT					
TaCslH1-2									
TaCslH1-3	-----	C.....	T..A.TGTT	..AGT.TTTTTTTTGAGCGAT.....		T.....			
	1720	1730	1740	1750	1760	1770	1780	1790	1800
									
HvCslH1	-----	AA.....		TG.....				A....T.....	
HvCslH1Him	-----	AA.....		TG.....				A....T.....	
HvCslH1g			AA.....	TG.....				A....T.....	
HvCslH1gHim			AA.....	TG.....				A....T.....	
TaCslH1-1	GTTC	AG AAAAATTGGAGGTGGGCTCGCCGGGATCCAAGGCACCTTCTACGGCGGCACGGGCTGTTTTCCACCGCAGGAAGGTCATCTACGGC							
TaCslH1-2				G.....				T.....	
TaCslH1-3	C.....		TG.....		G..A.....		A.G....A....T.....		
	1810	1820	1830	1840	1850	1860	1870	1880	1890
									
HvCslH1	A.T.....	A.....		-----					
HvCslH1Him	A.T.....	A.....		-----					
HvCslH1g	A..T.....	C....A.....	---G.....		A..AC....T.....		A.G....G....-		
HvCslH1gHim	A..T.....	A.....	---G.....		A..AC....T.....		A.G....G....-		
TaCslH1-1	ATGCCGCCTCCGGACACCGTCAAGCACGAGACAAGAG	GT AATAAAACTGGGCACGCACAAGATGAGATCATCCGACGTAAATTGAAGTAT							
TaCslH1-2				G.....				T.....	
TaCslH1-3	G.....	A..A.....	A.....	TG.A.....	A..A.....		G.....G.....		

FIGURE 17 (Cont.)

	1900	1910	1920	1930	1940	1950	1960	1970	1980
									
HvCslH1	-----								
HvCslH1Him	-----								
HvCslH1g	-----								
HvCslH1gHim	-----								
TaCslH1-1	TTGGTCAGTGCATTTCAGTTCGACTAGGGC-ATATCAAATGGC----								
TaCslH1-2	C.....								
TaCslH1-3	G.....								
	1990	2000	2010	2020	2030	2040	2050	2060	2070
									
HvCslH1	.T..G.....								
HvCslH1Him	.T..G.....								
HvCslH1g	.T..G.....								
HvCslH1gHim	.T..G.....								
TaCslH1-1	GCCAAAGTTTGGGAGCTCAAAGGAGTTGATCGAATCATCTAGGAACATCATCTCAGGGGACCTGCTCGCTAGACCAACCGTAGATATATCG								
TaCslH1-2									
TaCslH1-3									
	2080	2090	2100	2110	2120	2130	2140	2150	2160
									
HvCslH1	CA.....								
HvCslH1Him	CA.....								
HvCslH1g	CA.....								
HvCslH1gHim	CA.....								
TaCslH1-1	AGTCGTGTCGAAATGGCAAAACAAGTAGGCGACTGCAACTATGAGGCTGGCACATGTTGGGGCCAAGAGGTGTGCTTAGCT-TC-GTTGC								
TaCslH1-2	G.....								
TaCslH1-3	C.....								
	2170	2180	2190	2200	2210	2220	2230	2240	2250
									
HvCslH1	-----								
HvCslH1Him	-----								
HvCslH1g	-----								
HvCslH1gHim	C.....								
TaCslH1-1	CGTATTTTTCAGGTTTCTGCTACAGTACGGCCACATCTACACACCTTCTGCAGTTTCTCTCTA-TTACAGTTTCTTCC---ATGTATTT								
TaCslH1-2	A.....								
TaCslH1-3	AT.T..A.....								
	2260	2270	2280	2290	2300	2310	2320	2330	2340
									
HvCslH1	-----								
HvCslH1Him	-----								
HvCslH1g	-----								
HvCslH1gHim	T.....								
TaCslH1-1	TTGCAGATTGGGTGGGTCTATGGATCAATGACAGAGGACATTTTGACCGGTCAACGGATCCAGGCGGCGGGTTGGGAATCGGCCCTTGTTG								
TaCslH1-2	G.....								
TaCslH1-3	C.....								
	2350	2360	2370	2380	2390	2400	2410	2420	2430
									
HvCslH1	A.....								
HvCslH1Him	A.....								
HvCslH1g	A.....								
HvCslH1gHim	A.....								
TaCslH1-1	GACACCGACCCACCGGCATTCTGGGATGTGCTCCGACCGGTGGACCAGCCAGCTTGACCCAGTTCAAGAGATGGGCAACAGGGCTTCTG								
TaCslH1-2	T.....								
TaCslH1-3	G.....								
	2440	2450	2460	2470	2480	2490	2500	2510	2520
									
HvCslH1	T.....								
HvCslH1Him	T.....								
HvCslH1g	T.....								
HvCslH1gHim	T.....								
TaCslH1-1	GAGATACTCATCAGCCGGAACAGCCCCATCCTCGGCACCATCTTCAAGGGCCTCCAACCTCCGGCAATGCCTTGCTATCTCATCGTAGAC								
TaCslH1-2	G.C.....								
TaCslH1-3	A.....								

FIGURE 17 (Cont.)

	2530	2540	2550	2560	2570	2580	2590	2600	2610
									
HvCslH1				A..A.....			G.....	G.....	---
HvCslH1Him				A..A.....			G.....	G.....	---
HvCslH1g				A..A.....			G.....	G.....	
HvCslH1gHim				A..A.....			G.....	G.....	
TaCslH1-1	GCGTG	CCCCGTG	AGGGCGCCTTT	CGAGCTGTGCTATG	CGCTCTTG	GGACCTTTCTG	CCTTCTCACAAACCAATC	CCTTCTTACCAACG	GTA
TaCslH1-2		A.....	A.....	A.....	T..C.....	A.....		G.....	G.....
TaCslH1-3		C.....		AC.....			G.....	G.....	
	2620	2630	2640	2650	2660	2670	2680	2690	2700
									
HvCslH1	-----	-----	-----	-----	-----	-----	-----	-----	-----
HvCslH1Him	-----	-----	-----	-----	-----	-----	-----	-----	-----
HvCslH1g	..T...C...C...---	GTT.GCC.AG.T.CATTATGC....	A..CAAAA.TGCTGTGTGATTTGTTTGATAATGAAGCAGGACCTAGTT						
HvCslH1gHim	..T...C...C...---	GTT.GCC.AG.T.CATTATGC....	A..CAAAA.TGCTGTGTGATTTGTTTGATAATGAAGCAGGACCTAGTT						
TaCslH1-1	CACACATTTT	TGCCATGACCCATTACTACATTGCTCATAGCTG	AAATTTTA-----						
TaCslH1-2		T.....		C.GTACACGTGGTGATGTGGAAACACAAGTCTATGCAACTA					
TaCslH1-3	..T...C.....		ATATGC.A..A..CAAAA.TGTTGTGTGATTTGTTTGATAATGAAGCGCGACCTAGTT						
	2710	2720	2730	2740	2750	2760	2770	2780	2790
									
HvCslH1	-----	-----	-----	-----	-----	G.....	G.....	T	
HvCslH1Him	-----	-----	-----	-----	-----	G.....	G.....	T	
HvCslH1g	GGCTAATATGTATGTAAATTCAGATATTTTTTTTATGATTG..A.....TT.....	G.....	G.....	T					
HvCslH1gHim	GGCTAATATGTATGTAAATTCAGATATTTTTTTTATGATTG..A.....TT.....	G.....	G.....	T					
TaCslH1-1	-----	-----	-----	-----	GTGCATTTGCCGTTTTGC AG GCATCAGATGAAGGTTTTTCACATCCCAGC				
TaCslH1-2	AACAAAAATGTTTGTGTA-----	ATTTGTTTGATAAGATTT.....	T.....	A.....	TA.....	G.....			
TaCslH1-3	GGTTAATGTGTAAATATA-----	TATTTTCATGATTTT.A.....	T.....	G.....	C.G.....				
	2800	2810	2820	2830	2840	2850	2860	2870	2880
									
HvCslH1	A.....	C....G....C.....	T.....		T.C.....	C.....	C.....	C.....	C.....
HvCslH1Him	A.....	C....G....C.....	T.....		T.C.....	C.....	C.....	C.....	C.....
HvCslH1g	A.....	C....G....C.....	T.....		T.C.....	C.....	C.....	C.....	C.....
HvCslH1gHim	A.....	C....G....C.....	T.....		T.C.....	C.....	C.....	C.....	C.....
TaCslH1-1	GGCTCTATTTT	TGACTTACAACATATACCACTGATGGAGTACAAGGAGTGC	GGGCTCTCGGTCCGCGCCTGGTGAACAACCATAGGAT						
TaCslH1-2		C....G....C..G.....						C.....	C.....
TaCslH1-3		C....C.G.C.....					C.....	C.....	C.....
	2890	2900	2910	2920	2930	2940	2950	2960	2970
									
HvCslH1					G.....	A.....	T.....		
HvCslH1Him					G.....	A.....	T.....		
HvCslH1g					G.....	A.....	T.....		
HvCslH1gHim					G.....	A.....	T.....		
TaCslH1-1	GCAACGCATCACCTCGGCCTCCGCCTGGCTCCTCGCCTTCCTCACCGTCATCCTCAAGACGCTAGGGCTCTCCGAGACCGTGTTGAGGT								
TaCslH1-2									
TaCslH1-3					T.....				
	2980	2990	3000	3010	3020	3030	3040	3050	3060
									
HvCslH1		A.....	---		C.....	G.....	A..AC.....	A..C.....	
HvCslH1Him		A.....	---		C.....	G.....	A..AC.....	A..C.....	
HvCslH1g		A.....	---		C.....	G.....	A..AC.....	A..C.....	
HvCslH1gHim		A.....	---		C.....	G.....	A..AC.....	A..C.....	
TaCslH1-1	CACCCGCAAGGAGAGCAGCACGTCATCCGATGGCGGCGCGGGCACCGACGATGCCGATCCTGGGTGTTTCACCTTTGACTCGGCGCCCCGT								
TaCslH1-2			C.....	T.....		G.....	A.....	C.....	C.....
TaCslH1-3		A.....		C.....		G.....	C.C.....	C.....	
	3070	3080	3090	3100	3110	3120	3130	3140	3150
									
HvCslH1		G.....		G.....	G.....	G.....	A.....	T.C.....	T...
HvCslH1Him		G.....		G.....	G.....	G.....	A.....	T.C.....	T...
HvCslH1g		G.....		G.....	G.....	G.....	A.....	T.C.....	T...
HvCslH1gHim		G.....		G.....	G.....	G.....	A.....	T.C.....	T...
TaCslH1-1	TTTCATCCCAGTGACGGCGCTCTCAGTGTTGAACATTGTGCGCCCTCACCGTCGCGGCATGGCGCGCCGTCGTCGGGACGGTGGCGGGCGGT								
TaCslH1-2		G.....		A..C.....		G.....		T.....	C.....
TaCslH1-3		G.....	T.....	A..C.....		G.....	T..T..T.....	G..C.....	

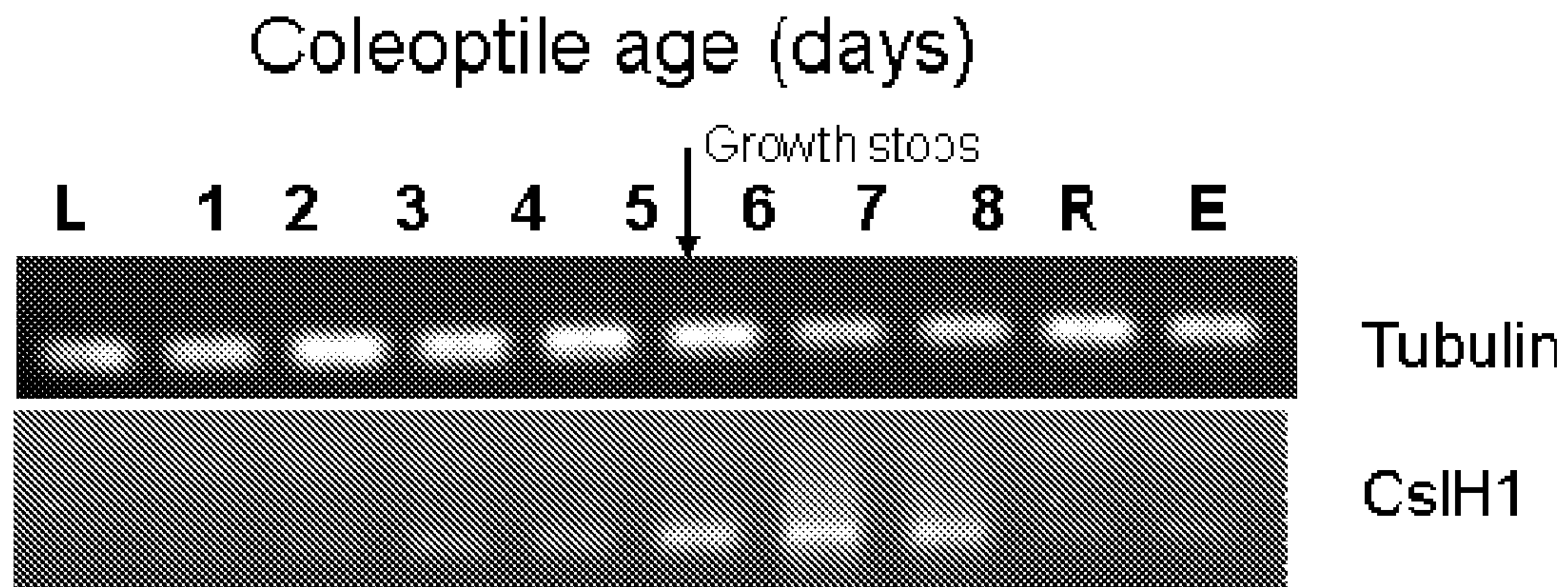
	3160	3170	3180	3190	3200	3210	3220	3230	3240
									
<i>HvCslH1</i>	A.....					G.....		C.....	G..
<i>HvCslH1Him</i>	A.....					G.....		C.....	G..
<i>HvCslH1g</i>	A.....					G.....		C.....	G..
<i>HvCslH1gHim</i>	A.....					G.....		C.....	G..
<i>TaCslH1-1</i>	TCATGGTGGCCCGGGCGTCGGAGAGTTCGTGTGCTGTGGCTGGATGGTGTGTGCTTCTGGCCATTTCGTGAGAGGGCTTGTTCAGTAGTGG								
<i>TaCslH1-2</i>	A.....					C.....	G..A.....	C.....	
<i>TaCslH1-3</i>	A.....G.....					C..C.....	G.....	C.....	G..
	3250	3260	3270	3280	3290	3300	3310	3320	3330
									
<i>HvCslH1</i>	C....A.....C....A.....		T.....						-----
<i>HvCslH1Him</i>	C....A.....C....A.....		T.....						-----
<i>HvCslH1g</i>	C....A.....C....AA.....		T.....						G
<i>HvCslH1gHim</i>	C....A.....C....A.....		T.....						-----
<i>TaCslH1-1</i>	AAAGTATGGGATCCCGTGGAGTGTCTCAGGGTGAAGGCTGGGTTGATTGTGGCTGCGTTTCGTGCACCTCTGCACAAGGAAC <u>TA</u> ACCGGCCG								
<i>TaCslH1-2</i>									G.-
<i>TaCslH1-3</i>	A.....		T.....			A.....			G.-
	3340	3350	3360	3370	3380	3390	3400	3410	3420
									
<i>HvCslH1</i>	-----								
<i>HvCslH1Him</i>	-----								
<i>HvCslH1g</i>	GCTGGCCATCGAAATATTGGAAGCGTAATTTTAGTTCTACCATTTGGAACATGTAACGAAGACTCAAGCAGGAATTAAAGCGTGTTATTAA								
<i>HvCslH1gHim</i>	-----								
<i>TaCslH1-1</i>	-----								
<i>TaCslH1-2</i>	-----								
<i>TaCslH1-3</i>	-----								
	3430	3440	3450	3460	3470	3480			
									
<i>HvCslH1</i>	-----								
<i>HvCslH1Him</i>	-----								
<i>HvCslH1g</i>	AGGAAGTTTGACCCAATGTATCTATCTATCTCTCCCAATCTCTCTCAATAATAAAGCAAAT								
<i>HvCslH1gHim</i>	-----								
<i>TaCslH1-1</i>	-----								
<i>TaCslH1-2</i>	-----								
<i>TaCslH1-3</i>	-----								

[illegible]

	460	470	480	490	500	510	520	530	540
HvCslHl1 (Him)		GWYCGSMTED ILT CQRIHAAGUKS ALLD TNP PAF LGCAPT GGPASLT QFKRMAT CVLEILISRN SPILGTIFQR LQLRQC LCYLIVEAMP							
TaCslHl1-3pro	L.....E.....E.....	L.....E.....E.....							
TaCslHl1-lpro	Q.....E.....D.....	Q.....E.....D.....							
TaCslHl1-2pro	Q.....D.....D.....	Q.....D.....D.....							
	550	560	570	580	590	600	610	620	630
HvCslHl1 (Him)		VRA PFELCYALLGPFCLLTNQSF LPTASDECFRIPV ALFLSYHIYHLMEYKE CGLSARAMMMNHRRMURITSA SAMLLAFLT WILKTLGLS							
TaCslHl1-3pro		A.....TC.....V.....							
TaCslHl1-lpro		H.....A.....TN.....V.....							
TaCslHl1-2pro	M.....M.....T.N.....A.....V.....V.....	M.....M.....T.N.....A.....V.....V.....							
	640	650	660	670	680	690	700	710	720
HvCslHl1 (Him)		ETVFEVTRKESST -SDCGACTDEADPC LFTFDSAPVFIPVTALSWLNIV ALAVCAMRAVICTA AVWHGCCPGICEFWCCGMWL CFMPFVR							
TaCslHl1-3pro	S.....S.....D.....S.....	S.....S.....D.....S.....							
TaCslHl1-lpro	S.....D.....D.....S.....	S.....D.....D.....S.....							
TaCslHl1-2pro	S.....T.....T.....M.....A.....V.....G.....V.....M.....	S.....T.....T.....M.....A.....V.....G.....V.....M.....							
	730	740	750						
HvCslHl1 (Him)		GLVSRGKHGCI PMSEVKAKACLI VAAAFUHLCTRN							
TaCslHl1-3pro	Y.....Y.....R.....I.....	Y.....Y.....R.....I.....							
TaCslHl1-lpro	S.....Y.....R.....R.....	S.....Y.....R.....R.....							
TaCslHl1-2pro	S.....Y.....R.....R.....	S.....Y.....R.....R.....							

FIGURE 19

A



B

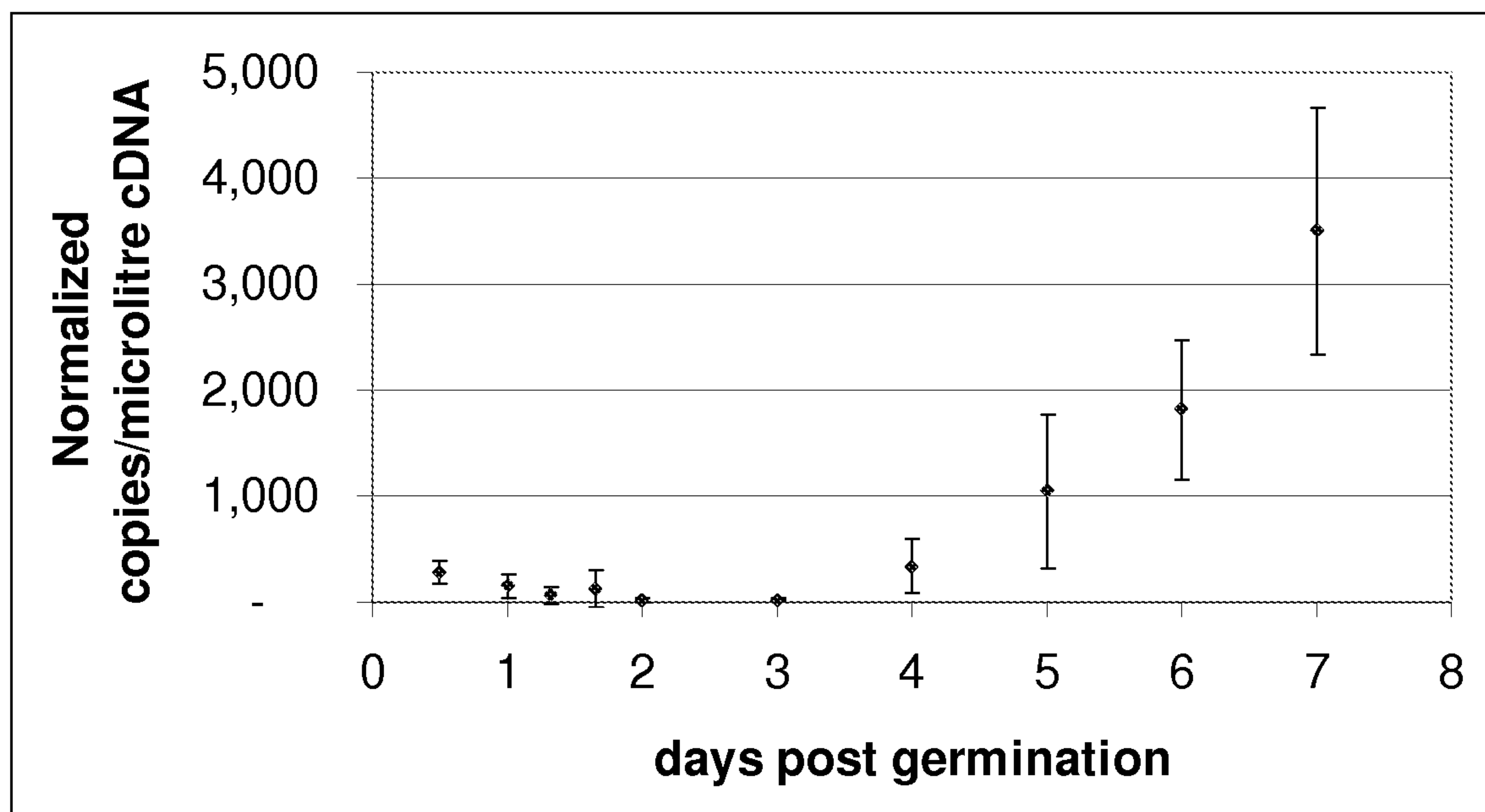


FIGURE 20

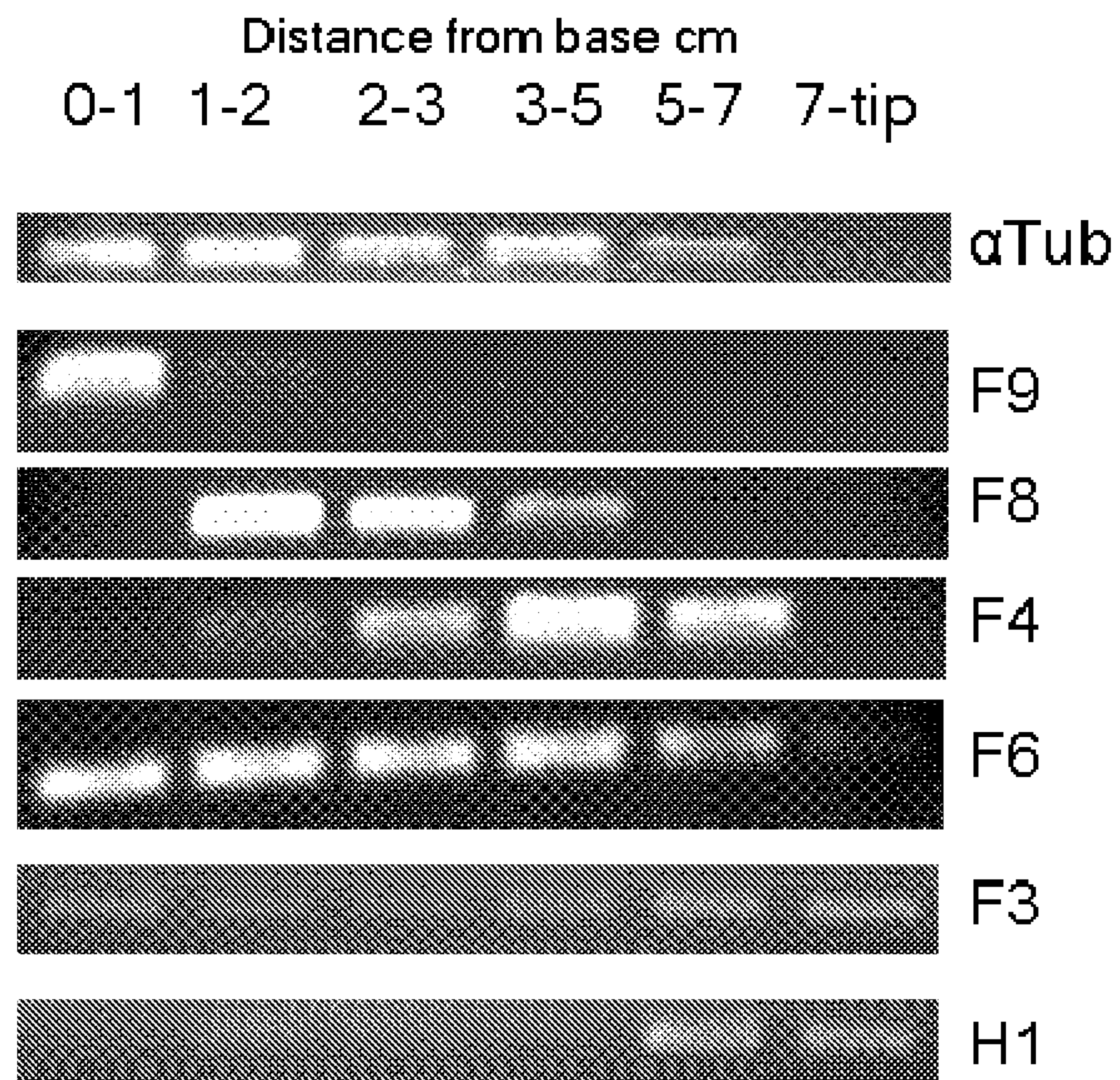


FIGURE 21

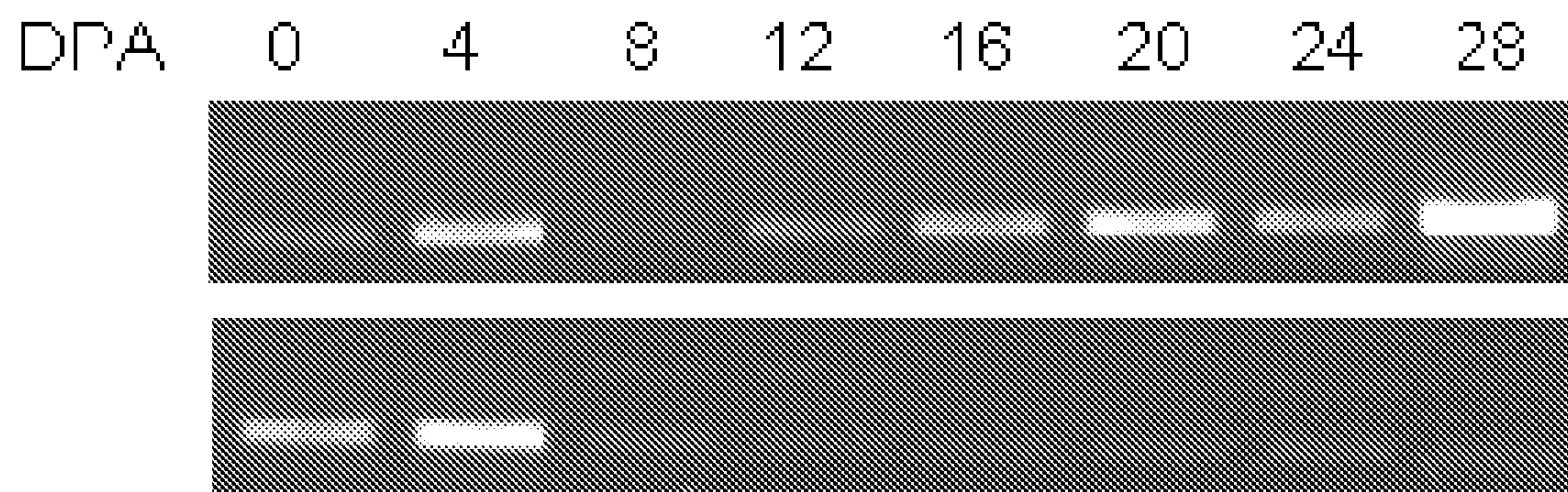


FIGURE 22

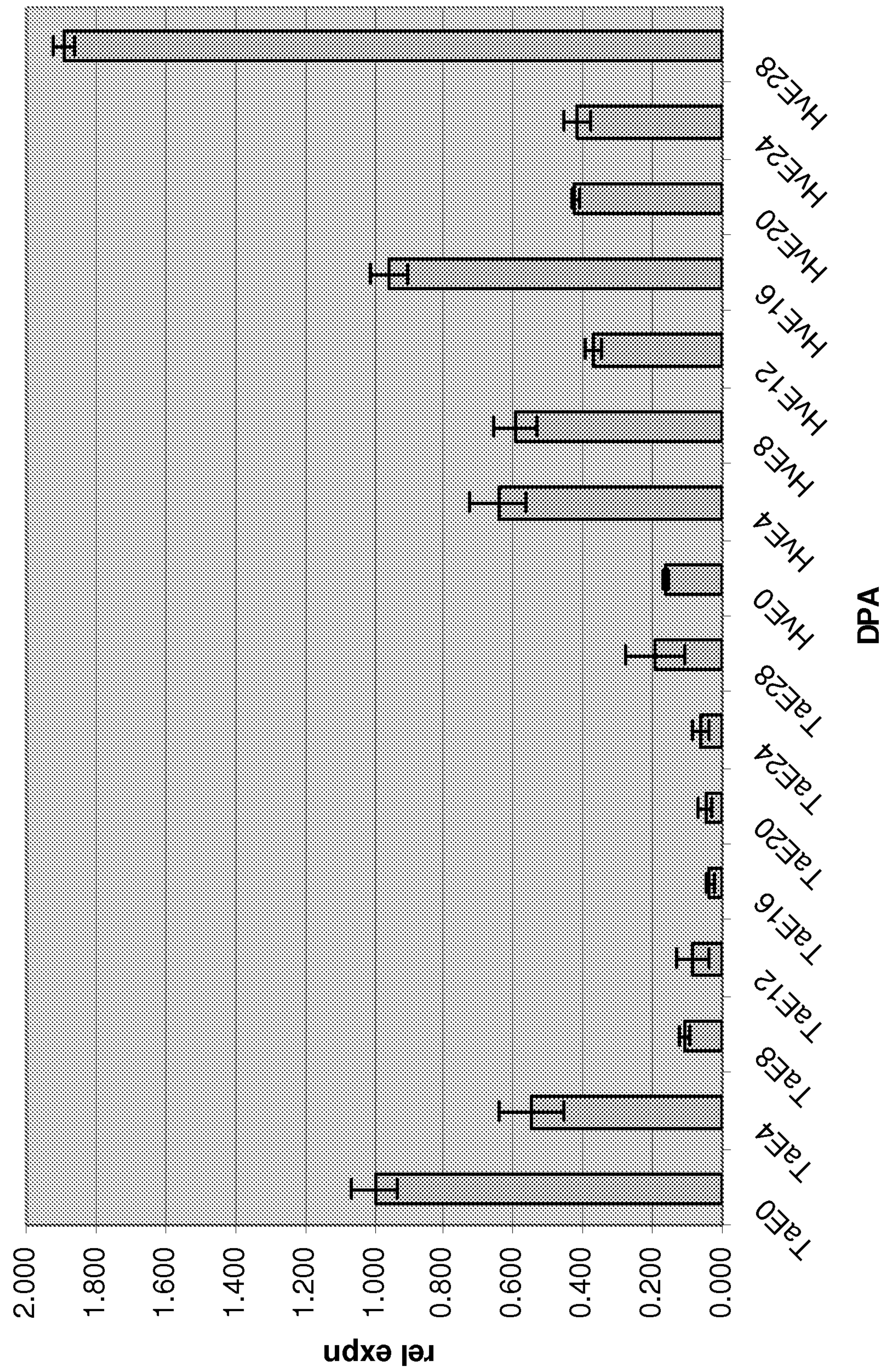


FIGURE 23

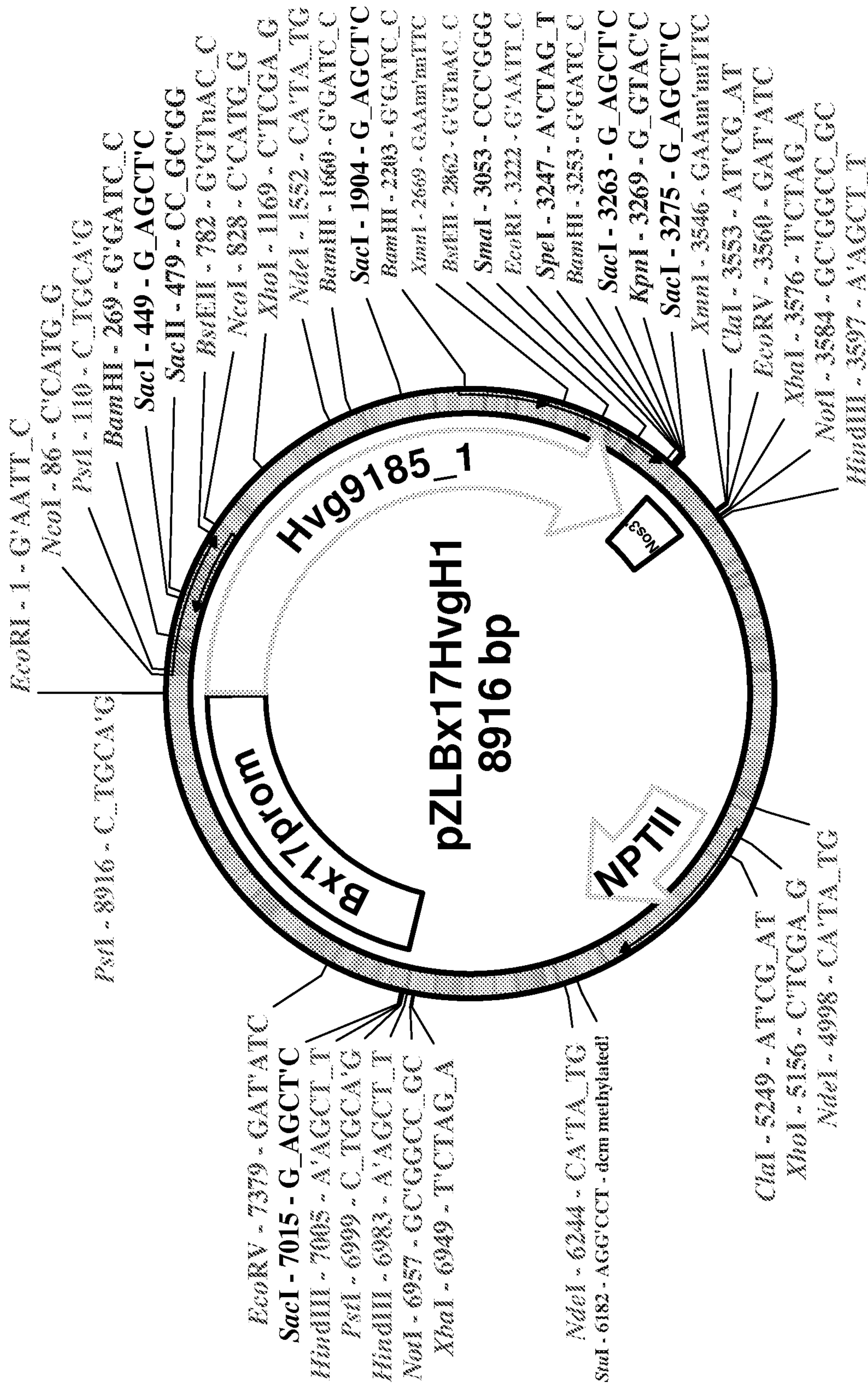


FIGURE 24

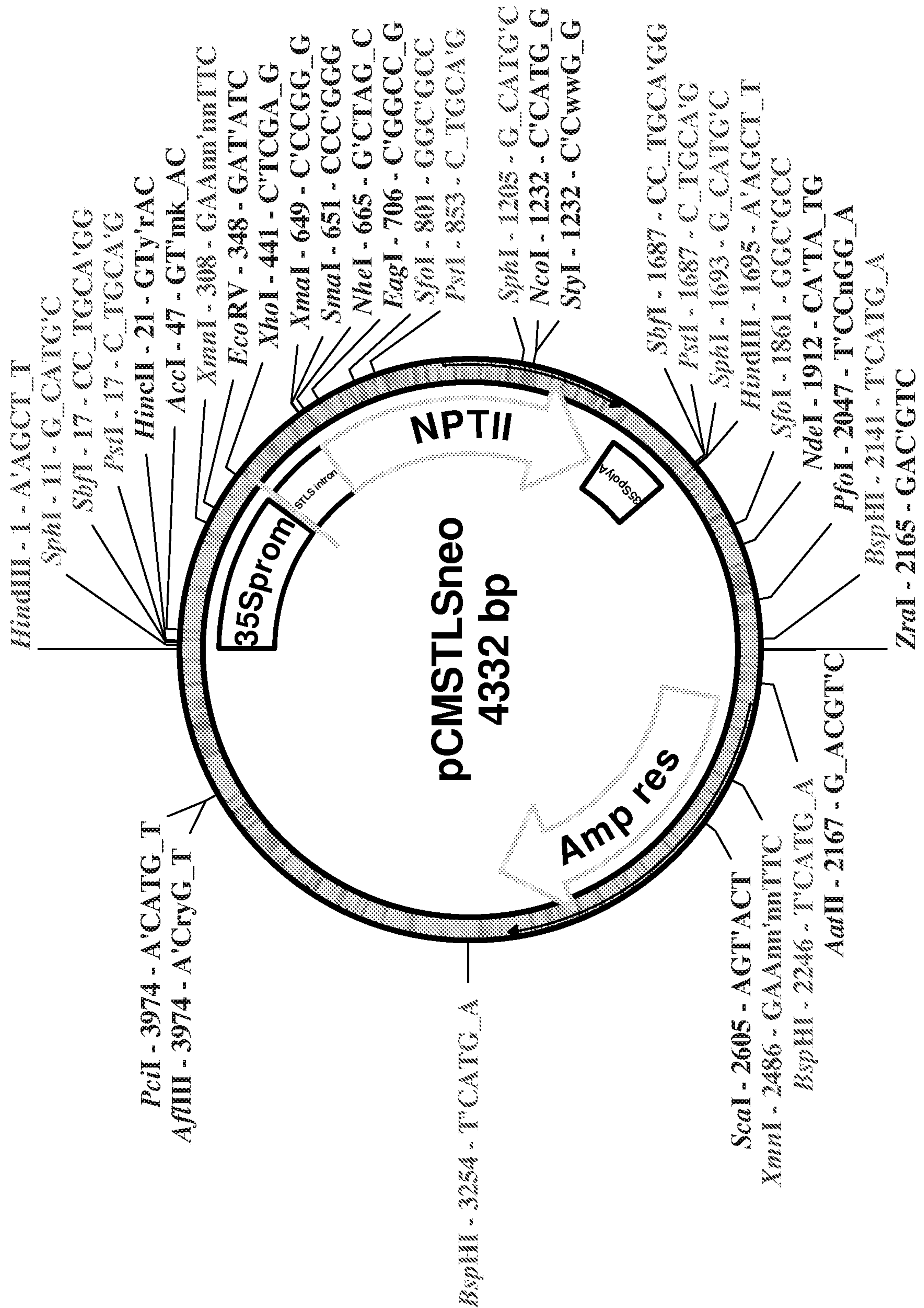


FIGURE 25

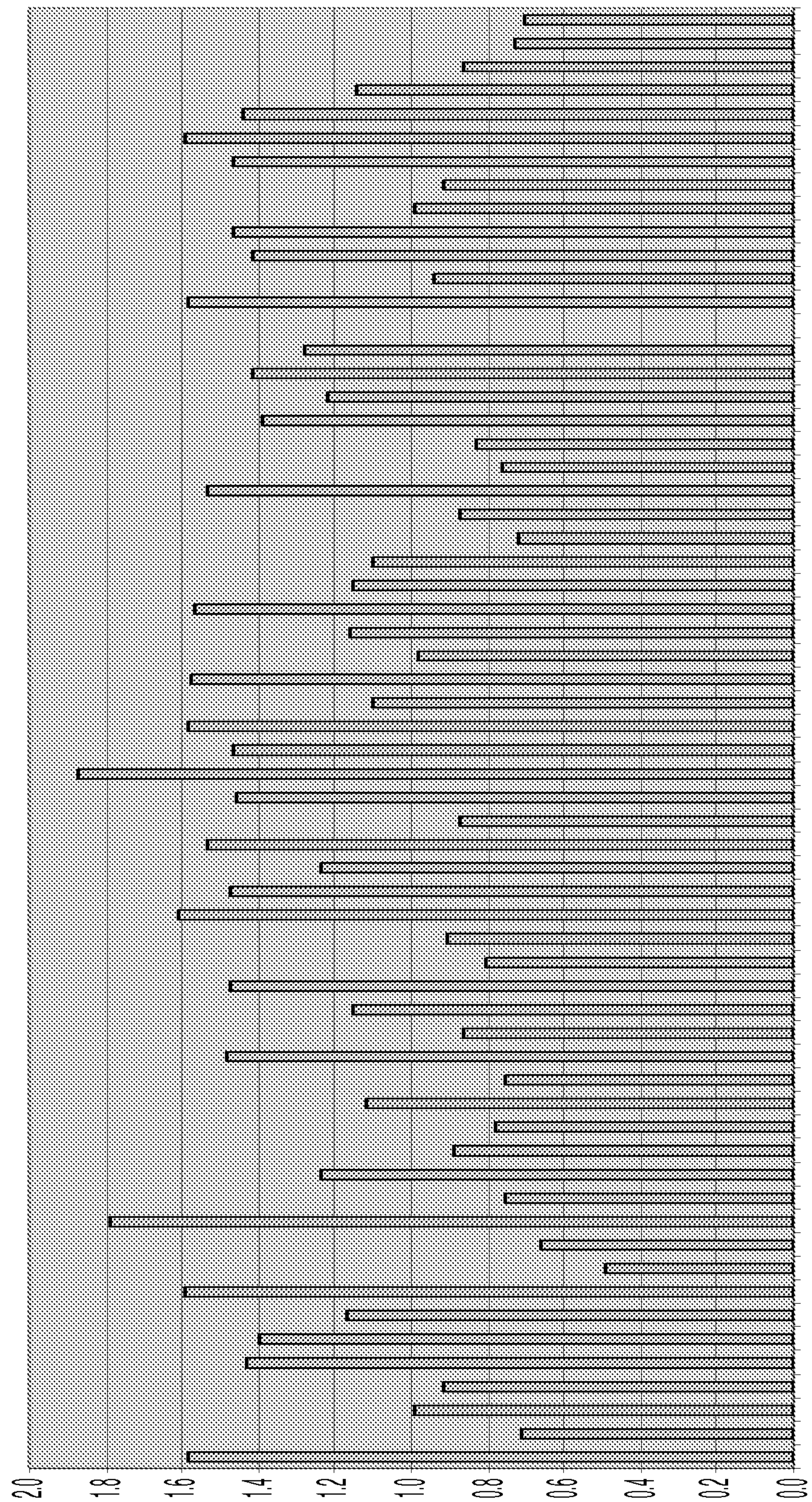


FIGURE 26

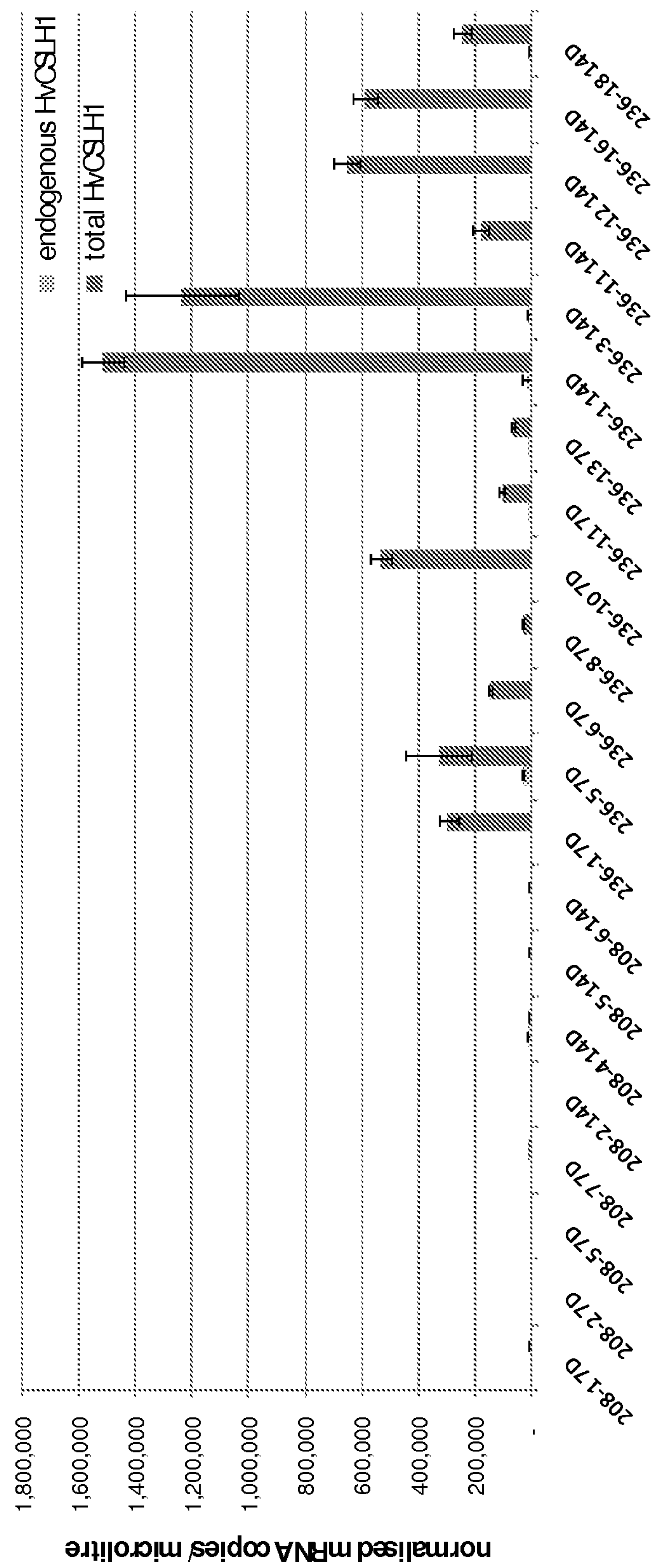


FIGURE 27

Seq->	HvCslH1		HvCslH1(Him)		TaCslH1-3		TaCslH1-1		TaCslH1-2	
HvCslH1	100		99.9		93.7		93.8		93.4	
	100	100	100	100	93.1	97.3	92.8	97.6	92.6	97.7
HvCslH1(Him)			100		93.8		93.9		93.5	
			100	100	93.1	97.3	92.8	97.6	92.6	97.7
TaCslH1-3					100		95.3		95.2	
					100	100	94.3	98.1	94.0	98.3
TaCslH1-1							100		96.1	
							100	100	95.1	99.1
TaCslH1-2	DNA % identity								100	
	aa % id	aa % sim							100	100