

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(12)

Oversættelse af europæisk patentskrift

(10) **DK/EP 2287300 T3**

-
- (51) Int.Cl.: **A 01 H 5/00 (2006.01)** **C 12 N 15/09 (2006.01)** **A 61 K 39/106 (2006.01)**
A 61 K 39/108 (2006.01) **A 61 K 39/112 (2006.01)** **A 61 P 31/04 (2006.01)**
C 07 K 14/245 (2006.01) **C 07 K 14/25 (2006.01)** **C 07 K 14/28 (2006.01)**
C 07 K 19/00 (2006.01) **C 12 N 5/10 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2018-09-17**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2018-06-27**
- (86) Europæisk ansøgning nr.: **09738819.3**
- (86) Europæisk indleveringsdag: **2009-04-28**
- (87) Den europæiske ansøgnings publiceringsdag: **2011-02-23**
- (86) International ansøgning nr.: **JP2009058345**
- (87) Internationalt publikationsnr.: **WO2009133882**
- (30) Prioritet: **2008-05-02 JP 2008120573**
- (84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK TR**
- (73) Patenthaver: **Idemitsu Kosan Co., Ltd., 1-1, Marunouchi 3-chome, Chiyoda-ku, Tokyo 100-8321, Japan**
National University Corporation Nara Institute of Science and Technology, 8916-5, Takayama-cho, Ikoma-shi, Nara 630-0192, Japan
- (72) Opfinder: **YOSHIDA, Kazuya, , deceased, Japan**
SAWADA, Kazutoshi, 1280, Kamiizumi, Sodegaura-shi, Chiba 299-0293, Japan
MATSUI, Takeshi, 1280, Kamiizumi, Sodegaura-shi, Chiba 299-0293, Japan
- (74) Fuldmægtig i Danmark: **Budde Schou A/S, Hausergade 3, 1128 København K, Danmark**
- (54) Benævnelse: **Bakterietoksinvaccine**
- (56) Fremdragne publikationer:
WO-A1-2005/011733
WO-A2-01/89456
GEORGE R A ET AL: "An analysis of protein domain linkers: their classification and role in protein folding", PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 15, no. 11, 1 November 2002 (2002-11-01), pages 871-879, XP002374925, ISSN: 0269-2139, DOI: DOI:10.1093/PROTEIN/15.11.871
RAN ET AL: "The immunogenicity of fusion protein linking the carboxyl terminus of the B subunit of Shiga toxin 2 to the B subunit of E. coli heat-labile enterotoxin", VETERINARY MICROBIOLOGY, ELSEVIER BV, NL, vol. 127, no. 1-2, 19 December 2007 (2007-12-19), pages 209-215, XP022393837, ISSN: 0378-1135, DOI: DOI:10.1016/J.VETMIC.2007.08.021
GAO X ET AL: "Immunogenicity of a novel Stx2B-Stx1B fusion protein in a mice model of Enterohemorrhagic Escherichia coli O157:H7 infection", VACCINE, ELSEVIER LTD, GB, vol. 27, no. 14, 23 March 2009 (2009-03-23), pages 2070-2076, XP025969109, ISSN: 0264-410X, DOI: DOI:10.1016/J.VACCINE.2009.01.115 [retrieved on 2009-

Fortsættes ...

02-02]

- SATOH J ET AL: "The 5'-untranslated region of the tobacco alcohol dehydrogenase gene functions as an effective translational enhancer in plant", JOURNAL OF BIOSCIENCE AND BIOENGINEERING, ELSEVIER, AMSTERDAM, NL, vol. 98, no. 1, 1 January 2004 (2004-01-01), pages 1-8, XP004544191, ISSN: 1389-1723
- MASON HUGH S ET AL: "Edible plant vaccines: Applications for prophylactic and therapeutic molecular medicine", TRENDS IN MOLECULAR MEDICINE, vol. 8, no. 7, July 2002 (2002-07), pages 324-329, XP002634139, ISSN: 1471-4914
- TSUJI T ET AL: "A nasal vaccine comprising B-subunit derivative of Shiga toxin 2 for cross-protection against Shiga toxin types 1 and 2", VACCINE, ELSEVIER LTD, GB, vol. 26, no. 17, 16 April 2008 (2008-04-16), pages 2092-2099, XP022590859, ISSN: 0264-410X, DOI: DOI:10.1016/J.VACCINE.2008.02.034 [retrieved on 2008-03-07]
- WEN SHARON X ET AL: "A plant-based oral vaccine to protect against systemic intoxication by Shiga toxin type 2", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 103, no. 18, May 2006 (2006-05), pages 7082-7087, XP002635191, ISSN: 0027-8424
- MATSUI T ET AL.: "Production of double repeated B subunit of Shiga toxin 2e at high levels in transgenic lettuce plants as vaccine material for porcine edema disease.", TRANSGENIC RES., 21 October 2010 (2010-10-21), XP002634124, DOI: DOI: 10.1007/s11248-010-9455-9
- MATSUI TAKESHI ET AL: "Transgenic Lettuce Producing a Candidate Protein for Vaccine against Edema Disease", BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY, vol. 73, no. 7, July 2009 (2009-07), pages 1628-1634, XP002635192, ISSN: 0916-8451
- KAZUTOSHI SAWADA ET AL.: 'Kumikae Lettuce ni yoru Kachikuyo Keiko Vaccine Tanpakushitsu Seisan no Kenkyu Kaihatsu' BIOTECHNOLOGY SYMPOSIUM 06 November 2007, pages 28 - 31, XP008128434
- KAZUTOSHI SAWADA ET AL.: 'Lettuce ni yoru Vaccine Seibun Seisan Gijutsu Kaihatsu (Sono 1) Kumikae Lettuce ni yoru Kachikuyo Keiko Vaccine Tanpakushitsu Seisan no Kenkyu Kaihatsu' PREPRINTS OF BIOTECHNOLOGY SYMPOSIUM 06 November 2007, pages 107 - 108, XP008128433
- TAKESHI MATSUI ET AL.: 'Buta Fushu Vaccine Tanpakushitsu Seisan no Tameno Lettuce Shoho Yuso Kogaku' ABSTRACT OF THE 25TH MEETING AND SYMPOSIUM IN CHIBA OF THE JAPANESE ASSOCIATION FOR PLANT TISSUE CULTURE 08 August 2007, page 180, XP008144517
- YASUDA, HIROSHI. ET AL.: 'The Correlation between Expression and Localization of a Foreign Gene Product in Rice Endosperm' PLANT AND CELL PHYSIOLOGY vol. 47, no. 6, 13 April 2006, pages 756 - 763, XP008112037
- O'DOWD A M ET AL: "Novel modifications to the C-terminus of LTB that facilitate site-directed chemical coupling of antigens and the development of LTB as a carrier for mucosal vaccines", VACCINE, ELSEVIER LTD, GB, vol. 17, no. 11-12, 1 March 1999 (1999-03-01), pages 1442-1453, XP004158272, ISSN: 0264-410X, DOI: 10.1016/S0264-410X(98)00375-2
- GUSTAVSSON MALIN ET AL: "Stable linker peptides for a cellulose-binding domain-lipase fusion protein expressed in Pichia pastoris", PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 14, no. 9, 1 September 2001 (2001-09-01), pages 711-715, XP009118009, ISSN: 0269-2139
- JIN LIANG ET AL: "A Th1-recognized peptide P277, when tandemly repeated, enhances a Th2 immune response toward effective vaccines against autoimmune diabetes in nonobese diabetic mice", JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, [Online] vol. 180, no. 1, 1 January 2008 (2008-01-01), pages 58-63, XP009155180, ISSN: 0022-1767 Retrieved from the Internet:
URL:<http://jimmunol.org/content/180/1/58.abstract>
- OSCHERWITZ J ET AL: "A V3 loop haptenic peptide sequence, when tandemly repeated, enhances immunogenicity by facilitating helper T-cell responses to a covalently linked carrier protein", VACCINE, ELSEVIER LTD, GB, vol. 17, no. 19, 14 May 1999 (1999-05-14), pages 2392-2399, XP004168970, ISSN: 0264-410X, DOI: 10.1016/S0264-410X(99)00030-4

DESCRIPTION

Technical Field

[0001] The present invention relates to a hybrid protein used for vaccines for diseases caused by bacterial toxins such as Shiga toxins, cholera toxins, and *Escherichia coli* heat-labile toxins, and a DNA construct for producing the hybrid protein.

Background Art

[0002] Shiga toxins (Stxs, verotoxins) are proteinous exotoxins produced by enterohemorrhagic *Escherichia coli* of pathogenic *Escherichia coli* species. The Shiga toxins cause hemorrhagic enteritis, hemolytic uremic syndrome, encephalopathy, and the like.

[0003] The Shiga toxins are broadly classified into Stx1 and Stx2, each of which is further classified into subclasses. An example of the Stx2 includes Stx2e which causes swine edema disease. The swine edema disease is known to frequently occur in baby pigs one to two weeks after weaning. A fatality due to infection with edema disease bacteria is 50 to 90%, which is extremely high.

[0004] Further, cholera toxins (CTs) are proteinous exotoxins produced by *Vibrio cholerae*. The CTs are known to cause severe diarrhea and emesis.

[0005] Still further, *Escherichia coli* heat-labile toxins (LTs) are proteinous endotoxins produced by enterotoxigenic *Escherichia coli*. The LTs are known to cause diarrhea and emesis.

[0006] The bacterial toxins, Stxs, LTs, and CTs are all known to include a B-subunit pentamer involved in adhesion to cells and an A-subunit monomer having a toxicity. The LTs and the CTs are also known to be similar structurally and functionally.

[0007] As a method of preventing the diseases caused by those bacterial toxins, methods of administering a vaccine by an injection or a nasal spray and administering the vaccine orally are known.

[0008] For example, a technology where an attenuated Stx2e protein is produced using recombinant *Escherichia coli* and administered to pigs by an injection is known (Non-patent Document 1). However, for example, an amount of the attenuated Stx2e protein produced by the recombinant *Escherichia coli* is not sufficient and the administration of the vaccine by an injection requires human labor. This has been a problem. Further, the method of administering the vaccine orally draws increasing attention in terms of reducing the labor in a stockbreeding

field. In such a context, a technology where the bacterial toxin protein is produced by plants using a transgenic technology has been being developed. For example, a transgenic plant containing DNA encoding an LT protein B subunit (LTB) and expressing the DNA has been described (Patent Documents 1 and 2). A transgenic plant expressing DNA encoding the LT protein or the CT protein has been also described (Patent Document 3). However, there has been a problem that the amount of the produced protein is not sufficient in those technologies. An example of producing the LTB in *Lactuca sativa* has been reported (Non-patent Document 2). In this study, a gene of the LT protein B subunit including modified codons is expressed in *Lactuca sativa* using both a cauliflower mosaic virus 35S RNA promoter (CaMV35S) which is a promoter expressed highly in a plant and Kozak sequence which is an enhancer. As a result, it has been reported that the LT protein B subunit is accumulated in an amount of about 2.0% by mass of a total soluble protein of *Lactuca sativa*. However, this extent of the accumulated protein is thought to be insufficient to efficiently prevent a bacterial disease by utilizing the transgenic plant. That is, it is necessary to efficiently produce and accumulate the target bacterial toxin protein in plant cells. A hybrid bacterial toxin subunit is provided by WO 2005/011733. A further hybrid comprising the A-subunit of LT-A and the B-subunit of CT-B is provided by WO 2001/89456. The immunogenicity of a hybrid comprising the B-subunit of Shiga toxin 2 and the B-subunit of *E. coli* heat-labile enterotoxin has been studied by Ran (2007) Veterinary Microbiology 127, no.1-2, pages 209-215.

[0009] The inventors of the present invention found that the Stx2e protein could be produced efficiently in a plant such as *Lactuca sativa* and accumulated at a high concentration in a plant body by expressing the Stx2e protein where a secretory signal peptide derived from a plant had been added to its amino terminus, using a 5'-untranslated region (ADH 5'-UTR) of an alcohol dehydrogenase gene derived from a plant, and filed the patent (Patent Document 4).

[0010]

[Patent Document 1] JP 10-507916 A

[Patent Document 2] JP 2000-166411 A

[Patent Document 3] JP 2002-533068 A

[Patent Document 4] WO 2009/004842 A1

[Non-patent Document 1] Makino et al., Microbial Pathogenesis, Volume 31, Number 1, July 2001, pp. 1-8(08)

[Non-patent Document 2] Kim et al., Protein Expression and Purification, Volume 51, Number 1, Jan 2006, pp. 22-27(06)

Summary of Invention

[0011] It is an object of the present invention to more efficiently produce a Stx protein and other bacterial toxin proteins having a conformation similar thereto using plant cells.

[0012] Through production of a hybrid protein in which two or three bacterial toxin proteins such as Stx2e and CT are tandemly linked through a peptide having a particular sequence in plant cells, the inventors of the present invention have succeeded in accumulating the bacterial toxin protein at a high concentration in the plant cells and completed the present invention.

[0013] The present invention is as follows:

1. (1) A hybrid protein comprising
 1. (a) two toxin proteins selected from the group consisting of Shiga toxin (Stx) protein, cholera toxin (CT) protein or *Escherichia coli* heat-labile toxin (LT) protein, wherein said toxin proteins are tandemly linked through a peptide having the amino acid sequence represented by SEQ ID NO: 2, 82 or 84, and
 2. (b) a peptide having the amino acid sequence represented by SEQ ID NO: 2, 82 or 84 linked to the C-terminus of the tandemly linked toxin proteins of (a), wherein the Stx proteins is a STx2eB protein, wherein the CT protein is a CT protein B subunit, and wherein the LT protein is a LT protein B subunit.
2. (2) The hybrid protein according to item 1, wherein said toxin proteins are tandemly linked through a peptide having the amino acid sequence represented by SEQ ID NO: 2.
3. (3) The hybrid protein according to item 1 or 2, comprising an amino acid sequence represented by SEQ ID NO: 14 or 16.
4. (4) The hybrid protein according to item 1, comprising an amino acid sequence represented by SEQ ID NO: 90, 92, 98, or 100.
5. (5) The hybrid protein according to any one of items 1 to 4, wherein a secretory signal peptide derived from a plant is added to its amino terminus.
6. (6) The hybrid protein according to any one of items 1 to 5, wherein an endoplasmic reticulum retention signal peptide is added to its carboxyl terminus.
7. (7) A DNA construct comprising DNA encoding the hybrid protein according to any one of items 1 to 6.
8. (8) The DNA construct according to item 7, comprising DNA having a base sequence represented by SEQ ID NO: 13, 15, 89, 91, 97, or 99.
9. (9) The DNA construct according to item 7 or 8, wherein DNA encoding a hybrid protein is operably-linked to a 5'-untranslated region of an alcohol dehydrogenase gene derived from a plant.
10. (10) The DNA construct according to item 9, wherein the 5'-untranslated region of an alcohol dehydrogenase gene derived from a plant is derived from *Nicotiana tabacum*.
11. (11) The DNA construct according to item 10, comprising a base sequence represented by any one of SEQ ID NOS: 28, 29, 101, 102, 103, 104, 108, 109, 110, or 111.
12. (12) A recombinant vector comprising the DNA construct according to any one of items 7 to 11.
13. (13) A transformant transformed with the recombinant vector according to item 12.
14. (14) The transformant according to item 13, wherein the transformant is a transformed

plant cell or a transformed plant.

15. (15) A seed, which is obtained from the transformant according to item 13 or 14.
16. (16) A peptide having an amino acid sequence represented by one of SEQ ID NO: 2, 82, or 84.

[0014] The disclosure relates to:

1. (1) a hybrid protein, in which two or three of Shiga toxin proteins, cholera toxin proteins, or *Escherichia coli* heat-labile toxin proteins are each tandemly linked through a peptide having the following characteristics (A) and (B) :
 1. (A) the number of amino acids is 12 to 30; and
 2. (B) the content of proline is 20 to 35%;
2. (2) the hybrid protein according to Item (1), in which the peptide further has the following characteristic (C):

(C) proline is allocated every two or three amino acids;
3. (3) the hybrid protein according to Item (2), in which the peptide has an amino acid sequence represented by SEQ ID NO: 2, 82, or 84;
4. (4) the hybrid protein according to Item (3), in which two of the Shiga toxin proteins, cholera toxin proteins, or *Escherichia coli* heat-labile toxin proteins are tandemly linked through the peptide having the amino acid sequence represented by SEQ ID NO: 2;
5. (5) the hybrid protein according to any one of Items (1) to (4), in which the Shiga toxin proteins are Shiga toxin protein B subunits;
6. (6) the hybrid protein according to any one of Items (1) to (5), in which the Shiga toxin proteins are Stx2e proteins;
7. (7) the hybrid protein according to any one of Items (1) to (4), in which the cholera toxin proteins are cholera toxin protein B subunits;
8. (8) the hybrid protein according to Item (4), including an amino acid sequence represented by SEQ ID NO: 10, 12, 14, or 16;
9. (9) the hybrid protein according to Item (3), including an amino acid sequence represented by SEQ ID NO: 86, 88, 90, 92, 94, 96, 98, or 100;
10. (10) the hybrid protein according to any one of Items (1) to (9), in which a secretory signal peptide derived from a plant is added to its amino terminus;
11. (11) the hybrid protein according to Item (10), in which an endoplasmic reticulum retention signal peptide is added to its carboxyl terminus;
12. (12) the hybrid protein according to any one of Items (1) to (9), in which a chloroplast transit signal peptide is added to its amino terminus;
13. (13) a DNA construct, including DNA encoding the hybrid protein according to any one of Items (1) to (12);
14. (14) the DNA construct according to Item (13), including DNA having a base sequence represented by SEQ ID NO: 9, 11, 13, or 15;
15. (15) the DNA construct according to Item (13), including DNA having a base sequence represented by SEQ ID NO: 85, 87, 89, 91, 93, 95, 97, or 99;
16. (16) the DNA construct according to any one of Items (13) to (15), in which DNA

encoding a hybrid protein is operably-linked to a 5'-untranslated region of an alcohol dehydrogenase gene derived from a plant;

17. (17) the DNA construct according to Item (16), in which the 5'-untranslated region of the alcohol dehydrogenase gene derived from the plant is derived from *Nicotiana tabacum*;
18. (18) a DNA construct according to Item (17), including a base sequence represented by any one of SEQ ID NOS: 24 to 29;
19. (19) a DNA construct according to Item (17), including a base sequence represented by any one of SEQ ID NOS: 101 to 111;
20. (20) a recombinant vector, including the DNA construct according to any one of Items (13) to (19);
21. (21) a transformant transformed with the recombinant vector according to Item (20);
22. (22) a transformant according to Item (21), in which the transformant is a transformed plant cell or a transformed plant;
23. (23) a seed, which is obtained from the transformant according to Item (21) or (22); and
24. (24) a peptide, having an amino acid sequence represented by SEQ ID NO: 2, 82, or 84.

Brief Description of Drawings

[0015] In the accompanying drawings:

FIG. 1 is a view illustrating a design of Stx2eB expression vectors, in which an arrow denotes a translation initiation site, and an inverted triangle denotes a site to be cleaved after the translation;

FIG. 2 is a photograph illustrating levels of accumulated Stx2eB obtained in a transient expression experiment using *Lactuca sativa* protoplasts;

FIG. 3 is a photograph illustrating levels of accumulated CTB obtained in a transient expression experiment using the *Lactuca sativa* protoplasts;

FIG. 4 is a view illustrating a design of DNA constructs encoding an Stx2eB-YFP fusion protein, in which an arrow denotes a translation initiation site, and an inverted triangle denotes a site to be cleaved after the translation;

FIG. 5 is a photograph illustrating localization of the Stx2eB-YFP fusion protein obtained in a transient expression experiment using cultured tobacco cell protoplasts;

FIG. 6 is a photograph illustrating localization of the Stx2eB-YFP fusion protein obtained in a transient expression experiment using the cultured tobacco cell protoplasts;

FIG. 7 is a photograph illustrating localization of vacuole-type GFP and the Stx2eB-YFP fusion protein in co-expression of ARF1pWT and ARF1pDN obtained in a transient expression experiment using the cultured tobacco cell protoplasts;

FIG. 8 is a photograph illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using cultured tobacco cells (BY2), in which a numeral in each lane denotes a clone number;

FIG. 9 is a photograph illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using the cultured tobacco cells (BY2), in which the numeral in each lane denotes the clone number;

FIG. 10 is photographs illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using the cultured Tobacco cells (BY2), in which the numeral in each lane denotes the clone number;

FIG. 11 is a graph illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using the cultured tobacco cells (BY2), in which each numeral denotes the clone number;

FIG. 12 is a graph illustrating a relationship between mRNA levels of Stx2eB and the levels of accumulated Stx2eB;

FIG. 13 is a view illustrating the design of DNA constructs encoding Stx2eB, in which A, B, and C denote the designs of an endoplasmic reticulum type, a cytoplasm type, and a chloroplast type of the DNA constructs, respectively;

FIG. 14 is a view illustrating the design of DNA constructs encoding CTB, in which A, B, and C denote the designs of an endoplasmic reticulum type, a cytoplasm type and a chloroplast type of the DNA constructs, respectively;

FIG. 15 is a photograph illustrating the levels of accumulated Stx2eB obtained in a transient expression experiment using the *Lactuca sativa* protoplasts;

FIG. 16 is a photograph illustrating the levels of accumulated CTB obtained in a transient expression experiment using the *Lactuca sativa* protoplasts;

FIG. 17 is photographs illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using the cultured tobacco cells (BY2), in which the numeral in each lane denotes the clone number;

FIG. 18 is photographs illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using a tobacco plant body, in which the numeral in each lane denotes the clone number;

FIG. 19 is a photograph illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using the tobacco plant body, in which the numeral in each lane denotes the clone number;

FIG. 20 is a view illustrating the design of DNA constructs encoding Stx2eB;

FIG. 21 is a view illustrating the design of DNA constructs encoding CTB;

FIG. 22 is photographs illustrating the levels of accumulated Stx2eB obtained in a transformation experiment using the cultured tobacco cells (BY2); and

FIG. 23 is a photograph illustrating the levels of accumulated CTB obtained in a transformation experiment using the cultured tobacco cells (BY2).

Description of Embodiments

[0016] In a hybrid protein of the present invention, two or three of Shiga toxin (Stx) proteins, cholera toxin (CT) proteins, or *Escherichia coli* heat-labile toxin (LT) proteins are each tandemly linked through a peptide having the following characteristics (A) and (B):

1. (A) the number of amino acids is 12 to 30; and
2. (B) the content of proline is 20 to 35%.

[0017] Shiga toxins (Stxs) are classified into type 1 (Stx1) and type 2 (Stx2). The Stx1 is further classified into subclasses a to d, and the Stx2 is further classified into subclasses a to g, respectively. The Shiga toxin includes one A subunit which is a toxin main body and five B subunits involved in invasion into intestinal mucosa.

[0018] Of those, for example, Stx2e is known as a swine edema disease toxin, and its A subunit (Stx2eA) is represented by an amino acid sequence of SEQ ID NO: 4 and its B subunit (Stx2eB) is represented by an amino acid sequence of SEQ ID NO: 6.

[0019] In Stx2eA and Stx2eB, one or several amino acids may be substituted, deleted, inserted, or added in the amino acid sequences represented by SEQ ID NO: 4 or 6 as long as they can cause an immune response by administering to pigs. For example, the term "several" means the number of preferably 2 to 30, more preferably 2 to 20, and still more preferably 2 to 10, in Stx2eA, and means the number of preferably 2 to 10, more preferably 2 to 5, and still more preferably 2 to 3, in Stx2eB.

[0020] Further, Stx2eA and Stx2eB may be those having an identity of preferably 85% or more, more preferably 90% or more, and still more preferably 95% or more to the amino acid sequences represented by SEQ ID NOS: 4 and 6, and being capable of causing the immune response by administering to the pig.

[0021] The cholera toxin (CT) includes one A subunit (CTA) which is the toxin main body and five B subunits (CTB) represented by SEQ ID NO: 8 and involved in the invasion into intestinal mucosa.

[0022] In CTB, one or several amino acids may be substituted, deleted, inserted, or added in the amino acid sequence represented by SEQ ID NO: 8 as long as CTB can cause the immune response by administering to animals. The term "several" means preferably 2 to 10, more preferably 2 to 5, and still more preferably 2 to 3.

[0023] Further, CTB may be those having an identity of preferably 85% or more, more preferably 90% or more, and still more preferably 95% or more to the amino acid sequences represented by SEQ ID NO: 8, and being capable of causing the immune response by administering to the animals.

[0024] The *Escherichia coli* heat-labile toxin (LT) protein includes one A subunit which is the toxin main body and five B subunits involved in the invasion into intestinal mucosa.

[0025] The Shiga toxin, the cholera toxin, and the *Escherichia coli* heat-labile toxin are also collectively referred to as "bacterial toxins" herein.

[0026] The number of the amino acids in the peptide is preferably 12 to 25 and more preferably 12 to 22. The content of proline in the peptide is preferably 20 to 27% and more preferably 20 to 25%.

[0027] Further, proline is allocated preferably every two or three residues in the peptide. But, in this case, the amino acids other than proline may be consecutive within 5 residues and preferably 4 residues in the terminus of the peptide.

[0028] In addition, the total content of serine, glycine, arginine, lysine, threonine, glutamine, asparagine, histidine, and aspartic acid in the amino acids other than proline is preferably 70% or more, more preferably 80% or more, and still more preferably 90% or more in the peptide. Further, the total content of serine, glycine, and asparagine in the amino acids other than proline is preferably 70% or more, more preferably 80% or more, and still more preferably 90% or more in the peptide. Still further, the total content of serine and glycine in the amino acids other than proline is preferably 70% or more, more preferably 80% or more, and still more preferably 90% or more in the peptide. This is because the peptide containing those amino acids abundantly is hard to form a secondary structure (β -sheet structure and helix structure).

[0029] Meanwhile, the total content of alanine, methionine, and glutamic acid in the amino acids other than proline is preferably 30% or less, more preferably 20% or less, and still more preferably 10% or less in the peptide. This is because the peptide containing those amino acids abundantly easily forms the helix structure. The total content of tryptophan, leucine, isoleucine, tyrosine, phenylalanine, and valine in the amino acids other than proline is preferably 20% or less, more preferably 10% or less, and still more preferably 5% or less in the peptide. This is because the peptide containing those amino acids abundantly easily forms the β -sheet structure and the helix structure.

[0030] The peptide is preferably selected from the peptide (PG12) having the amino acid

sequence represented by SEQ ID NO: 2, the peptide (PG17) having the amino acid sequence represented by SEQ ID NO: 82, and the peptide (PG22) having the amino acid sequence represented by SEQ ID NO: 84.

[0031] In the hybrid protein of the present invention, two or three hybrid proteins of the A subunit and the B subunit may be tandemly linked through the above peptide, or two or three A subunits may be tandemly linked through the peptide, or two or three B subunits may be tandemly linked through the peptide. When the hybrid protein contains the A subunit, the A subunit is preferably attenuated. In the hybrid protein of the present invention, preferably two or three B subunits are tandemly linked through the peptide. In the hybrid protein of the present invention, preferably two B subunits are tandemly linked through PG12.

[0032] Further, in the hybrid protein of the present invention, the peptide is preferably added to its C terminus. PG12 is particularly preferably added to its C terminus in the hybrid protein of the present invention.

[0033] The hybrid protein of the present invention has the amino acid sequence represented by SEQ ID NO: 10, 12, 14, 16, 86, 88, 90, 92, 94, 96, 98, or 100, for example. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 10, two Stx2eBs are tandemly linked through PG12. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 12, two CTBs are tandemly linked through PG12. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 14, two Stx2eBs are tandemly linked through PG12 and further PG12 is linked to the C terminus thereof. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 16, two CTBs are tandemly linked through PG12 and further PG12 is linked to the C terminus thereof. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 86, three Stx2eBs are each tandemly linked through PG12. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 88, three Stx2eBs are each tandemly linked through PG12 and further PG12 is linked to the C terminus thereof. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 90, two Stx2eBs are tandemly linked through PG17 and further PG12 is linked to the C terminus thereof. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 92, two Stx2eBs are tandemly linked through PG22 and further PG12 is linked to the C terminus thereof. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 94, three CTBs are each tandemly linked through PG12. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 96, three CTBs are each tandemly linked through PG12 and further PG12 is linked to the C terminus thereof. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 98, two CTBs are tandemly linked through PG17 and further PG12 is linked to the C terminus thereof. In the hybrid protein having the amino acid sequence represented by SEQ ID NO: 100, two CTBs are tandemly linked through PG22 and further PG12 is linked to the C terminus thereof.

[0034] By using the peptide such as PG12, PG17, or PG22 as a linker for linking the bacterial toxin proteins, the level of the bacterial toxin protein accumulated in the plant cell is increased.

[0035] In the hybrid protein of the present invention, a secretory signal peptide derived from a plant or a chloroplast transit signal peptide is preferably added to its amino terminus. Here, the term "addition" is a concept including both the case where the secretory signal peptide is directly bound to the amino terminus of the two or three bacterial toxin proteins linked through the peptide and the case where the secretory signal peptide is bound thereto through another peptide.

[0036] The secretory signal peptide is derived from preferably a plant belonging to the family *Solanaceae*, *Brassicaceae*, or *Asteraceae*, further preferably a plant belonging to the genus *Nicotiana*, *Arabidopsis*, *Lactuca*, etc., and more preferably *Nicotiana tabacum* or *Arabidopsis thaliana*, *Lactuca sativa*, etc.

[0037] Moreover, it is preferably derived from a β -D-glucan exohydrolase of *Nicotiana tabacum* or a 38-kDa peroxidase of *Nicotiana tabacum* (GenBank ACCESSION D42064).

[0038] An example of the secretory signal peptide includes a peptide that is derived from a β -D-glucan exohydrolase of *Nicotiana tabacum* and has the amino acid sequence represented by SEQ ID NO: 18.

[0039] An example of the chloroplast transit signal peptide includes a chloroplast transit signal peptide (transit peptide, T.P., SEQ ID NO: 79) derived from *Lactuca sativa* Rbcs (Rubisco small subunit) (GenBank ACCESSION D14001). A base sequence of DNA which encodes the chloroplast transit signal peptide derived from *Lactuca sativa* Rbcs is represented by SEQ ID NO: 80. Herein, the hybrid protein including the chloroplast transit signal peptide added to its amino terminus is also referred to as a chloroplast-type (Chl) hybrid protein, and a DNA construct encoding the chloroplast-type (Chl) hybrid protein is also referred to as a chloroplast-type DNA construct. The chloroplast-type hybrid protein is efficiently accumulated particularly in a plant whose chloroplast is developed well such as *Nicotiana tabacum*.

[0040] The hybrid protein in which neither the secretory signal peptide nor the chloroplast transit signal protein is added to its amino terminus is referred to as a cytoplasm-type (Cyt) hybrid protein, and the DNA construct encoding the cytoplasm-type hybrid protein is referred to as a cytoplasmic-type DNA construct. In the cytoplasm-type hybrid protein, particularly preferably three bacterial toxin protein B subunits are tandemly linked through the peptide.

[0041] In the hybrid protein of the present invention, the signal peptide such as an endoplasmic reticulum retention signal peptide and a vacuolar transport signal peptide may be added to its carboxyl terminus. Here, the term "addition" is the concept including both the case where the signal peptide is directly bound to the carboxyl terminus of the hybrid protein and the case where the signal peptide is bound thereto through another peptide. Herein, the hybrid protein in which the secretory signal peptide is added to its amino terminus and the endoplasmic reticulum retention signal peptide is added to the carboxyl terminus is also referred to as an endoplasmic reticulum-type (ER) hybrid protein, and the DNA construct

encoding the endoplasmic reticulum-type hybrid protein is also referred to as an endoplasmic reticulum-type DNA construct. The endoplasmic reticulum-type hybrid protein is efficiently accumulated particularly in a plant such as *Lactuca sativa*.

[0042] In the hybrid protein of the present invention, the endoplasmic reticulum retention signal peptide is preferably added to its carboxyl terminus. Examples of the endoplasmic reticulum retention signal peptide include an endoplasmic reticulum retention signal peptide including KDEL sequence (SEQ ID NO: 19), HDEL sequence (SEQ ID NO: 20), KDEF sequence (SEQ ID NO: 21), or HDEF sequence (SEQ ID NO: 22).

[0043] The vacuolar transport signal peptide is derived from preferably a plant belonging to the family *Solanaceae*, *Brassicaceae*, or *Asteraceae*, further preferably a plant belonging to the genus *Nicotiana*, *Arabidopsis*, *Armoracia*, etc., and more preferably *Nicotiana tabacum*, *Arabidopsis thaliana*, *Armoracia rusticana*, etc. In addition, the peptide is preferably derived from a chitinase. The amino acid sequence of a vacuolar transport signal peptide derived from a tobacco chitinase is represented by SEQ ID NO: 76. Meanwhile, the base sequence of DNA encoding a vacuolar transport signal peptide derived from a tobacco chitinase is represented by SEQ ID NO: 75, for example.

[0044] Moreover, the peptide is preferably derived from a horseradish peroxidase C1a isozyme. The amino acid sequence of a vacuolar transport signal peptide derived from a horseradish peroxidase C1a isozyme is represented by SEQ ID NO: 78. Meanwhile, the base sequence of DNA encoding a vacuolar transport signal peptide derived from a horseradish peroxidase C1a isozyme is represented by SEQ ID NO: 77, for example. Herein, the hybrid protein in which the secretory signal peptide is added to its amino terminus, and the vacuolar transport signal peptide is added to its carboxyl terminus is also referred to as a vacuole-type (Vac) hybrid protein, and a DNA construct encoding the vacuole-type hybrid protein is also referred to as a vacuole-type DNA construct.

[0045] The hybrid protein of the present invention may be synthesized chemically, or may be produced by genetic engineering. A method of producing by the genetic engineering is described later.

[0046] The DNA construct of the present invention is characterized by containing DNA encoding the hybrid protein of the present invention.

[0047] That is, the DNA construct of the present invention includes DNA in which DNAs encoding the two or three bacterial toxin proteins are tandemly linked through DNA encoding the peptide. The DNA encoding the peptide is represented by, for example, SEQ ID NO: 1 (PG12), SEQ ID NO: 81 (PG17), and SEQ ID NO: 83 (PG22). Examples of the DNA encoding the bacterial toxin protein include DNA (SEQ ID NO: 3) encoding Stx2eA, DNA (SEQ ID NO: 5) encoding Stx2eB, and DNA (SEQ ID NO: 7) encoding CTB. The DNA encoding the peptide and the DNA encoding the bacterial toxin protein are linked by matching their reading frames except stop codons.

[0048] The DNA encoding the bacterial toxin protein can be obtained by a common genetic engineering technique based on the base sequence of SEQ ID NO: 3, 5, or 7, for example. Specifically, a cDNA library is prepared from a bacterium which produces each bacterial toxin according to a conventional method, and a desired clone is selected using a probe prepared from the library based on the base sequence. Alternatively, the DNA can also be synthesized chemically based on the base sequence, or synthesized by PCR with genomic DNA as a template using a 5'- and 3'- terminal base sequence of the base sequence as primers, for example.

[0049] The DNA encoding the hybrid protein of the present invention is represented by SEQ ID NO: 9, 11, 13, 15, 85, 87, 89, 91, 93, 95, 97, or 99.

[0050] In the DNA encoding the hybrid protein, preferably a codon corresponding to an amino acid which composes the hybrid protein is appropriately modified so that the amount of the translated hybrid protein is increased depending on a host cell in which the hybrid protein is produced.

[0051] As the method of modifying the codon, a method of Kang et al. (2004) may serve as a reference, for example. And, the method of selecting the codon frequently used in the host cell, the method of selecting the codon in which the content of GC is high, or the method of selecting the codon frequently used in house keeping genes in the host cell is exemplified.

[0052] Further, the DNA encoding the hybrid protein may be DNA which hybridizes with DNA having the base sequence of SEQ ID NO: 9, 11, 13, 15, 85, 87, 89, 91, 93, 95, 97, or 99 under a stringent condition. The term "stringent condition" refers to the condition where a so-called specific hybrid is formed whereas no non-specific hybrid is formed. There is exemplified the condition where two DNAs with high identity, e.g., two DNAs having the identity of preferably 80% or more, more preferably 90% or more, and particularly preferably 95% or more are hybridized with each other whereas two DNAs with lower identity than that are not hybridized. Further, there is exemplified the condition of 2×SSC (330 mM NaCl, 30 mM citric acid) at 42°C and preferably 0.1×SSC (330 mM NaCl, 30 mM citric acid) at 60°C.

[0053] In the DNA construct of the present invention, preferably the DNA encoding the hybrid protein is operably-linked to an enhancer. The term "operably" refers to the fact that the hybrid protein is produced in host cells when a vector obtained by inserting the DNA construct of the present invention into a vector including a suitable promoter is introduced into suitable host cells. In addition, the term "linked" refers to both a case where two DNAs are directly linked and a case where two DNAs are linked via another base sequence.

[0054] Examples of the enhancer include Kozak sequence and a 5'-untranslated region of an alcohol dehydrogenase gene derived from a plant. Particularly preferably, the DNA encoding the hybrid protein is operably-linked to the 5'-untranslated region of an alcohol dehydrogenase gene derived from a plant.

[0055] The 5'-untranslated region of an alcohol dehydrogenase gene refers to a region including a sequence between the base at the transcription initiation site of a gene encoding an alcohol dehydrogenase and the base before the translation initiation site (ATG, methionine). The region has a function to increase a translation level. The phrase "function to increase a translation level" refers to a function to increase an amount of a protein produced by translation when the information encoded in a structural gene is transcribed and then translated to produce a protein. The region may be derived from a plant, and it is preferably derived from a plant belonging to the family *Solanaceae*, *Brassicaceae*, or *Asteraceae*, further preferably derived from a plant belonging to the genus *Nicotiana*, *Arabidopsis*, *Lactuca*, etc., and more preferably derived from *Nicotiana tabacum*, *Arabidopsis thaliana*, *Lactuca sativa*, etc.

[0056] The 5'-untranslated region of an alcohol dehydrogenase gene is particularly preferably a region including the base sequence represented by SEQ ID NO: 23, which is the 5'-untranslated region of an alcohol dehydrogenase gene (NtADH 5'-UTR) derived from *Nicotiana tabacum*, for example.

[0057] The 5'-untranslated region of an alcohol dehydrogenase gene derived from a plant can be isolated from an alcohol dehydrogenase gene of a plant cultured cell where an alcohol dehydrogenase is highly expressed, for example (see JP 2003-79372 A). Meanwhile, in the case of a region having a determined base sequence, such as the 5'-untranslated region of an alcohol dehydrogenase gene derived from *Nicotiana tabacum*, the region can also be synthesized by chemical synthesis, PCR using a genomic DNA as a template and using the base sequences of the 5'- and 3'-termini of the region as primers, or the like. In addition, if a part of the region having a determined base sequence is used as a probe, the 5'-untranslated region of an alcohol dehydrogenase gene derived from another plant can be searched and isolated.

[0058] The 5'-untranslated region of an alcohol dehydrogenase gene represented by the base sequence of SEQ ID NO: 23 may have substitution, deletion, insertion, or addition of one or several bases as long as the region has a function to increase a translation level. The term "several" means the number of preferably 2 to 10, further preferably 2 to 5, and particularly preferably 2 to 3.

[0059] In addition, DNA having an identity of preferably 85% or more and particularly preferably 90% or more to the 5'-untranslated region of an alcohol dehydrogenase gene and having an ability to increase a translation level may be used.

[0060] Whether the region has an intended function to increase a translation level or not can be confirmed by, for example, a transient assay using a GUS (β -glucuronidase) gene or a luciferase gene as a reporter gene in tobacco cultured cells, or an assay in transformed cells engineered to carry those genes in a chromosome.

[0061] The DNA construct of the present invention has the base sequence represented by any one of SEQ ID NOS: 24 to 29 and SEQ ID NOS: 101 to 111, for example.

[0062] The DNA construct having the base sequence represented by SEQ ID NO: 24 is the DNA construct in which DNA (SEQ ID NO: 9) encoding the hybrid protein in which two Stx2eB proteins are tandemly linked through PG12 is linked to the 5'-untranslated region (NtADH 5'-UTR, SEQ ID NO: 23) of an alcohol dehydrogenase gene derived from *Nicotiana tabacum*. Further, the DNA construct having the base sequence represented by SEQ ID NO: 25 is the DNA construct in which DNA (SEQ ID NO: 11) encoding the hybrid protein in which two CTB proteins are tandemly linked through PG12 is linked to NtADH 5'-UTR.

[0063] The DNA construct having the base sequence represented by SEQ ID NO: 26 is the DNA construct in which DNA encoding the hybrid protein in which two Stx2eB proteins are tandemly linked through PG12, the secretory signal peptide is added to its amino terminus, and the endoplasmic reticulum retention signal peptide is added to its carboxyl terminus is linked to NtADH 5'-UTR. Further, the DNA construct having the base sequence represented by SEQ ID NO: 27 is the DNA construct in which DNA encoding the hybrid protein in which two CTB proteins are tandemly linked through PG12, the secretory signal peptide is added to its amino terminus, and the endoplasmic reticulum retention signal peptide is added to its carboxyl terminus is linked to NtADH 5'-UTR.

[0064] The DNA construct having the base sequence represented by SEQ ID NO: 28 is the DNA construct in which DNA encoding the hybrid protein in which two Stx2eB proteins are tandemly linked through PG12, the secretory signal peptide is added to its amino terminus, PG12 is linked to its carboxyl terminus, and further the endoplasmic reticulum retention signal peptide is added to its carboxyl terminus is linked to NtADH 5'-UTR. Further, the DNA construct having the base sequence represented by SEQ ID NO: 29 is the DNA construct in which DNA encoding the hybrid protein in which two CTB proteins are tandemly linked through PG12, the secretory signal peptide is added to its amino terminus, PG12 is linked to its carboxyl terminus, and further the endoplasmic reticulum retention signal peptide is added to its carboxyl terminus is linked to NtADH 5'-UTR.

[0065] The DNA construct having the base sequence represented by SEQ ID NO: 101 is the DNA construct in which DNA encoding the hybrid protein in which two Stx2eB proteins are tandemly linked through PG12 and PG12 is linked to its carboxyl terminus is linked to NtADH 5'-UTR.

[0066] The DNA construct having the base sequence represented by SEQ ID NO: 102 is the DNA construct in which DNA encoding the hybrid protein in which two Stx2eB proteins are tandemly linked through PG17 to NtADH 5'-UTR, the secretory signal peptide is added to its amino terminus, PG12 is linked to its carboxyl terminus, and further the endoplasmic reticulum retention signal peptide is added to its carboxyl terminus is linked to NtADH 5'-UTR. Further, the DNA construct having the base sequence represented by SEQ ID NO: 103 is the DNA construct in which DNA encoding the hybrid protein in which two Stx2eB proteins are tandemly linked through PG22, the secretory signal peptide is added to its amino terminus, PG12 is linked to its carboxyl terminus, and further the endoplasmic reticulum retention signal peptide is

added to its carboxyl terminus is linked to NtADH 5'-UTR.

[0067] The DNA construct having the base sequence represented by SEQ ID NO: 104 is the DNA construct in which DNA encoding the hybrid protein in which two Stx2eB proteins are tandemly linked through PG12, the chloroplast transit signal peptide is added to its amino terminus, and PG12 is linked to its carboxyl terminus is linked to NtADH 5'-UTR.

[0068] The DNA construct having the base sequence represented by SEQ ID NO: 105 is the DNA construct in which DNA encoding the hybrid protein in which three Stx2eB proteins are each tandemly linked through PG12 and PG12 is linked to its carboxyl terminus is linked to NtADH 5'-UTR.

[0069] The DNA construct having the base sequence represented by SEQ ID NO: 106 is the DNA construct in which DNA encoding the hybrid protein in which three Stx2eB proteins are each tandemly linked through PG12, the secretory signal peptide is added to its amino terminus, and PG12 is linked to its carboxyl terminus, and further the endoplasmic reticulum retention signal peptide is added to its carboxyl terminus is linked to NtADH 5'-UTR.

[0070] The DNA construct having the base sequence represented by SEQ ID NO: 107 is the DNA construct in which DNA encoding the hybrid protein in which three Stx2eB proteins are each tandemly linked through PG12, the chloroplast transit signal peptide is added to its amino terminus, and PG12 is linked to its carboxyl terminus is linked to NtADH 5'-UTR.

[0071] The DNA construct having the base sequence represented by SEQ ID NO: 108 is the DNA construct in which DNA encoding the hybrid protein in which two CTB proteins are tandemly linked through PG12 and PG12 is linked to its carboxyl terminus is linked to NtADH 5'-UTR.

[0072] The DNA construct having the base sequence represented by SEQ ID NO: 109 is the DNA construct in which DNA encoding the hybrid protein in which two CTB proteins are tandemly linked through PG17, the signal peptide is added to its amino terminus, PG12 is linked to its carboxyl terminus, and further the endoplasmic reticulum retention signal peptide is added to the its carboxyl terminus is linked to NtADH 5'-UTR. Further, the DNA construct having the base sequence represented by SEQ ID NO: 110 is the DNA construct in which DNA encoding the hybrid protein in which two CTB proteins are tandemly linked through PG22, the signal peptide is added to its amino terminus, PG12 is linked to its carboxyl terminus, and further the endoplasmic reticulum retention signal peptide is added to the its carboxyl terminus is linked to NtADH 5'-UTR.

[0073] The DNA construct having the base sequence represented by SEQ ID NO: 111 is the DNA construct in which DNA encoding the hybrid protein in which two CTB proteins are tandemly linked through PG12, the chloroplast transit signal peptide is added to its amino terminus, and PG12 is linked to its carboxyl terminus is linked to NtADH 5'-UTR.

[0074] The DNA construct of the present invention can be prepared by a general genetic engineering technique, which includes the following procedures: digesting DNAs including the 5'-untranslated region of an alcohol dehydrogenase gene derived from a plant, a DNA encoding a secretory signal peptide derived from a plant, a DNA encoding a chloroplast transit signal peptide, a DNA encoding a bacterial toxin protein, and a DNA encoding an endoplasmic reticulum retention signal peptide with suitable restriction enzymes; and ligating the resultant fragments with a suitable ligase.

[0075] The recombinant vector of the present invention is characterized by including the DNA construct of the present invention. The recombinant vector of the present invention may be a vector obtained by inserting a DNA encoding a hybrid protein of the present invention into a vector so that the DNA can be expressed in host cells to be introduced with the vector. The vector is not particularly limited as long as it can replicate in host cells, and examples thereof include a plasmid DNA and a viral DNA. In addition, the vector preferably includes a selective marker such as a drug resistance gene. The plasmid DNA can be prepared from *Escherichia coli* or *Agrobacterium* by the alkaline extraction method (Birnboim, H. C. & Doly, J. (1979) *Nucleic acid Res* 7: 1513) or a modified method thereof. Commercially available plasmids such as pBI221, pBI121, pBI101, and pLG121Hm may be used. The viral DNA may be, for example, pTB2 (Donson et al., 1991) (see Donson J., Kerney CM., Hilf ME., Dawson WO. Systemic expression of a bacterial gene by a tobacco mosaic virus-based vector. *Proc. Natl. Acad. Sci.* (1991) 88: 7204-7208).

[0076] Promoters to be used in vectors may be appropriately selected depending on host cells to be introduced with vectors. The promoters are preferably a cauliflower mosaic virus 35S promoter (Odell et al., 1985, *Nature* 313:810), a rice actin promoter (Zhang et al. 1991 *Plant Cell* 3:1155), a corn ubiquitin promoter (Cornejo et al., 1993, *Plant Mol. Biol.*, 23:567), etc. for example. Meanwhile, terminators to be used in vectors may be appropriately selected depending on host cells to be introduced with vectors. The terminators are preferably a nopaline synthase gene transcription terminator, a cauliflower mosaic virus 35S terminator, etc.

[0077] The recombinant vector of the present invention may be prepared as follows.

[0078] First, a DNA construct of the present invention is digested with a suitable restriction enzyme, or a restriction enzyme site is added to the DNA construct by PCR. Subsequently, the DNA construct is inserted into the restriction enzyme site or multicloning site of a vector.

[0079] The transformant of the present invention is characterized by being transformed with the recombinant vector of the present invention. The host cells to be used for transformation may be eukaryotic cells or prokaryotic cells.

[0080] The eukaryotic cells are preferably plant cells, particularly preferably cells of plants belonging to the family *Asteraceae*, *Solanaceae*, *Brassicaceae*, and *Chenopodiaceae*. Moreover, the eukaryotic cells are preferably cells of plants belonging to the genus *Lactuca*, particularly preferably *Lactuca sativa* cells. In the case of using *Lactuca sativa* cells as host

cells, a cauliflower mosaic virus 35S RNA promoter or the like may be used in the vector.

[0081] The prokaryotic cells may be cells of *Escherichia coli*, *Agrobacterium tumefaciens*, etc.

[0082] The transformant of the present invention can be prepared by a general genetic engineering technique by introducing a vector of the present invention into host cells. For example, the transformant can be prepared by the introduction method using *Agrobacterium* (Hood, et al., 1993, Transgenic, Res. 2:218, Hiei, et al., 1994 Plant J. 6:271), an electroporation method (Tada, et al., 1990, Theor. Appl. Genet, 80:475), a polyethylene glycol method (Lazzeri, et al., 1991, Theor. Appl. Genet. 81:437), a particle gun method (Sanford, et al., 1987, J. Part. Sci. tech. 5:27), a polycation method (Ohtsuki), etc.

[0083] After introduction of the vector of the present invention into host cells, a transformant of the present invention can be selected based on the phenotype of a selective marker. If the selected transformant is cultured, the bacterial toxin protein can be produced. The culture medium and conditions for culture may be suitably selected depending on the type of a transformant.

[0084] In addition, in the case of using a plant cell as a host cell, culture of selected plant cell in accordance with a conventional method can regenerate a plant and accumulate the bacterial toxin protein in the plant cells or outside the cell membranes of the plant cells. The method depends on the type of the plant cell, and examples thereof include the method of Visser et al. (Theor. Appl. Genet, 78:594(1989)) for potato and the method of Nagata and Takebe (Planta, 99:12(1971)) for *Nicotiana tabacum*.

[0085] In the case of *Lactuca sativa*, a shoot can be regenerated in MS medium containing 0.1 mg/l NAA (naphthalene acetic acid), 0.05 mg/l BA (benzyladenine), and 0.5 g/l polyvinylpyrrolidone, and culture of the regenerated shoot in a 1/2 MS medium containing 0.5 g/l polyvinylpyrrolidone may cause rooting.

[0086] The seed of the present invention can be obtained by collecting a seed from a plant regenerated as above. If the seed of the present invention are sown and cultivated by a suitable method, a plant capable of producing a bacterial toxin protein can be obtained and is included in the transformant of the present invention.

EXAMPLES

<1> Transient expression experiment

(1) Construction of Stx2eB transient expression vector

[0087] A vector containing a DNA construct in which DNA (SEQ ID NO: 5) encoding an Stx2e protein B subunit (Stx2eB) was linked to a 5'-untranslated region (NtADH 5'-UTR) of a tobacco alcohol dehydrogenase gene was prepared as follows.

[0088] A design of the vector is shown in FIG. 1.

[0089] 1×Stx2eB (PG12) denotes a DNA construct containing DNA in which DNA encoding PG12 is linked to DNA encoding Stx2eB. 2×Stx2eB (PG12) denotes a DNA construct containing DNA in which two DNAs encoding Stx2eB are linked using DNA encoding PG12 as a spacer.

[0090] In addition, a DNA construct, 3×Stx2eB (PG12) in which three DNAs encoding Stx2eB are linked using DNA encoding PG12 as a spacer, and a DNA construct, 4×Stx2eB (PG12) in which four DNAs encoding Stx2eB are linked using DNA encoding PG12 as a spacer were prepared as well.

[0091] Specific techniques are shown below.

[0092] PCR using a Kozak-stx2eb-F primer (SEQ ID NO: 30) and an stx2eb-R primer (SEQ ID NO: 31) was performed to amplify a DNA fragment encoding a mature region (except for a secretory signal peptide to periplasm, Ala 19 to Asn 87) of Stx2eB. The resulting DNA fragment was cloned into an EcoRV gap in pBluescript II SK. The resulting plasmid was cleaved with HindIII, and treated with T4 DNA polymerase, followed by self-ligation to convert a HindIII site to a NheI site (plasmid 1).

[0093] Stx2eB was inserted as follows into the multicloning site (MCS) of a transient expression vector in plant cells, pBI221 (Clontech).

[0094] In order to introduce Sall, KpnI, and SmaI sites into the MCS, SalkpnSma-F (SEQ ID NO: 32) and SalkpnSma-R (SEQ ID NO: 33) were annealed and phosphorylated with T4 polynucleotide kinase (T4 PNK) (TaKaRa) and inserted into the SacI gap of pBI221 (plasmid 2). Stx2eB was cleaved out from plasmid 1 using XbaI and KpnI to insert into plasmid 2, and the resultant product was arranged between a cauliflower mosaic virus 35S RNA promoter (35S pro.) and a nopaline synthase gene transcription terminator (NOS-T) (plasmid 3).

[0095] The 5'-untranslated region (NtADH 5'-UTR, SEQ ID NO: 23) of a tobacco alcohol dehydrogenase gene was amplified by PCR with ADH-221 (Sato et al., 2004, (see below)) as a template using ADH XbaI-F primer (SEQ ID NO: 34) and ADH NsiI-R primer (SEQ ID NO: 35). A DNA region (SEQ ID NO: 17) encoding a secretory signal peptide (SEQ ID NO: 18) of β -D glucan exohydrolase (GenBank ACCESSION AB017502) was amplified with a tobacco genomic DNA as a template using β D NsiI-F primer (SEQ ID NO: 36) and β D BamHI-R primer (SEQ ID NO: 37). The obtained respective DNA fragments of NtADH 5'-UTR and the secretory signal peptide were treated with NsiI (manufactured by Toyobo Co., Ltd.), ligated using Ligation High (manufactured by Toyobo Co., Ltd.), followed by being blunted, and cloned into the

EcoRV gap in pBluescript II SK (manufactured by Stratagene) (plasmid 4).

[0096] Satoh et al., The 5'-untranslated region of the tobacco alcohol dehydrogenase gene functions as an effective translational enhancer in plant. *J. Biosci. Bioeng.* (2004) 98,1-8

[0097] Plasmid 4 was treated with NsiI, and blunted with T4 DNA polymerase (manufactured by Toyobo Co., Ltd.), followed by performing self-ligation to be ligated so that the initiation codon (atg) of NtADH was matched to the initiation codon of the secretory signal peptide (plasmid 5).

[0098] A DNA obtained by ligating an NtADH 5'-UTR fragment and a secretory signal peptide was amplified using plasmid 5 as a template and using ADH XbaI-F primer (SEQ ID NO: 34) and β D BamHI-R primer (SEQ ID NO: 35). The resultant DNA fragment was treated with XbaI and BamHI and inserted into the XbaI-BamHI gap of plasmid 3 (plasmid 6).

[0099] In order to add an endoplasmic reticulum retention signal (SEQ ID NO: 38), an HDEL-F primer (SEQ ID NO: 39) and an HDEL-R primer (SEQ ID NO: 40) were annealed and phosphorylated with T4 PNK, and the resultant product was inserted into the BglII gap of plasmid 6, which had been dephosphorylated with alkaline phosphatase (AP) (TakaRa) (plasmid 7).

[0100] An HA tag was added as a peptide tag for detecting Stx2eB. In order to add the HA tag, an HA-F primer (SEQ ID NO: 41) and an HA-R primer (SEQ ID NO: 42) were annealed and phosphorylated with T4 PNK. The resultant phosphorylated HA fragment was inserted into the BglII gap of plasmid 7 (plasmid 8) .

[0101] A PG12 spacer (SEQ ID NO: 2) was inserted between Stx2eB and the HA tag. A PG12-F primer (SEQ ID NO: 43) and a PG12-R primer (SEQ ID NO: 44) were annealed and phosphorylated with T4 PNK. The resulting phosphorylated DNA fragment was inserted into the BglII gap of plasmid 8 (1×Stx2eB (PG12)).

[0102] 2×Stx2eB (PG12) was obtained by cleaving a 2eB-PG12 fragment out from 1×Stx2eB (PG12) with BamHI and BglII and then inserting the fragment into the BamHI gap of the 1×Stx2eB (PG12). 3×Stx2eB (PG12) was obtained by cleaving an Stx2eB-PG12 fragment out from 1×Stx2eB (PG12) with BamHI and BglII and then inserting the fragment into the BamHI gap of 2×Stx2eB (PG12). Further, 4×Stx2eB (PG12) was obtained by cleaving a 2x (Stx2eB-PG12) fragment out from 2×Stx2eB (PG12) with BamHI and BglII and then inserting the fragment into the BamHI gap of the 2×Stx2eB (PG12).

(2) Construction of CTB transient expression vector

[0103] A vector containing a DNA construct (2×CTB (PG12)) in which DNA encoding a CT protein B subunit (CTB) had been linked to the 5'-untranslated region of a tobacco alcohol

dehydrogenase gene was prepared as follows.

[0104] A DNA fragment (SEQ ID NO: 7) encoding the mature region (except for the secretory signal to the periplasm, Thr 22 to Asn 124) (SEQ ID NO: 8) of CTB was prepared. First, the following ten primers were prepared.

CTB1: SEQ ID NO: 45

CTB2: SEQ ID NO: 46

CTB3: SEQ ID NO: 47

CTB4: SEQ ID NO: 48

CTB5: SEQ ID NO: 49

CTB6: SEQ ID NO: 50

CTB7: SEQ ID NO: 51

CTB8: SEQ ID NO: 52

CTB9: SEQ ID NO: 53

CTB10: SEQ ID NO: 54

[0105] PCR using the primers synthesized above was performed under the condition described in Kang et al. (2004). That is, PCR was performed in combination of CTB1 and CTB2, CTB3 and CTB4, CTB5 and CTB6, CTB7 and CTB8, and CTB9 and CTB10, and DNA fragments of 72 bp (1+2), 74 bp (3+4), 67 bp (5+6), 82 bp (7+8) and 68 bp (9+10) were synthesized, respectively. Subsequently, the second PCR was performed in combination of CTB1+2 and CTB3+4, CTB3+4 and CTB5+6, CTB5+6 and CTB7+8, and CTB7+8 and CTB9+10, and DNA fragments of 135 bp (1+2+3+4), 132 bp (3+4+5+6), 138 bp (5+6+7+8), and 141 bp (7+8+9+10) were synthesized, respectively. Then, the third PCR was performed in combination of CTB1+2+3+4 and CTB3+4+5+6, and CTB5+6+7+8 and CTB7+8+9+10, and DNA fragments of 194 bp (1+2+3+4+5+6) and 198 bp (5+6+7+8+9+10) were synthesized, respectively. Finally, PCR was performed in combination of CTB1+2+3+4+5+6 and CTB5+6+7+8+9+10, and a DNA fragment of 315 bp in which a BamHI site and a BglII site were added to a CTB coding region was synthesized.

[0106] The DNA fragment prepared above was treated with BamHI and BglII, and inserted into a BamHI-BglII gap in plasmid 8 (plasmid 9).

[0107] The PG12 spacer (SEQ ID NO: 2) was inserted between CTB and the HA tag. A PG12-F primer (SEQ ID NO: 43) and a PG12-R primer (SEQ ID NO: 44) were annealed and phosphorylated with T4 PNK. The resulting phosphorylated DNA fragment was inserted into

the BglII gap of plasmid 9 (1×CTB (PG12)).

[0108] A CTB-PG12 fragment was cut out from 1×CTB (PG12) using BamHI and BglII, and inserted into the BamHI gap of 1×CTB (PG12) (2×CTB (PG12)).

(3) Transient expression test using *Lactuca sativa* protoplast

[0109] A leaf of potted *Lactuca sativa* (green wave) (about 1 g) was cut into 0.5-cm square pieces using a surgical knife, to thereby prepare leaf discs. The leaf discs were immersed in 500 mM mannitol, and shaken for 1 hour. The leaf discs were immersed in 50 ml of a protoplastization enzyme solution (1.0% cellulose RS (Yakult Honsha Co., Ltd.), 0.25% macerozyme R-10 (Yakult Honsha Co., Ltd.), 400 mM mannitol, 8 mM CaCl₂, and 5 mM Mes-KOH, pH 5.6), and the whole was shaken at room temperature for 2 hours. The protoplast suspension was passed through meshes of 100 μm and 40 μm to remove the leaf discs. The protoplast suspension was centrifuged at 60 g for 5 minutes to precipitate the protoplast. The protoplast was resuspended in an aqueous solution containing 167 mM mannitol and 133 mM CaCl₂, and the suspension was centrifuged at 40 g for 5 minutes. The protoplast was resuspended in an aqueous solution containing 333 mM mannitol and 66.7 mM CaCl₂, and the suspension was centrifuged at 40 g for 5 minutes. The protoplast was suspended in W5 solution (154 mM NaCl, 125 mM CaCl₂, 5 mM KCl, 2 mM Mes-KOH, pH 5.6), and the suspension was allowed to stand on ice for 1 hour. The protoplast suspension was centrifuged at 40 g for 5 minutes, and the protoplast was suspended in an MaMg solution (400 mM mannitol, 15 mM MgCl₂, and 4 mM Mes-KOH, pH 5.6) to have a protoplast concentration of 2×10⁶ cells/ml.

[0110] Each of the Stx2eB transient expression vector and the CTB transient expression vector prepared above was mixed with 120 μL of a protoplast suspension, subsequently 140 μL of PEG solution (400 mM mannitol, 100 mM Ca(NO₃)₂ and 40% PEG) was added thereto, and the resulting mixture was blended gently and incubated for 7 minutes. Then, 1 mL of W5 solution was added to the protoplast suspension over about 20 minutes. A solution (1 mL) obtained by mixing 400 mM mannitol and the W5 solution at a ratio of 4:1 was added to the protoplast precipitated by centrifugation. LS medium (1 mL) containing 1% sucrose, 400 mM mannitol, and 0.3 mM carbenicillin was added to the protoplast precipitated by centrifugation, and the mixture was then cultured in a dark place at 25°C for 24 hours.

(4) Western analysis

[0111] To the protoplast collected by centrifugation were added 30 μl of SDS-sample buffer (4% (w/v) SDS, 20% (w/v) glycerol, 0.05% (w/v) bromophenol blue, 300 mM β-mercaptoethanol, 125 mM Tris-HCl, pH 6.8), followed by thermal denaturation at 95°C for 2

minutes, to thereby prepare samples. Proteins were separated using a 15% acrylamide gel and blotted on a PVDF membrane (Hybond-P; Amersham) using an electro transfer system. An anti-HA antibody (No. 11 867 423 001, Roche) was used to detect Stx2eB and CTB.

(a) Effects of linking number of Stx2eB

[0112] A result is shown in FIG. 2. When 1×Stx2eB (PG12) was expressed, a signal was detected at a position of about 8.5 kDa. When 2×Stx2eB (PG12) was expressed, a signal was detected at a position of about 17 kDa at the same level as when 1×Stx2eB (PG12) was expressed. When 3×Stx2eB (PG12) was expressed, a signal was detected at a position of about 26 kDa, the signal being smaller than that when 1×Stx2eB (PG12) was expressed. These corresponded to the molecular weights estimated from the design of the DNA constructs. When 4×Stx2eB (PG12) was expressed, the specific signal was below a detection limit.

[0113] From the above result, it was demonstrated that when 2×Stx2eB (PG12) and 3×Stx2eB (PG12) were expressed, hybrid proteins in which multiple Stx2eB proteins were linked could be produced.

[0114] Since each of the above DNA constructs contains one molecule of the HA tag (see FIG. 1), it is conceivable that a protein amount corresponding to about 2 times an Stx2eB protein amount when 1×Stx2eB (PG12) is expressed is accumulated when 2×Stx2eB (PG12) is expressed. That is, it has been found that when two DNAs encoding the Stx2eB protein are linked through DNA encoding PG12, the Stx2eB protein can be produced with very high efficiency.

[0115] Meanwhile, it has been also found that when three DNAs encoding the Stx2eB protein or four DNAs encoding the Stx2eB protein are linked through DNA encoding PG12, the amount of the produced Stx2eB protein is equal to or less than the amount when one Stx2eB protein is linked to PG12.

[0116] It has been found that the level of accumulated proteins tends to be higher in the Stx2eB protein (1×Stx2eB (PG12)) having PG12 added at its carboxyl terminus prepared in this experiment, compared with the Stx2eB protein having no PG12. This speculates that it is a favorable form that PG12 is added to the carboxyl terminus in the hybrid protein of the present invention.

(b) Effects of linking number of CTB

[0117] The results are shown in FIG. 3.

[0118] When 1×CTB (PG12) was expressed, a signal was detected at the position of about 20kDa. When 2×CTB (PG12) was expressed, a larger signal than that when 1×CTB (PG12) was expressed was detected at the positions of about 33 kDa and about 35 kDa.

[0119] From the above results, it was demonstrated that the hybrid protein in which two CTB proteins were linked could be produced when 2×CTB (PG12) was expressed. These corresponded to the molecular weights estimated from the design of the DNA constructs.

[0120] Since each of the above DNA constructs contains one molecule of the HA tag, it is conceivable that the protein amount corresponding to the CTB protein amount which is larger than two times the protein amount when 1×CTB (PG12) is expressed is accumulated when 2×CTB (PG12) is expressed. That is, it has been found that when two DNAs encoding the CTB protein are linked through DNA encoding PG12, the CTB protein can be produced with very high efficiency.

(5) Analysis of localization of Stx2eB

[0121] In order to analyze the localization of Stx2eB in the cell, a transient expression vector for the hybrid protein of Stx2eB and a yellow fluorescent protein YFP was prepared. The design for the vector is shown in FIG. 4. 1×Stx2eB (PG12)-YFP denotes a DNA construct in which DNA encoding the Stx2eB protein is linked to DNA encoding YFP. 2×Stx2eB (PG12)-YFP denotes a DNA construct in which DNA encoding the hybrid protein in which two Stx2eB proteins are linked through PG12 is linked to DNA encoding YFP. 2×Stx2eB (RS)-YFP using RS (Arg Ser) in place of PG12 as a spacer was also prepared.

[0122] A specific technique is shown below.

[0123] First, a DNA fragment of YFP was amplified by PCR with pEYFP (Clontech) as a template using a YFP-F primer (SEQ ID NO: 55) and a YFP-R primer (SEQ ID NO: 56). The resulting DNA fragment was treated with BamHI and BglII, and inserted into the BamHI-BglII gap of plasmid 8 (ER-YFP).

[0124] An Stx2eB fragment was cleaved out from plasmid 1 with BamHI and BglII and then inserted into the BamHI gap of the 1×Stx2eB (PG12) (2×Stx2eB (RS)).

[0125] An Stx2eB-PG12 fragment, a 2×(Stx2eB-RS) fragment, and a 2×(Stx2eB-PG12) fragment were cleaved out from 1×Stx2eB (PG12), 2×Stx2eB (RS), and 2×Stx2eB (PG12) with BamHI-BglII, respectively, and each fragment was inserted into the BamHI gap of ER-YFP (1×Stx2eB (PG12)-YFP, 2×Stx2eB (RS)-YFP, and 2×Stx2eB (PG12)-YFP).

[0126] Meanwhile, an expression vector for a red fluorescent protein (mRFP, Campbell R. E. et al., 2002, (see below)) localized in the endoplasmic reticulum was prepared as a vector for visualizing the endoplasmic reticulum. PCR was performed using an mRFP-F primer (SEQ ID

NO: 57) and an mRFP-R primer (SEQ ID NO: 58). The resulting DNA fragment was treated with BamHI and BglII, and inserted into the BamHI-BglII gap of plasmid 8 (ER-mRFP).
Campbell R. E. et al., A monomeric red fluorescent protein (2002), Proc. Nat. Acad. Sci., 99: 7877-7882.

[0127] The Stx2eB expression vector and the mRFP expression vector were introduced into the protoplasts of cultured tobacco cells (BY2) in the same way as the above methods, and observed using a confocal microscope observation system (LSM510, Zeiss).

[0128] The results are shown in FIGS. 5 and 6.

[0129] FIG. 5 shows the localization of the hybrid protein of Stx2eB-YFP. When 2×Stx2eB (PG12)-YFB was expressed, it was observed that the hybrid protein of Stx2eB-YFP was localized granularly at about 100 granules/cell. When 1×Stx2eB (PG12)-YFP and 2×Stx2eB (RS)-YFP were expressed, no granule was observed.

[0130] In FIG. 6, an image A in a leftmost column shows the localization of mRFP in a certain protoplast. The localization of mRFP reflects the location of the endoplasmic reticulum. An image B in a middle column shows the localization of the hybrid protein of Stx2eB-YFP in the identical protoplast. An image in a rightmost column is a composite image of the image A and the image B. It is found from this composite image that the hybrid protein of Stx2eB-YFP is localized granularly in the endoplasmic reticula.

(6) Effect of vesicular transport function

[0131] It was examined in which process after the protein translation the accumulation and aggregation of 2×Stx2eB (PG12) occur.

[0132] 2×Stx2eB (PG12)-YFP was co-expressed with an *Arabidopsis* vesicular transport regulation protein ARF1 or a dominant negative mutant thereof ARF1 (Q71L) (ARF1DN). As a reporter for inhibition of protein transport to Golgi apparatus by the co-expression with ARF1DN, vacuole-GFP was co-expressed with each ARF1. An expression vector for each ARF1 was constructed as follows. An expression vector for the vacuole-GFP can be prepared with reference to the following document.

[0133] Di Sansebastiano et. al., Specific accumulation of GFP in a non-acidic vacuolar compartment via a C-terminal propeptide-mediated sorting pathway. Plant J. (1998) 15, 449-457

[0134] As the vesicular transport regulation protein, expression vectors for *Arabidopsis* ARF1 (GenBank ACCESSION No. M95166) and for the dominant negative mutant thereof ARF1 (Q71L) (Misaki Takeuchi et al., 2002, (see below)) were constructed. PCR using cDNA prepared from *Arabidopsis* embryo plant as a template was performed using an ARF1-F

primer (SEQ ID NO: 59) and an ARF1-R primer (SEQ ID NO: 60). The resulting DNA fragment was subcloned into the EcoRV gap in pBluescript (Stratagene). Another PCR was performed using an ARFQL-F primer (SEQ ID NO: 61) and an ARFQL-R primer (SEQ ID NO: 62) to substitute a glutamine residue at position 71 with a leucine residue. Each resulting ARF1 fragment was subcloned into a transient expression vector pBI221,

[0135] Each of the vectors prepared was introduced into the protoplast of the cultured tobacco cell in the same manner as that described above, and the respective proteins were co-expressed to examine the localization of 2×Stx2eB (PG12).

[0136] The result is shown in FIG. 7.

[0137] Both when 2×Stx2eB (PG12)-YFP was co-expressed with ARF1 and co-expressed with ARF1 (Q71L), granules were formed as observed in an expression of 2×Stx2eB (PG12)-YFP alone. On the other hand, co-expression of ARF1 (Q71L) inhibited the exit of vacuole-GFP from ER. It was speculated from these that the granule formation is not dependent on vesicular transport process from the ER to Golgi apparatus.

<2> Transformation experiments using cultured tobacco cells

(1) Construction of vectors for transformation

[0138] 1×Stx2eB (PG12), 2×Stx2eB (PG12), 3×Stx2eB (PG12), and 4×Stx2eB (PG12) were prepared in the same way as above.

[0139] Further, by using RS (Arg, Ser), PG7 (SEQ ID NO: 63), or SG12 (SEQ ID NO: 64) as a spacer instead of PG12, DNA constructs, 2×Stx2eB (RS), 2×Stx2eB (PG7), and 2×Stx2eB (SG12) were prepared by the following methods.

[0140] A PG7 spacer (SEQ ID NO: 63) was inserted between Stx2eB and the HA tag in the plasmid 8. A PG7-F primer (SEQ ID NO: 65) and a PG7-R primer (SEQ ID NO: 66) were annealed and phosphorylated with T4 PNK. The obtained phosphorylated DNA fragment was inserted into the BglII gap of plasmid 8 (plasmid 10).

[0141] An SG12 spacer (SEQ ID NO: 64) was inserted between Stx2eB and the HA tag. An SG12-F primer (SEQ ID NO: 67) and an SG12-R primer (SEQ ID NO: 68) were annealed and phosphorylated with T4 PNK. The obtained phosphorylated DNA fragment was inserted into the BglII gap of plasmid 8 (plasmid 11).

[0142] An Stx2eB fragment was cleaved out from plasmid 1 with BamHI and BglII and then inserted into the BamHI gap of 1×Stx2eB (PG12) (2×Stx2eB (RS)). An Stx2eB-PG7 fragment

was cleaved out from plasmid 10 with BamHI and BglII and then inserted into the BamHI gap of 1×Stx2eB (PG12) (2×Stx2eB (PG7)). An Stx2eB-SG12 fragment was cleaved out from plasmid 11 with BamHI and BglII and then inserted into the BamHI gap of 1×Stx2eB (PG12) (2×Stx2eB (SG12)).

[0143] In order to produce Stx2eB using a stable transformant of the plant, each of the DNA constructs of the above Stx2eB was subcloned into a vector for transformation (for the design of the vectors, see FIG. 1). Each of 1×Stx2eB (PG12), 2×Stx2eB (PG12), 3×Stx2eB (PG12), 4×Stx2eB (PG12), 2×Stx2eB (RS), 2×Stx2eB (PG7), and 2×Stx2eB (SG12) was inserted into pBI121 (Clontech) using XbaI and SacI, and allocated between a cauliflower mosaic virus 35S RNA promoter (35S pro.) and a nopaline synthetase gene transcription terminator (NOS-T).

(2) Transformation of cultured tobacco cells

[0144] The produced vector for transformation was introduced into *Agrobacterium tumefaciens* EHA105 using an electroporation method. An *Agrobacterium* medium (100 µL) cultured in 5 mL of LB medium containing 100 mg/L of kanamycin at 28°C for 2 nights was mixed with 5 to 10 mL of a suspension of cultured tobacco cells (*Nicotiana tabacum*, cv BY2) on the fourth day of the culture in a petri-dish, and the mixture was co-cultured by being left to stand in a dark place at 25°C for 2 nights. In order to remove *Agrobacterium*, the medium in the petri-dish was transferred into a 15-mL centrifuging tube, which was then centrifuged (1,000 rpm, 5 minutes, 4°C), and a supernatant was removed. A modified LS medium was charged, and the tube was centrifuged (1,000 rpm, 5 minutes, 4°C) to wash the cells. This washing operation was repeated four times to remove *Agrobacterium*. The resultant BY2 cells were then placed in a modified LS agar medium containing kanamycin (100 mg/L), and cultured by being left to stand in the dark place at 25°C. After about 2 to 3 weeks, cells which has formed a callus were transferred to a new plate, and a growing clone was selected.

(3) Semi-quantification of Stx2eB protein using western analysis

[0145] Cultured tobacco cells cultured in a plate medium were collected in a centrifuging tube, and 1 µL of SDS sample buffer per mg of cell weight was added. The cells were denatured at 95°C for 2 minutes to make a sample for electrophoresis. Proteins were separated using 15% acrylamide gel, and then blotted onto a PVDF membrane (Hybond-P; Amersham) using an electrotransfer apparatus. An Stx2eB protein was detected using an anti-HA antibody (No. 11 867 423 001, Roche). Serial dilution of Stx2eB having the HA tag at known concentrations was prepared, and loaded on the gel. A calibration curve was prepared based on their signal intensities, and the amount of Stx2eB proteins in each sample was calculated.

[0146] The results are shown below.

(a) Effects of linking number of Stx2eB

[0147] The results are shown in FIGS. 8 and 9.

[0148] When 1×Stx2eB (PG12) was expressed, signals were detected at the positions of about 10 kDa and about 17 kDa. They are presumed to be Stx2eB in which the signal peptide was cleaved and Stx2eB in which the signal peptide is not cleaved, respectively. When 2×Stx2eB (PG12) was expressed, a signal was detected at the position of about 19 kDa at the similar level to that when 1×Stx2eB (PG12) was expressed. When 3×Stx2eB (PG12) was expressed, a signal was detected at the position of about 26 kDa, the signal being smaller than that when 1×Stx2eB (PG12) was expressed. These corresponded to the molecular weights estimated from the design of the DNA constructs. When 4×Stx2eB (PG12) was expressed, a specific signal was below the detection limit (data not shown).

[0149] It has been found from the above that 2×Stx2eB (PG12) accumulates the larger amount of Stx2eB than 1×Stx2eB (PG12) and 3×Stx2eB (PG12).

(b) Effects of spacer

[0150] The results are shown in FIGS. 10 and 11.

[0151] Based on the intensity of the signal corresponding to Stx2eB, the level of accumulated Stx2eB was the highest when PG12 was used as a spacer. The levels were secondly high when PG7 and SG12 were used, which were in the similar degree. The level was lowest when RS was used. This indicates that the length and the amino acid sequence of the spacer between two Stx2eBs affect the level of the accumulated 2×Stx2eB protein.

(4) Quantification of mRNA by real-time PCR

[0152] It was examined whether the level of the accumulated protein was influenced by a transcription level or not.

[0153] RNA was prepared using RNeasy Mini Kit (Qiagen) from each of the transformed BY2 cells obtained above. The resulting RNA was treated with DNase, and then reversely transcribed using Transcriptor Reverse Transcriptase (Roche). Real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems). A primer set (SEQ ID NO: 69 and SEQ ID NO: 70) which amplified the region containing NtADH 5'-UTR and the signal peptide which were common in the respective constructs was used for the quantification of Stx2eB mRNA. The amount of an expressed BY2 ubiquitin gene was quantified using a UBQ-F primer (SEQ ID NO: 71) and a UBQ-R primer (SEQ ID NO: 72) to compensate the mRNA level of an Stx2eB gene. Note that the mRNA level of 1×Stx2eB (PG12) was calculated by multiplying a

quantified value by 1/2.

[0154] The results are shown in FIG. 12.

[0155] The accumulation levels of Stx2eB protein per mRNA tend to be higher in cells expressing 2×Stx2eB (PG12) than those in cells expressing 2×Stx2eB (RS) or 1×Stx2eB (PG12). This indicates that the difference of the spacer does not influence on the transcription level but influences on a translation level or stability of the protein after the translation. Considering together the result that the 2×Stx2eB protein was localized granularly, it is conceivable that the spacer influences on the stability of the protein after the translation.

<3> Transient expression experiments

(1) Construction of Stx2eB transient expression vectors

[0156] Transient expression vectors for 1×Stx2eB (PG12), 2×Stx2eB (PG12), 3×Stx2eB (PG12), and 4×Stx2eB (PG12) were constructed by the method in above <1> (1) (FIG. 13-A). These vectors are referred to as ER-1×Stx2eB (PG12), ER-2×Stx2eB (PG12), ER-3×Stx2eB (PG12), and ER-4×Stx2eB (PG12), respectively. Note that "ER" means the endoplasmic reticulum type.

[0157] Further, transient expression vectors containing the cytoplasm (Cyt) type of DNA construct (FIG. 13-B) and expression vectors containing the chloroplast (Chl) type of DNA construct (FIG. 13-C) were constructed by the following method. These DNA constructs were designed to contain DNA encoding the endoplasmic reticulum retention signal peptide, for the purpose of expressing the hybrid protein having as close a structure as possible to that of the endoplasmic reticulum type of hybrid protein. But, since these DNA constructs do not contain DNA encoding the secretory signal peptide, the endoplasmic reticulum retention signal peptide does not exert its function (retention of the protein in the endoplasmic reticulum) in the produced hybrid protein.

[0158] An NtADH 5'-UTR fragment was amplified by PCR using an ADH XbaI-F primer (SEQ ID NO: 34) and an ADH BamHI-R primer (SEQ ID NO: 112), and the resulting DNA fragment was treated with XbaI and BamHI. The XbaI-BamHI fragment of NtADH 5'-UTR was inserted into the XbaI-BamHI gap of each of ER-1×Stx2eB (PG12), ER-2×Stx2eB (PG12), ER-3×Stx2eB (PG12), and ER-4×Stx2eB (PG12) to prepare Cyt-1×Stx2eB (PG12), Cyt-2×Stx2eB (PG12), Cyt-3×Stx2eB (PG12), and Cyt-4×Stx2eB (PG12) which were cytoplasm type Stx2eB vectors.

[0159] The NtADH 5'-UTR fragment was amplified by PCR using an ADH XbaI-F primer (SEQ ID NO: 34) and an ADH NsiI-R primer (SEQ ID NO: 35). A DNA fragment (SEQ ID NO: 80) encoding the transit signal peptide, the chloroplast being derived from *Lactuca sativa* Rbcs

(Rubisco small subunit) (GenBank ACCESSION D14001) (transit peptide, T.P.), was amplified by PCR with cDNA of a *Lactuca sativa* leaf as a template using a TP NsiI-F primer (SEQ ID NO: 113) and a TP BamHI-R primer (SEQ ID NO: 114). Each resulting DNA fragment of NtADH 5'-UTR and each DNA fragment of the secretory signal peptide was treated with NsiI (manufactured by Toyobo Co., Ltd.), ligated using Ligation High (Toyobo Co., Ltd.) followed by being blunted, and cloned into the EcoRV gap of pBluescript II SK (manufactured by Stratagene) (plasmid 12). Plasmid 12 was treated with NsiI, blunted with T4 DNA polymerase (Toyobo Co., Ltd.), and then self-ligated to be fused so that the initiation codon of NtADH and the initiation codon of Rbcs were matched (plasmid 13). An NtADH 5'-UTR-T.P. fusion fragment was cut out from plasmid 13 using XbaI and BamHI, and inserted into the XbaI-BamHI gap of each of ER-1×Stx2eB (PG12), ER-2×Stx2eB (PG12), ER-3×Stx2eB (PG12), and ER-4×Stx2eB (PG12) to prepare Chl-1×Stx2eB (PG12), Chl-2×Stx2eB (PG12), Chl-3×Stx2eB (PG12), and Chl-4×Stx2eB (PG12), which were chloroplast type Stx2eB vectors.

(2) Production of CTB transient expression vectors

[0160] Transient expression vectors for 1×CTB (PG12) and 2×CTB (PG12) were constructed by the method in above <1> (2) (FIG. 14-A). Hereinafter, these vectors are referred to as ER-1×CTB (PG12) and ER-2×CTB (PG12).

[0161] Transient expression vectors containing the cytoplasm (Cyt) type of DNA construct (FIG. 14-B) and expression vectors containing the chloroplast (Chl) type of DNA construct (FIG. 14-C) were constructed by the following methods.

[0162] A CTB-PG12 fragment was cut out from ER-1×CTB (PG12) using BamHI and BglII, and inserted into the BamHI-BglII gap in Cyt-1×Stx2eB (PG12) and the BamHI-BglII gap in Chl-1×Stx2eB (PG12) produced in above <3> (1) to prepare cytoplasm type of 1×CTB (PG12) and chloroplast type of 1×CTB (PG12) (Cyt-1×CTB (PG12), Chl-1×CTB (PG12)).

[0163] Subsequently, a 2×(CTB-PG12) fragment was cut out from ER-2×CTB (PG12) using BamHI and BglII, and inserted into the BamHI-BglII gap of Cyt-1×Stx2eB (PG12) and the BamHI-BglII gap of Chl-1×Stx2eB (PG12) to prepare cytoplasm type of 2×CTB (PG12) and chloroplast type of 2×CTB (PG12) (Cyt-2×CTB (PG12), Chl-2×CTB (PG12)).

(3) Transient expression experiments and western analysis

[0164] Transient expression experiments were carried out using *Lactuca sativa* protoplasts in the same way as in above <1> (3). Subsequently, Stx2eB and CTB were detected in the same way as in <1> (4).

(a) Effects of linking number of Stx2eB

[0165] The results are shown in FIG. 15.

[0166] When ER-1×Stx2eB (PG12) was expressed, a signal was detected at the position of about 10 kDa. When ER-2×Stx2eB (PG12) was expressed, a signal was detected at the position of about 19 kDa, the signal being larger than that when ER-1×Stx2eB (PG12) was expressed. When ER-3×Stx2eB (PG12) was expressed, a signal was detected at the position of about 27 kDa, the signal being larger than that when ER-1×Stx2eB (PG12) was expressed. These corresponded to the molecular weights estimated from the design of the DNA constructs. When ER-4×Stx2eB (PG12) was expressed, a specific signal was below the detection limit.

[0167] When Cyt-1×Stx2eB (PG12) was expressed, a specific signal was below the detection limit. When Cyt-2×Stx2eB (PG12) was expressed, a signal was detected at the position of about 20 kDa. When Cyt-3×Stx2eB (PG12) was expressed, a signal was detected at the position of about 30 kDa. These corresponded to the molecular weights estimated from the design of the DNA constructs. Further, when Cyt-4×Stx2eB (PG12) was expressed, a specific signal was below the detection limit.

[0168] When Chl-1×Stx2eB (PG12) was expressed, a signal was detected faintly at the position of about 14 kDa. When Chl-2×Stx2eB (PG12) was expressed, a signal was detected at the position of about 22 kDa, the signal being at the similar level to that when ER-3×Stx2eB (PG12) was expressed. When Chl-3×Stx2eB (PG12) was expressed, a signal was detected at the position of about 30 kDa, the signal being at the similar level to that when Chl-2×Stx2eB (PG12) was expressed. These corresponded to the molecular weights estimated from the design of the DNA constructs. When Chl-4×Stx2eB (PG12) was expressed, a signal was detected faintly at the position of about 34 kDa.

[0169] Since each of the above DNA constructs contains one molecule of the HA tag (see FIG. 13), when DNA encoding two Stx2eBs is expressed and when DNA encoding three Stx2eBs is expressed, the amounts of the accumulated proteins are thought to correspond to about two times and about three times, respectively, the amount when DNA containing one Stx2eB is expressed.

[0170] Therefore, it has been found that when any of the endoplasmic reticulum type (ER), cytoplasm type (Cyt), and chloroplast type (Chl) of DNA constructs is expressed, the Stx2eB protein can be more efficiently accumulated when the protein in which two or three Stx2eBs are tandemly linked through the spacer is expressed than when one Stx2eB protein is expressed.

(b) Effects of linking number of CTB

[0171] The results are shown in FIG. 16.

[0172] When ER-1×CTB (PG12) was expressed, a signal was detected at the position of about 17 kDa. When ER-2×CTB (PG12) was expressed, a larger signal than that when ER-1×Stx2eB (PG12) was expressed was detected at the positions of about 28 kDa and about 30 kDa. These corresponded to the molecular weights estimated from the design of the DNA constructs.

[0173] When Cyt-1×CTB (PG12) was expressed, a signal was faintly detected at the position of about 14 kDa. When Cyt-2×CTB (PG12) was expressed, a signal was detected at the position of about 26 kDa, the signal being at the similar level to that when ER-2×CTB (PG12) was expressed. These corresponded to the molecular weights estimated from the design of the DNA constructs.

[0174] When Chl-1×CTB (PG12) was expressed, a signal was detected at the position of about 14 kDa. When Chl-2×CTB (PG12) was expressed, a signal was detected at the position of about 26 kDa, the signal being at the similar level to that when ER-2×CTB (PG12) was expressed. These corresponded to the molecular weights estimated from the design of the DNA constructs.

[0175] From the above, it has been found that when any of the endoplasmic reticulum type (ER), cytoplasm type (Cyt), and chloroplast type (Chl) of DNA constructs is expressed, CTB proteins can be more efficiently accumulated when the protein in which two CTBs are tandemly linked through the spacer is expressed than when one CTB protein is expressed.

<4> Transformation experiments using cultured tobacco cells

(1) Construction of vectors for transformation

[0176] Transformation experiments were performed using ER-2×Stx2eB (PG12), Cyt-1×Stx2eB (PG12), Cyt-2×Stx2eB (PG12), and Cyt-3×Stx2eB (PG12) prepared above.

[0177] Vectors for transformation were prepared in the same way as the method in above <2> (1).

(2) Transformation and western analysis of cultured tobacco cells

[0178] Transformation experiments and the western analysis were carried out in the same ways as the methods in above <2> (2) and (3).

[0179] The results are shown in FIG. 17.

[0180] When ER-2×Stx2eB (PG12) was expressed, a signal was detected at the positions of about 19, 21, and 23 kDa. When Cyt-1×Stx2eB (PG12) was expressed, a specific signal was below the detection limit. When Cyt-2×Stx2eB (PG12) was expressed, a signal was detected at the position of about 19 kDa. When Cyt-3×Stx2eB (PG12) was expressed, a signal was detected at the position of about 27 kDa, the signal being larger than that when Cyt-2×Stx2eB (PG12) was expressed.

[0181] From the above, it has been found that also in the transformant of the cultured tobacco cell, Stx2eB proteins can be more efficiently accumulated when the protein in which two or three Stx2eBs are tandemly linked through the spacer is expressed than when one Stx2eB protein is expressed. It has been also found that when the cytoplasm type of DNA construct is expressed in the transformant of the cultured tobacco cell, in particular when the protein in which three Stx2eBs are tandemly linked through the spacer is expressed, the Stx2eB proteins can be efficiently accumulated.

[0182] It has been also found that in the transformant of the cultured tobacco cell, the Stx2eB proteins can be more efficiently accumulated when the endoplasmic reticulum type of DNA construct is expressed than when the cytoplasm type of DNA construct is expressed.

<5> Transformation experiments using tobacco plant body

(1) Construction of vectors for transformation

[0183] Transformation experiments were performed using ER-2×Stx2eB (PG12), Chl-1×Stx2eB (PG12), Chl-2×Stx2eB (PG12), and Chl-3×Stx2eB (PG12) prepared above.

[0184] Vectors for transformation were prepared in the same way as the method in above <2> (1).

(2) Transformation of tobacco plant body

[0185] Tobacco plant bodies were transformed by the following method using the vectors prepared above.

[0186] Seeds of the tobacco plant body (*Nicotiana tabacum* L. cv. Petit habana SR1) were sterilized and seeded on an MS medium. A leaf portion of the sterilized *Nicotiana tabacum* was cut into pieces each having a size of about 1×1 cm without including leaf veins, and placed to face up the backside of the leaf in a petri-dish containing sterile water. An *Agrobacterium*

suspension cultured for two nights in the LB medium containing 100 mg/L of kanamycin and obtained in above <2> (2) was poured in the petri-dish, and the leaf piece was immersed therein for 3 to 5 minutes. The leaf piece was picked up, and an extra bacterial medium on the leaf piece was wiped with sterile kim-towel. The leaf piece was placed on a callus formation medium and cultured at 25°C. After 2 to 3 days, when *Agrobacterium* became visible on the medium, the leaf piece was transferred into a 50-mL tube, washed five times with sterile water, placed on a callus formation medium (containing 100 mg/L of kanamycin and 250 mg/L of carbenicillin), and cultured at 25°C for 1 to 2 weeks. When the leaf piece curled up compared with the original and showed a concavoconvex surface, the leaf piece was transferred into a shoot formation medium (containing 100 mg/L of kanamycin and 250 mg/L of carbenicillin). After additional 4 to 6 weeks, a shoot having a developed stem and leaf portion was cut off, transferred to a root formation medium (containing 100 mg/L of kanamycin and 250 mg/L of carbenicillin), and cultured at 25°C until rhizogenesis was observed. A plant body grown to a certain size was grown as a pot plant.

(3) Western analysis

[0187] The leaf of the genetically engineered tobacco plant body produced above was sampled, and an SDS sample buffer was added thereto in the proportion of 1 μ L of SDS to 1 mg of leaf. The sample was thermally denatured at 95°C for 2 minutes to serve as the sample for electrophoresis. Proteins were separated using 15% acrylamide gel, and then blotted onto a PVDF membrane (Hybond-P; Amersham) using an electrotransfer apparatus. The Stx2eB protein was detected using an anti-HA antibody (No. 11 867 423 001, Roche).

[0188] The results are shown in FIGS. 18 and 19.

[0189] Clones in which Stx2eB was accumulated with high efficiency were obtained in the plant body transformed with one of ER-2 $\times$ Stx2eB (PG12), Chl-1 $\times$ Stx2eB (PG12), Chl-2 $\times$ Stx2eB (PG12), and Chl-3 $\times$ Stx2eB (PG12). Also in the chloroplast type of DNA constructs, it has been found that the clone accumulating Stx2eB efficiently is obtained with a higher probability when the protein in which two or three Stx2eBs are tandemly linked through the spacer is expressed than when one Stx2eB protein is expressed.

[0190] When ER-2 $\times$ Stx2eB (PG12) was expressed, signals were detected at the positions of about 15 kDa, about 19 kDa, and about 22 kDa. When Chl-1 $\times$ Stx2eB (PG12) was expressed, a signal was detected at the position of about 12 kDa. When Chl-2 $\times$ Stx2eB (PG12) was expressed, a signal was detected at the position of about 19 kDa. When Chl-3 $\times$ Stx2eB (PG12) was expressed, a signal was detected at the position of about 27 kDa. These corresponded to the molecular weights estimated from the design of the DNA constructs.

<6> Transformation experiments using cultured tobacco cells

(1) Construction of vectors for Stx2eB transformation

[0191] ER-2×Stx2eB (PG12) was prepared by the method in above <1> (1). ER-2×Stx2eB (PG17) and ER-2×Stx2eB (PG22) were prepared by the following method. The design of the DNA constructs is shown in FIG. 20.

[0192] A PG7-F primer (SEQ ID NO: 65) and a PG7-R primer (SEQ ID NO: 66) were annealed and phosphorylated with T4 PNK. The resulting phosphorylated DNA fragment was inserted into the BglII gap of ER-1×Stx2eB (PG12) obtained in <1> (1) (plasmid 14) .

[0193] A PG12-F primer (SEQ ID NO: 43) and a PG12-R primer (SEQ ID NO: 44) were annealed and phosphorylated with T4 PNK. The resulting phosphorylated DNA fragment was inserted into the BglII gap of ER-1×Stx2eB (PG12) (plasmid 15).

[0194] An Stx2eB-PG17 fragment was cut out from plasmid 14 using BamHI and BglII, and inserted into the BamHI gap of ER-1×Stx2eB (PG12) (2×Stx2eB (PG17)). An Stx2eB-PG22 fragment was cut out from plasmid 15 using BamHI and BglII, and inserted into the BamHI gap of ER-1×Stx2eB (PG12) (ER-2×Stx2eB (PG22)).

[0195] In order to produce Stx2eB using the stable transformant of the plant, each of the above DNA constructs for Stx2eB was subcloned into a vector for transformation. That is, each of ER-2×Stx2eB (PG12), ER-2×Stx2eB (PG17), and ER-2×Stx2eB (PG22) was inserted into pBI121 (Clontech) using XbaI and SacI, and allocated between the cauliflower mosaic virus 35S RNA promoter (35S pro.) and the nopaline synthetase gene transcription terminator (NOS-T).

(2) Construction of vectors for CTB transformation

[0196] ER-2×CTB (PG12) was prepared by the method in above <1> (2). ER-2×CTB (PG17) and ER-2×CTB (PG22) were prepared by the following method. The design of the DNA constructs is shown in FIG. 21.

[0197] A PG7-F primer (SEQ ID NO: 65) and a PG7-R primer (SEQ ID NO: 66) were annealed and phosphorylated with T4 PNK. The resulting phosphorylated DNA fragment was inserted into the BglII gap of ER-1×CTB (PG12) obtained in <1> (2) (plasmid 16). A PG12-F primer (SEQ ID NO: 43) and a PG12-R primer (SEQ ID NO: 44) were annealed and phosphorylated with T4 PNK. The resulting phosphorylated DNA fragment was inserted into the BglII gap of ER-1×CTB (PG12) (plasmid 17).

[0198] A CTB-PG17 fragment was cut out from plasmid 16 using BamHI and BglII, and inserted into the BamHI gap of ER-1×CTB (PG12) (ER-2×CTB (PG17)). A CTB-PG22 fragment

was cut out from plasmid 17 using BamHI and BglII, and inserted into the BamHI gap of ER-1×CTB (PG12) (ER-2×CTB (PG22)).

[0199] In order to produce CTB using the stable transformant of the plant, each of the above DNA constructs for CTB was subcloned into a vector for transformation. That is, each of ER-2×CTB (PG12), ER-2×CTB (PG17), and ER-2×CTB (PG22) was inserted into pBI121 (Clontech) using XbaI and SacI, and allocated between the cauliflower mosaic virus 35S RNA promoter (35S pro.) and the nopaline synthetase gene transcription terminator (NOS-T).

(3) Transformation experiments and western analysis

[0200] Transformation experiments and western analysis were carried out by the methods in above <2> (2) and (3).

(a) Effects of length of spacer on tandem linking of Stx2eB

[0201] The results are shown in FIG. 22.

[0202] When one of ER-2×Stx2eB (PG17) and ER-2×Stx2eB (PG22) was expressed, a signal was detected at the similar level to that when ER-2×Stx2eB (PG12) was expressed. This indicates that any of PG17 and PG22 exhibits the same effect as PG12.

[0203] When ER-2×Stx2eB (PG12) was expressed, signals were detected at the positions of about 19 kDa and about 22 kDa. When ER-2×Stx2eB (PG17) was expressed, signals were detected at the positions of about 19 kDa and about 22 kDa. When ER-2×Stx2eB (PG22) was expressed, signals were detected at the positions of about 20 kDa and about 23 kDa. These corresponded to the molecular weights estimated from the design of the DNA constructs.

(b) Effects of length of spacer on tandem linking of CTB

[0204] The results are shown in FIG. 23.

[0205] When one of ER-2×CTB (PG17) and ER-2×CTB (PG22) was expressed, a signal was detected at the similar level to that when ER-2×CTB (PG12) was expressed. This indicates that any of PG17 and PG22 exhibits the same effect as PG12.

[0206] When ER-2×CTB (PG12) was expressed, signals were detected at the positions of about 32 kDa, about 34 kDa, and about 36 kDa. When ER-2×CTB (PG17) was expressed, signals were detected at the positions of about 32 kDa, about 34 kDa, and about 36 kDa. When ER-2×CTB (PG22) was expressed, signals were detected at the positions of about 32

kDa, about 34 kDa, and about 36 kDa. These corresponded to the molecular weights estimated from the design of the DNA constructs.

INDUSTRIAL APPLICABILITY

[0207] The hybrid protein of the present invention is highly stable and accumulated at a high level in plant cells. Besides, by producing the hybrid protein of the present invention in the plant using the DNA construct of the present invention, it is possible to efficiently produce oral vaccines for Shiga toxin, cholera toxin, and *Escherichia coli* heat-labile toxin.

[0208] The present invention enables to express a bacterial antigen in the plant at the level which is enough to induce immunity. The present invention enables to give the immunity against the bacterial antigen to the animal at low cost by giving a transgenic plant as food to the animal. For example, the present invention is useful for developing swine edema disease vaccine and cholera vaccine.

SEQUENCE LISTING

[0209]

<110> IDEMITSU KOSAN Co., Ltd.

<120> BACTERIAL TOXIN VACCINE

<130> S3062 EP S3

<150> JP 08/120573

<151> 2008-05-02

<160> 114

<170> Patent In version 3.5

<210> 1

<211> 36

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA

<400> 1

agatccccctg gttctgggtcc tggttctcct agatcc 36

<210> 2

<211> 12
 <212> PRT
 <213> Artificial

<220>

<223> an artificially synthesized peptide

<400> 2

Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg Ser
 1 5 10

<210> 3

<211> 959

<212> DNA

<213> Escherichia coli

<400> 3

```
atgaagtgtatattgttaaa gttgatactg tgtctgttac tgggtttttc ttcggtatcc 60
tattcccagg agtttacgat agacttttcg acitcaacaaa gttatgtatc ttcgttaaat 120
agtatacggg cagcgatata gacccctctt gaacatataat ctacgggagc tacatcggta 180
tccgttattat aicatacacc accaggaagt tatatttcgg tagglatacg agggcttgat 240
gttlaicagg agcgttttga ccatcttcgt ctgattatig aacgaaataa ttataatgtg 300
gctggatttg itaatagac aacaaatact ttctacagat tttcagattt gcacatataat 360
cattgccccg tgtgacaact atttccatga caacggacag cagttatacc actctgcaac 420
gtgtcgccag gctggaaact tccggaatgc aaatcagtcg tcactcactg gtttcatcat 480
atctggcggt aatggagttc agtggtaata caatgaccag agatgcatac agagcagttc 540
tgcgttttgt cactgtcaca gcagaagcct tacggttcag gcaaatacag agagaatttc 600
gtctggcact gctcgaact gctcctgttt atacgatgac gccggaagac gttggacctca 660
ctctgaactg ggggagaatc agcaatgtgc ttccggagta tggggagag gctgggtgtca 720
gagtggggag aatatctttt aataatataf cagcgatact tggtagctgt gccgttatac 780
tgaattgcca tcatcagggc gcacgttctg ttccgcccgt gaatgaagag agtcaaccag 840
aatgtcagat aactggcgac agggccggtt taaaaataaa caatacatta tgggaagta 900
atacagcagc agcgtttctg aacagaaagt cacagccttt atatacaact ggtgaatga 959
```

<210> 4

<211> 304

<212> PRT

<213> Escherichia coli

<400> 4

Met Lys Cys Ile Leu Leu Lys Trp Ile Leu Cys Leu Leu Leu Gly Phe
 1 5 10 15
 Ser Ser Val Ser Tyr Ser Gln Glu Phe Thr Ile Asp Phe Ser Thr Gln
 20 25 30

Gln Ser Tyr Val Ser Ser Leu Asn Ser Ala Ile Ser Thr Pro Leu Glu
 35 40 45
 His Ile Ser Gln Gly Ala Thr Ser Val Ser Val Ile Asn His Thr Pro
 50 55 60
 Pro Gly Ser Tyr Ile Ser Val Gly Ile Arg Gly Leu Asp Val Tyr Gln
 65 70 75 80
 Glu Arg Phe Asp His Leu Arg Leu Ile Ile Glu Arg Asn Asn Leu Tyr
 85 90 95
 Phe Val Asn Thr Thr Asn Thr Phe Tyr Arg Phe Ser Asp Phe Ala
 100 105 110
 His Ile Ser Leu Pro Gly Val Thr Thr Ile Ser Met Thr Thr Asp Ser
 115 120 125
 Ser Tyr Thr Thr Leu Gln Arg Val Ala Ala Leu Glu Arg Ser Gly Met
 130 135 140
 Gln Ile Ser Arg His Ser Leu Tyr Leu Ala Leu Met Glu Phe Ser Gly
 145 150 155 160
 Asn Thr Met Thr Arg Asp Ala Ser Arg Ala Val Leu Arg Phe Val Thr
 165 170 175
 Val Thr Ala Glu Ala Leu Arg Phe Arg Gln Ile Gln Arg Glu Phe Arg
 180 185 190
 Leu Ala Leu Ser Glu Thr Ala Pro Val Tyr Thr Met Thr Pro Asp Leu
 195 200 205
 Thr Leu Asn Trp Gly Arg Ile Ser Asn Val Leu Pro Glu Tyr Arg Gly
 210 215 220

```

Glu Ala Gly Val Arg Val Gly Arg Ile Ser Phe Asn Asn Ile Ser Ala
225          230          235          240
Ile Leu Gly Thr Val Ala Val Ile Leu Asn Cys His His Gln Gly Ala
          245          250          255
Arg Ser Val Arg Ala Glu Ser Gln Pro Glu Cys Gln Ile Thr Gly Asp
260          265          270
Arg Pro Val Ile Lys Ile Asn Asn Thr Leu Trp Glu Ser Asn Thr Ala
275          280          285
Ala Ala Phe Leu Asn Arg Lys Ser Gln Pro Leu Tyr Thr Thr Gly Glu
290          295          300

```

<210> 5

<211> 210

<212> DNA

<213> Escherichia coli

<400> 5

```

gcggcggatt gtgctaaagg taaaattgag ttttccaagt ataatgagga taataccttt    60
actgtgaagg tgtcaggaag agaatactgg acgaacagat ggaatttgca gccatigtta    120
caaagtgtct agctgacagg gatgactgta acaatcatal ctaatacctg cagttcaggc    180
tcaggcttgg cccaggtgaa gttaaaciga                                210

```

<210> 6

<211> 69

<212> PRT

<213> Escherichia coli

<400> 6

```

Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn Glu
1          5          10          15
Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr Asn
20          25          30
Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly Met
35          40          45
Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe Ala
50          55          60
Gln Val Lys Phe Asn
65

```

<210> 7

<211> 312

<212> DNA

<213> Vibrio cholerae

<400> 7

```

accctccaga acatcacaga cctctgagcc gagagccaca acacccaaat ctacacccct    60
aagacaaga ttttcagcta caccgagagc ctgcctggca agaggagat ggcatcctc    120
acctcaaga acggcgccat cticcaggtc gaggtccccc gcagccagca catcgacagc    180

cagaagaagg ccctcgagag gatgaaggac accctcagga tcgcctacct caccgagccc    240
aaggctcaga agctctgcgt ctggaacaac aagaccccc acgcatcgc cgccatcagc    300
atggccaact ga                                312

```

<210> 8

<211> 103

<212> PRT

<213> Vibrio cholerae

<400> 8

```

Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His Asn Thr Gln
1          5          10          15
Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser Tyr Thr Glu Ser Leu Ala
20          25          30
Gly Lys Arg Glu Met Ala Ile Ile Thr Phe Lys Asn Gly Ala Ile Phe
35          40          45

```

```

Gln Val50 Glu Val55 Pro Gly Ser Gln His Ile Asp Ser60 Gln Lys Lys Ala
Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Ala Tyr Leu Thr Glu Ala
65 Lys Val Glu Lys Leu Cys Val Trp Asn Asn Lys Thr Pro His Ala Ile
80 Ala Ala Ile Ser Met Ala Asn
100

```

<210> 9

<211> 453

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 9

```

gcggcggatt gtgctaaagg taaaattgag ttttccaagt ataatgagga taataccttt 60
actgtgaagg tctcaggaag agaatactgg acgaacagat ggaatttgca gccattgtta 120
caaagtgcct agctgacagg gatgactgta acaatcatac ctaataacctg cagttcaggc 180
tcaggctttg cccaggtgaa gtttaacaga tccccgtggt cttgtctctg tctctctaga 240
tcgcgcgcgg attgtgctaa aggttaaaatt gagttttcca agtataatga ggataatacc 300
tttactgtga aggtgtcagg aagagaatac tggacgaaca gatggaattt gcagccattg 360
ttacaaagtg ctacagctgac agggatgact gtaacaatca tatctaatac ctgcagttca 420
ggctcaaggct ttgccagggt gaagtttaac tga 453

```

<210> 10

<211> 150

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 10

```

Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn Glu
1 5 10 15
Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr Asn
20 25 30
Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly Met
35 40 45
Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe Ala
50 55 60
Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg
65 70 75 80
Ser Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn
85 90 95
Glu Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr
100 105 110
Asn Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly
115 120 125
Met Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe
130 135 140
Ala Gln Val Lys Phe Asn

```

145

150

<210> 11

<211> 657

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 11

```

acccccaga acatcacga cctctgccc gagagccaca acacccaaat ctacaccctc   60
aacgacaaga ttctcagcta caccgagagc ctgcgccgca agaggagat ggccatcatc  120
accttcaaga acggcgccat ctccaggtc gaggtccccg gcagccagca catcgacagc  180
cagaagaagg ccatcgagag gatgaaggac accctcagga tcgcttacct caccgaggcc  240
aaggtcgaga agctctgctt ctggaacaac aagacccccg acgccaatgc cggcatcagc  300
atggccaaca gatcccttgg ttctggtcct ggttctccta gatccacccc ccagacatc  360
accgacctct gcgccgagag ccacaacacc caaatctaca cctcaacga caagattttc  420
agctacaccg agagcctcgc cggcaagagg gagatggcca tcatcacctt caagaacggc  480
gccatcttcc aggtcgaggc cccccgcagc cagcacatcg acagccagaa gaaggccatc  540
gagaggatga aggacacctt caggatcgcc tacctcaccg aggccaaagt cgagaagctc  600
tgcgtctgga acaacaagac cccccacgcc atcgccgcca tcagcatggc caactga   657

```

<210> 12

<211> 218

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 12

```

Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His Asn Thr Gln
1      5      10      15
Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser Tyr Thr Glu Ser Leu Ala
20     25     30
Gly Lys Arg Glu Met Ala Ile Ile Thr Phe Lys Asn Gly Ala Ile Phe
35     40     45
Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln Lys Lys Ala
50     55     60
Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Ala Tyr Leu Thr Glu Ala
65     70     75     80
Lys Val Glu Lys Leu Cys Val Trp Asn Asn Lys Thr Pro His Ala Ile
85     90     95
Ala Ala Ile Ser Met Ala Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser
100    105    110
Pro Arg Ser Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His
115    120    125
Asn Thr Gln Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser Tyr Thr Glu
130    135    140
Ser Leu Ala Gly Lys Arg Glu Met Ala Ile Ile Thr Phe Lys Asn Gly
145    150    155    160
Ala Ile Phe Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln
165    170    175
Lys Lys Ala Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Ala Tyr Leu
180    185    190
Thr Glu Ala Lys Val Glu Lys Leu Cys Val Trp Asn Asn Lys Thr Pro
195    200    205
His Ala Ile Ala Ala Ile Ser Met Ala Asn
210    215

```

<210> 13

<211> 489

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 13

```

gcggcggatt gtcctaaagg taaaattgag ttctccaagt ataattgagga taataccttt   60
actgtgaagg tgcagggaag agaatactgg acgaacagat ggaatttgca gccattgtta  120

caaagtgtct agctgacagg gatgactgta acaatcatat ctaatacctg cagttcaggc  180
tcaggctttg cccaggtgaa gtttaacaga tccccgtgtt ctggtccttg ttctcctaga  240
tcgcggcggc attgtgctaa aggtaaaaatt gagttttcca agtataatga ggataatacc  300
tttactgtga aggtgtcagg aagagaatac tggacgaaca gatggaattt gcagccattg  360
ttacagatga ctacagtgag aggaatgaat ctacagatga tctatctatc ctacagatga  420

```

```

ttacaaatg ttacgtgat agggatgatt gtaataatc tatltaaat ttgtagitc 420
ggctcaggct ttgccaggt gaagttaaac agatccccctg gtctcggfoc tggttctcct 480
agatcttga 489

```

<210> 14

<211> 162

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 14

```

Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn Glu
1      5      10      15
Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr Asn
20     25     30
Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly Met
35     40     45
Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe Ala
50     55     60
Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg
65     70     75     80
Ser Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn
85     90     95
Glu Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr
100    105    110
Asn Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly
115    120    125
Met Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe
130    135    140
Ala Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro
145    150    155    160
Arg Ser

```

<210> 15

<211> 693

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 15

```

acccccaga acatcacgga cctctgcgcc gagagccaca acacccaaat ctacacccctc 60
aacgacaaga ttctcagcta caccgagagc ctgcgccgca agaggagat ggccatcatc 120
acctcaaga acggcgccat ctccaggtc gaggtcccg gcagccagca catcgacagc 180
cagaagaagg ccacgcagag gatgaaggac accctcagga tcgctacct caccgaggcc 240
aaggtcgaga agctctgctt ctggaacaac aagaccccc acgcatcgc cggcatcagc 300
atggccaaca gatccctgg ttctggctct gggtctccta gatccaccc ccagaacatc 360
accgacctct gcgccgagag ccacaacacc caaatctaca cctcaacga caagattttc 420
agctacaccg agagcctcgc cggcaagagg gagatggcca tcatcacctt caagaacggc 480
gcatcttcc aggtcgaggt ccccgccagc cagcacaicg acagccagaa gaaggccatc 540
gagaggatga aggacaccct caggatcgcc tacctcaccg aggccaaagt cgagaagctc 600
tgcgtctgga acaacaagac cccccacgcc atcgccgcca tcagcatggc caacagatcc 660
cttggttctg gtcciggttc tcttagatct tga 693

```

<210> 16

<211> 230

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 16

Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His Asn Thr Gln

```

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

```

<210> 17

<211> 72

<212> DNA

<213> Nicotiana tabacum

<400> 17

```

atggggagaa tgtcaatacc catgatgggt tttgtggtgt tatgtctatg ggcagtggta 60
gcagaaggat cc                                     72

```

<210> 18

<211> 24

<212> PRT

<213> Nicotiana tabacum

<400> 18

```

Met Gly Arg Met Ser Ile Pro Met Met Gly Phe Val Val Leu Cys Leu
1       5       10      15
Trp Ala Val Val Ala Glu Gly Ser
20

```

<210> 19

<211> 4

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized peptide

<400> 19

```

Lys Asp Glu Leu
1

```

<210> 20
 <211> 4
 <212> PRT
 <213> Artificial

<220>
 <223> an artificially synthesized peptide

<400> 20
 His Asp Glu Leu
 1

<210> 21
 <211> 4
 <212> PRT
 <213> Artificial

<220>
 <223> an artificially synthesized peptide

<400> 21
 Lys Asp Glu Phe
 1

<210> 22
 <211> 4
 <212> PRT
 <213> Artificial

<220>
 <223> an artificially synthesized peptide

<400> 22
 His Asp Glu Phe
 1

<210> 23
 <211> 91
 <212> DNA
 <213> Nicotiana tabacum

<400> 23
 tatTTaactc agtattcaga aacaacaaaa gtTctTctct acatanaatt tTcctatttt 60
 agTgatcagT gaaggaaatc aagaaaaata a 91

<210> 24
 <211> 547
 <212> DNA
 <213> Artificial

<220>
 <223> an artificially synthesized DNA construct

<400> 24

tattttaactc	agtattcaga	aacaacaaaa	gttccttctct	acataaaaatt	ttcctatttt	60
agtgatcagt	gaaggaaaac	aagaaaaata	aatggcggcg	gatigtgcta	aaggtaaaat	120
tgagttttcc	aagtataatg	aggataatac	ctttactgtg	aagggttcag	gaagagaata	180
ctggaccgaac	agatgggaatt	tgcagccatt	gttacaaagt	gctcagctga	cagggaatgac	240
tgtacaacac	atacttaata	cctgcagttc	aggctcaggc	tttgcccagg	tgaagtttaa	300
cagatccccct	ggttctgggtc	ciggcttctcc	tagatccgcg	gcggattgtg	ctaaaggtaa	360
aattgagttt	tccaagtata	atgaggataa	taccittact	gtgaagggtg	cagggaagaga	420
atactggacg	aacagatgga	atttgcagcc	attgttacaa	agtgcctcagc	tgacagggat	480
gactgttaaca	atcatactca	atacctgcag	ttcaggctca	ggctttgccc	aggatgaagt	540
taactga						547

<210> 25

<211> 751

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 25

tattttaactc	agtattcaga	aacaacaaaa	gttccttctct	acataaaaatt	ttcctatttt	60
agtgatcagt	gaaggaaaac	aagaaaaata	aatgaccccc	cagaacatca	ccgacctctg	120
cgccgagagc	cacaacaccc	aaatctacac	cctcaacgac	aagattttca	gctacaccga	180
gagcctcgcc	ggcaagaggg	agatggccat	catcaccttc	aagaacggcg	ccatcttcca	240
ggtcagggtc	cccggcagcc	agcacatcga	cagccagaag	aaggccaicg	agaggatgaa	300
ggacacccctc	aggatcgccct	acctcaccga	ggccaaggtc	gagaagctct	gcgtctggaa	360
caacaagacc	ccccacgcca	tgcgcccat	cagcatggcc	aacagatccc	ctggttctgg	420
tcttggttct	cttagatcca	ccccccagaa	catcacccag	ctctgcgccg	agagccacaa	480
cacccaaatc	tacacctca	acgacaagat	tttcagctac	accgagagcc	tgcgcggcaa	540
gagggagatg	gccatcatca	ccttcaagaa	cggcgccatc	ttccaggtcg	aggctcccgg	600
cagccagcac	atcgacagcc	agaagaaggc	catcgagagg	atgaaggaca	ccctcaggat	660
cgcttacctc	accgagccca	aggctcgaga	gctctgcgtc	tggaaacaca	agacccccca	720
cgccatcgcc	gccatcagca	tggccaactg	a			751

<210> 26

<211> 637

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 26

tattttaactc	agtattcaga	aacaacaaaa	gttccttctct	acataaaaatt	ttcctatttt	60
agtgatcagt	gaaggaaaac	aagaaaaata	aatggggaga	atgtcaatac	ccatgatggg	120
ttttgtgggt	ttatgtctat	gggcagtggt	agcagaagga	tccgcggcgg	attgtgctaa	180
aggtaaaatt	gagttttcca	agtataatga	ggataatacc	tttactgtga	agggttcagg	240
aagagaatac	tggacgaaca	gatggaaatt	gcagccattg	ttacaaagtg	ctcagctgac	300
agggaatgact	gtacaatatc	tatctaatat	cctgcagttc	ggctcaggct	ttgcccagggt	360
gaagtttaac	agatccccctg	gttctgggtcc	tgtttctcct	agatccgcgg	cggatttgtgc	420
taaaggtaaa	attgagtttt	ccaagtataa	tgaggataat	acctttactg	tgaagggtgc	480
aggaagagaa	tactggacga	acagaatgaa	tttgcagcca	ttgttacaaa	gtgctcagct	540
gacagggatg	acigttaacaa	tcatatctaa	tacctgcagt	tcaggctcag	gctttgcccc	600
ggtgaagttt	aacagatctg	aacatgatga	attgtga			637

<210> 27

<211> 841

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 27

```
tatttaactc agtattcaga aacaacaaaa gtcttctct acataaaati ttctatttt 60
agtgatcagt gaaggaatc aagaaaaata aatggggaga atgtcaatc ccatgatggg 120
ttttgtggg ttatgtctat gggcagtggt agcagaagga tccaccccc agaacaatc 180
cgacctctgc gccgagagcc acaacaccca aatctacacc ctcaacgaca agattttcag 240
ctacaccgag agcctcgcgc gcaagaggga gatggccatc atcaccttca agaacggcgc 300
catcttccag gtccaggtcc cggcagcca gcacatcgac agccagaaga aggccatcga 360
gaggatgaag gacacccctc ggaicgccta cctcaccgag gccaaaggtcg agaagctctg 420
cgtctggaac aacaagacc cccacgccat cgcgcgcctc agcatggcca acagatcccc 480
tggttctggt cctgggttct ctgatatccac cccccagaac atcaccgacc tctgcgcga 540
gagccacaac acccaaatct acacccctcaa cgacaagatt tttagctaca ccgagagcct 600
cgccggcaag agggagatgg ccatcatcac ctcaagaac ggcccatctt tccaggtcga 660
ggtcccccgc agccagcaca tcgacagcca gaagaaggcc atcgagagga tgaaggacac 720
cctcaggatc gccctaccca ccgaggccaa ggtcgagaag ctctgcgtct ggaacaacaa 780
gacccccac gccatcgccg ccatcagcat ggccaacaga tctgaacatg atgaattgtg 840
a
```

<210> 28

<211> 667

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 28

```
tatttaactc agtattcaga aacaacaaaa gtcttctct acataaaati ttctatttt 60
agtgatcagt gaaggaatc aagaaaaata aatggggaga atgtcaatc ccatgatggg 120
ttttgtggg ttatgtctat gggcagtggt agcagaagga tccaccccc agtattgctaa 180
aggtaaaatt gagttttcca agtataatga ggaataatc tttactgtga aggtgtcagg 240
aagagaatc tggacgaaca gatggaatit gcagccattt ttacaagatg ctacagctgac 300
agggatgact gtaacaatca tatctaatac ctgcagttca ggctcaggct ttgcccagggt 360

gaagttaaac agatcccttg gtctctgtcc tggttctcct agatccggcg cggattgtgc 420
taaaagttaa attgagtttt ccaagtataa tgaggataat acccttactg tgaagggtgc 480
aggaagagaa tactggacga acagatggaa ttgacgcca ttgtttacaa gtgctcagct 540
gacagggatg actgtaacaa tcatatctaa tacctgcagt tcaggctcag gctttgcccc 600
ggtgaagtit aacagatccc ctggttcttg tctgtgttct cctagatctg aacatgatga 660
attgtga
```

<210> 29

<211> 871

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 29

```
tatttaactc agtattcaga aacaacaaaa gtcttctct acataaaati ttctatttt 60
agtgatcagt gaaggaatc aagaaaaata aatggggaga atgtcaatc ccatgatggg 120
ttttgtggg ttatgtctat gggcagtggt agcagaagga tccaccccc agaacaatc 180
cgacctctgc gccgagagcc acaacaccca aatctacacc ctcaacgaca agattttcag 240
ctacaccgag agcctcgcgc gcaagaggga gatggccatc atcaccttca agaacggcgc 300
catcttccag gtccaggtcc cggcagcca gcacatcgac agccagaaga aggccatcga 360
gaggatgaag gacacccctc ggaicgccta cctcaccgag gccaaaggtcg agaagctctg 420
cgtctggaac aacaagacc cccacgccat cgcgcgcctc agcatggcca acagatcccc 480
tggttctggt cctgggttct ctgatatccac cccccagaac atcaccgacc tctgcgcga 540
gagccacaac acccaaatct acacccctcaa cgacaagatt tttagctaca ccgagagcct 600
cgccggcaag agggagatgg ccatcatcac ctcaagaac ggcccatctt tccaggtcga 660
ggtcccccgc agccagcaca tcgacagcca gaagaaggcc atcgagagga tgaaggacac 720
cctcaggatc gccctaccca ccgaggccaa ggtcgagaag ctctgcgtct ggaacaacaa 780
gacccccac gccatcgccg ccatcagcat ggccaacaga tccccgtgtt ctggtcctgg 840
ttctcctaga tctgaacatg atgaattgtg a
```

<210> 30

<211> 38
 <212> DNA
 <213> Artificial

<220>
 <223> an artificially synthesized primer

<400> 30
 tatctagagc caccatggga tccgcggcgg attgtgct 38

<210> 31
 <211> 30
 <212> DNA
 <213> Artificial

<220>
 <223> an artificially synthesized primer

<400> 31
 ttcaagatct gttaaacttc acctgggcaa 30

<210> 32
 <211> 23
 <212> DNA
 <213> Artificial

<220>
 <223> an artificially synthesized primer

<400> 32
 gtcgacggta cccccgggga gct 23

<210> 33
 <211> 23
 <212> DNA
 <213> Artificial

<220>
 <223> an artificially synthesized primer

<400> 33
 cccccgggggt accgtcgaca gct 23

<210> 34
 <211> 41
 <212> DNA
 <213> Artificial

<220>

<223> an artificially synthesized primer

<400> 34

aatctagagt ctatttaact cagtattcag aaacaacaaa a 41

<210> 35

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 35

aaatgcatta ttttcttga ttccttcac 30

<210> 36

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 36

aaatgcatgg ggagaatgac aatacccatg 30

<210> 37

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 37

tataggatcc cattatcttt cttgatttcc 30

<210> 38

<211> 7

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized peptide

<400> 38

Gly Ser Glu His Asp Glu Leu

1

b

<210> 39

<211> 21

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 39

gatctgaaca tgatgaattg t 21

<210> 40

<211> 21

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 40

gatcacaatt catcatgttc a 21

<210> 41

<211> 33

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 41

gatcttatcc ttatgattat cctgattatg ctg 33

<210> 42

<211> 33

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 42

gatccagcat aatcaggata atcataagga taa 33

<210> 43

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 43

gatcccctgg ttctggtcct ggttctccta 30

<210> 44

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 44

gatctaggag aaccaggacc agaaccaggg 30

<210> 45

<211> 40

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 45

ttggatccac cccccagaac atcacgacc tctgcgccga 40

<210> 46

<211> 39

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 46

cgttgaggggt gtagattgg gtgtgtggc tctcggcgc 39

<210> 47

<211> 40

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 47
ccctcaacga caagattttc agctacaccg agagcctcgc 40

<210> 48
<211> 40
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer

<400> 48
cttgaagggtg atgatggcca tctccctctt gccggcgagg 40

<210> 49
<211> 32
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer

<400> 49
caccttcaag aacggcgcca tctccaggt cg 32

<210> 50
<211> 45
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer

<400> 50
cttctggctg tcgatgtgct ggctgccggg gacctcgacc tggaa 45

<210> 51
<211> 45
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer

<400> 51
agccagaaga aggccatcga gaggatgaag gacaccctca ggatc 45

<210> 52

<211> 43

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 52

gcagagcttc tcgacctgg cctcgggtgag gtaggcgatc ctg 43

<210> 53

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 53

aagctctgcg tctggaacaa caagaccccc 30

<210> 54

<211> 45

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 54

aaagatctgt tggccatgct gatggcggcg atggcgtggg gggtc 45

<210> 55

<211> 31

<212> DNA

<213> Artificial

<220>

<223> an artificially primer

<400> 55

tttgatcca gcaagggcga ggagctgttc a 31

<210> 56

<211> 33

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 56

tttagatctc ttgtacagct cgtccatgcc gag 33

<210> 57

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 57

aaaggatccg cctcctccga ggacgtcatc 30

<210> 58

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 58

aaaagatctg gcgccggtgg agtggcggcc 30

<210> 59

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 59

gatcaaaaatg gggttgtcat tcgaaaagtt 30

<210> 60

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 60

attccatcta tgccttgctt gcgatgtgt 30

<210> 61

<211> 24

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 61

ctagacaaga tccgtccatt gtgg 24

<210> 62

<211> 24

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 62

acccccaaca tccacacgg tgaa 24

<210> 63

<211> 7

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized peptide

<400> 63

Arg Ser Pro Gly Ser Arg Ser
1 5

<210> 64

<211> 12

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized peptide

<400> 64

Arg Ser Gly Ser Gly Ser Gly Ser Gly Ser Arg Ser
1 5 10

<210> 65

<211> 15

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 65

gatcccctgg ttcca 15

<210> 66

<211> 15

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 66

gatctggaac caggg 15

<210> 67

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 67

gatccggttc tggttctggt tctggtcca 30

<210> 68

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 68

gatctggaac cagaaccaga accagaaccg 30

<210> 69

<211> 25

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 69

gtgatcagtg aaggaaatca agaaa 25

<210> 70

<211> 23

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 70

cataacacca caaaacccat cat 23

<210> 71

<211> 22

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 71

ccaagccaaa gaagatcaag ca 22

<210> 72

<211> 24

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 72

ccctgaatca tcgacctgt agaa 24

<210> 73

<211> 700

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 73

```
tatttaactc agtattcaga aacaacaaaa gtctctctct acataaaatt ttcctatatt 60
agtatcagtg gaaggaaatc aagaaaaata aatggggaga atgtcaatac ccatgatggg 120
ttttgtggtg ttatgtctat gggcagtggt agcagaagga tccgcggcgg atgtgtctaa 180
--ttt--ttt-- --ttt--ttt-- --ttt--ttt-- --ttt--ttt-- --ttt--ttt-- 240
```

```

aggtaaaat gagtttcca agtataatga ggataatacc ttatcttga aggtgtcagg 240
aagagaatag tggacgaaca gatggaattt gcagccattg ttacaaagt ctcagctgac 300
aggaatgact gtaacaaatc tatctaatac ctgcagttca ggcicaggct ttgcccagg 360
gaagttaaac agatccccct gttcttggtc ttgttctctt agatccgagg cggattgtgc 420
taaaggtaaa attgagtttt ccaagtataa tgaggataat acctttactg tgaagggtgc 480
aggaagagaa tactggacga acagatggaa ttgcagcca ttgttacaaa gtgtcagct 540
gacagggaag actgtaacaa tcatatctaa tacctgcagt tcaggctcag gctttgccca 600
ggtaagttt aacagatccc ctggttctgg tcciggttct cctagatctt atccttatga 660
ttatctgat tatgttgat ctgaacatga tgaattgtga 700

```

<210> 74

<211> 904

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 74

```

tatttaac agtattcaga aacaacaaaa gtcttctct acataaaatt ttctatttt 60
agtgtcagt gaaggaaatc aagaaaaata aatggggaga atgtcaatac ccatgatggg 120
ttttgtgtg ttatgtctat gggcagtggt agcagaagga tccaccccc agaactcac 180
cgacctctgc gccgagagcc acaacaccca aaictacacc ctcaacgaca agattttcag 240
ctacaccgag agcctcggcg gcaagaggga gatggccatc atcaccttca agaaccggcg 300
caicttccag gtccaggtcc cgggcagcca gcacatcgac agccagaaga aggccatcga 360
gaggatgaag gacaccccca ggaatcgcta cctcaccgag gccaaagtcg agaagctctg 420
cgtctggaac aacaagaccc ccacgccat cgcggccatc agcatggcca acagatcccc 480
tggttctggt cciggttctc ctgatccac cccccagaac atcaccgacc tctgcgccga 540
gagccacaac acccaaatct acaccctcaa cgacaagatt tttagctaca ccgagagcct 600
cgccggcaag agggagatgg ccatcatcac ctccaagaac ggcgccatct tccaggtcga 660
ggtccccggc agccagcaca tcgacagcca gaagaaggcc atcgagagga tgaaggacac 720
cctcaggaat gccctaccct cggaggccaa ggtcgagaag ctctgcgtct ggaacaacaa 780
gacccccca gccaatcgcc ccatcagcat ggccaacaga tccccctggt ctggtctctg 840
ttctctaga tcttatctt atgattatcc tgattatgct ggatctgaac atgatgaatt 900
gtga 904

```

<210> 75

<211> 21

<212> DNA

<213> Nicotiana tabacum

<400> 75

gatttgttgg ttgatactat g 21

<210> 76

<211> 7

<212> PRT

<213> Nicotiana tabacum

<400> 76

```

Asp Leu Leu Val Asp Thr Met
1 5

```

<210> 77

<211> 45

<212> DNA

<213> Armoracia rusticana

<400> 77

ctactccatg atatggtgga ggtcgttgac ttgttagct ctatg 45

<210> 78

<211> 15

<212> PRT

<213> *Armoracia rusticana*

<400> 78

Leu	Leu	His	Asp	Met	Val	Glu	Val	Val	Asp	Phe	Val	Ser	Ser	Met
1				5					10					15

<210> 79

<211> 75

<212> PRT

<213> *Lactuca sativa*

<400> 79

Met	Ala	Ser	Ile	Ser	Ser	Ser	Ala	Ile	Ala	Thr	Val	Asn	Arg	Thr	Thr
1			5						10					15	
Ser	Thr	Gln	Ala	Ser	Leu	Ala	Ala	Pro	Phe	Thr	Gly	Leu	Lys	Ser	Asn
		20						25					30		
Val	Ala	Phe	Pro	Val	Thr	Lys	Lys	Ala	Asn	Asn	Asp	Phe	Ser	Ser	Leu
		35				40						45			
Pro	Ser	Asn	Gly	Gly	Arg	Val	Gln	Cys	Met	Lys	Val	Trp	Pro	Pro	Ile
		50			55						60				
Gly	Leu	Lys	Lys	Tyr	Glu	Thr	Leu	Ser	Tyr	Leu					
65				70					75						

<210> 80

<211> 225

<212> DNA

<213> *Lactuca sativa*

<400> 80

atggcctc	ca tctcctc	ctc agccatc	gcc accgtca	acc ggaccac	ctc cacc	caagct	60
agcttggc	ag ctccatt	cac cggcctc	aag tctaacg	tag ctttcc	cagt tacca	agaag	120
gctaaca	atg acttttc	atc cctacc	cagc aacggt	ggaa gagtaca	atg catga	agggtg	180
tggccac	caa ttgggtt	gaa gaagtac	gag actcttc	cat accta			225

<210> 81

<211> 51

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA

<400> 81

agatcccc	tg gttctg	tcc tgggtct	cct agatccc	ctg gttccag	atc t	51
----------	-----------	-------------	-------------	-------------	-------	----

<210> 82

<211> 17

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized peptide

<400> 82

Arg	Ser	Pro	Gly	Ser	Gly	Pro	Gly	Ser	Pro	Arg	Ser	Pro	Gly	Ser	Arg
1			5					10					15		
Ser															

<210> 83

<211> 66

<212> DNA

<213> Artificial Sequence

<220>

<223> an artificially synthesized DNA

<400> 83

aga	tcccc	g	gttc	gg	tcc	tgg	tct	cct	aga	tcccc	g	gttc	gg	tcc	tgg	tct	cct	60
aga	tct																	66

<210> 84

<211> 22

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized peptide

<400> 84

Arg	Ser	Pro	Gly	Ser	Gly	Pro	Gly	Ser	Pro	Arg	Ser	Pro	Gly	Ser	Gly
1			5					10					15		
Pro	Gly	Ser	Pro	Arg	Ser										
			20												

<210> 85

<211> 696

<212> DNA

<213> Artificial Sequence

<220>

<223> an artificially synthesized DNA construct

<400> 85

gcg	gcg	gatt	gtg	ctaa	aagg	taaa	attg	ag	ttt	cca	agt	ata	atg	agga	taata	acct	tt	60	
act	gtg	aagg	tg	tcag	gaag	aga	tact	gg	acg	aac	agat	gga	att	ggc	gca	att	gtta	120	
caa	agt	gtc	tc	gac	ag	gat	act	gt	aca	at	cat	at	cta	at	acct	g	cag	tcag	180
tc	agg	ct	ttg	ccc	agg	tga	gtt	ta	caga	tccc	ct	ggt	ct	ctg	tt	ct	ctaga	240	
tc	gcg	gcg	att	gtc	taa	aggt	aaa	att	gag	ttt	cca	agt	ata	atg	gga	ta	at	acc	300
ttt	act	gtg	aga	agg	gtc	agg	aga	at	gg	aca	ga	gat	gga	att	gc	ag	ccatt	g	360
tt	aca	aag	gt	ctc	ag	ctg	ac	agg	at	gact	gta	aca	at	ca	tat	ca	at	ctg	420
gg	ctc	agg	ct	ttg	ccc	agg	t	ga	ag	ttt	aac	aga	tccc	ctg	gtt	ctg	tcc	t	480
aga	tccc	gcg	cgg	att	gtc	taaa	gg	taaa	att	gag	ttt	cca	agt	ata	taa	tg	agg	ata	540
acc	ttt	act	tga	agg	gtg	tc	agg	ag	aaa	tact	gg	acg	a	cag	atg	gaa	ttt	gc	600
ttg	tt	acaaa	gtg	ctc	ag	ctg	acg	atg	gga	atg	act	gt	aaca	tcat	at	ctaa	tac	ctg	660
tc	agg	ctc	ag	gctt	gtccc	gg	tga	ag	ttt	aact	ga								696

<210> 86

<211> 231

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 86

```

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

```

<210> 87

<211> 732

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 87

```

gcggcggatt gtgctaaagg taaaattgag ttttccaagt ataatgagga taataccttt      60
actgtgaagg igtcaggaag agaatactgg acgaacagat ggaatttgca gccattgtta      120
caaaagtgtc agctgaacagg gatgactgta acaatcatai ciaatacttg cagttcaggc      180
tcaggctttg cccaggtgaa gtttaacaga tccccctggt ctggtcctgg ttctcctaga      240
tcgcgcgcgg attgigctaa aggtaaaaat gagttttcca agtataatga ggataatacc      300
tttactgtga aggtgtcagg aagagaatac tggacgaaca gatggaattt gcagccattg      360
ttacaagtg ctcagctgac agggatgact gtaacaatca tatctaatac ctgcagtica      420
ggctcaggct ttgcccaggt gaagtttaac agatccccct gttctggtcc tggttctcct      480
agatccgcgg cggattgtgc taaaggtaaa attgagtttt ccaagtataa tgaggataat      540
acctttactg tgaagggtgc aggaagagaa tactggacga acagatggaa ttgcagccca      600
ttgttacaaa gtgctcagct gacagggatg actgtaacaa tcatatctaa tacctgcagt      660
tcaggctcag gccttgccca ggtgaagttt aacagatccc ctggttctgg tctgtgttct      720
cctagatcct ga                                     732

```

<210> 88

<211> 243

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 88

```

Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn Glu
1      5      10      15
Asn Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr Asn

```

```

Asp Asn Thr Thr Val Lys Val Ser Arg Arg Glu Tyr Thr Thr Asn
20 25 30
Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly Met
35 40 45
Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe Ala
50 55 60
Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg
65 70 75 80
Ser Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn
85 90 95
Glu Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr
100 105 110
Asn Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly
115 120 125
Met Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe
130 135 140
Ala Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro
145 150 155 160
Arg Ser Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr
165 170 175
Asn Glu Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp
180 185 190
Thr Asn Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr
195 200 205
Gly Met Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly
210 215 220
Phe Ala Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser
225 230 235 240
Pro Arg Ser

```

<210> 89

<211> 504

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 89

```

ggcgccgatt gtgctaaagg taaaattgag ttctccaagt ataagagga taataccttt      60
actgigaagg tctcaggaag agaatactgg acgaacagat ggaatttgca gccatgltta      120
caaagtgtct agctgacagg gatgactgta acaatcatat ctaatacctg cagttcaggc      180
tcaggcttgg ccagggtgaa gttaacaga tcccctggtt ctggtcctgg ttctcctaga      240
tcccctggtt ccagatctgc ggcggattgt gctaaaggta aaattgagtt ttccaagtat      300
aatgaggata atacccttac tctgaagggt tcaggaagag aatactggac gaacagatgg      360
aatctgcagc catctgtaca aagtgtctcag ctgacagga tgactglaac aatcatactt      420
aatacctgca gtccagctc agcctttgcc caggtgaagt ttaacagatc cctgtgtct      480
ggctctggtt ctcctagatc ttaga                                     504

```

<210> 90

<211> 167

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 90

```

Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn Glu
1 5 10 15
Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr Asn
20 25 30
Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly Met
35 40 45
Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe Ala
50 55 60
Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg
65 70 75 80
Ser Pro Gly Ser Arg Ser Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu
85 90 95
Phe Ser Lys Tyr Asn Glu Asp Asn Thr Phe Thr Val Lys Val Ser Gly
100 105 110

```

100 105 110
 Arg Glu Tyr Trp Thr Asn Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser
 115 120 125
 Ala Gln Leu Thr Gly Met Thr Val Thr Ile Ile Ser Asn Thr Cys Ser

130 135 140
 Ser Gly Ser Gly Phe Ala Gln Val Lys Phe Asn Arg Ser Pro Gly Ser
 145 150 155 160
 Gly Pro Gly Ser Pro Arg Ser
 165

<210> 91

<211> 519

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 91

```

gcggcggatt gtgctaaagg taaaattgag ttttccaagt ataatgagga taataccitt      60
actgtgaagg tgtcaggaag agaatactgg acgaacagat ggaatttgca gccattgita      120
caaagtgcct agctgacagg gatgactgta acaatcatat ctaatacccg cagttcaggc      180
tcaggctttg cccagggtgaa gttaaacaga tccccctggt cttgttcctg tttctctaga      240
tccccctggt ctggttcctg tttctctaga tctgcggcgg attgtgctaa aggtaaaatt      300
gagttttcca agtataatga ggataatacc ttactctgta aggtgtcagg aagagaatac      360
tggacgaaca gatggaattt gcagccattg ttacaaagtg ctCagctgac agggatgact      420
gtacaatca tatctaatac ctgcagttca ggctcaggct ttgccagggt gaagtttaac      480
agatccctcg gttctggtcc tggttctcct agatcttga                               519
  
```

<210> 92

<211> 172

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 92

```

Ala Ala Asp Cys Ala Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn Glu
1      5      10
Asp Asn Thr Phe Thr Val Lys Val Ser Gly Arg Glu Tyr Trp Thr Asn
20     25     30
Arg Trp Asn Leu Gln Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly Met
35     40     45
Thr Val Thr Ile Ile Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe Ala
50     55     60
Gln Val Lys Phe Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg
65     70     75     80
Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg Ser Ala Ala Asp Cys Ala
85     90     95
Lys Gly Lys Ile Glu Phe Ser Lys Tyr Asn Glu Asp Asn Thr Phe Thr
100    105    110
Val Lys Val Ser Gly Arg Glu Tyr Trp Thr Asn Arg Trp Asn Leu Gln
115    120    125
Pro Leu Leu Gln Ser Ala Gln Leu Thr Gly Met Thr Val Thr Ile Ile
130    135    140
Ser Asn Thr Cys Ser Ser Gly Ser Gly Phe Ala Gln Val Lys Phe Asn
145    150    155    160
Arg Ser Pro Gly Ser Gly Pro Gly Ser Pro Arg Ser
165    170
  
```

<210> 93

<211> 1002

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 93

```

acccccccaga acatcaccca cctctgcgcc gagagccaca acacccaaat ctacaccctc    60
aacgacaaga ttttcagcta caccgagagc ctgcgccgca agaggagat ggccatcatc    120
accttaaga acggcgccat cticcaggtc gagggtcccc gcagccagca catcgacagc    180
cagaagaagg ccatcgagag gatgaaggac accctcagga tcgcctacct caccgaggcc    240
aaggtcgaga agctctgcgt ctggaacaac aagaccccc acgccatgcg cgccatcagc    300
atggccaaca gatccccctg ttctggtcct ggttctccta gatccacccc ccagaacatc    360

```

```

accgacctct gcgcgagag ccacaacacc caaatctaca cctcaacga caagattttc    420
agctacacgg agagcctcgc cggcaagagg gagatggcca tcatcacctt caagaacggc    480
gccatcttcc aggtcgaggt ccccgccagc cagcacatcg acagccagaa gaaggccatc    540
gagaggatga aggcacccct caggatcgcc tacctcaccg aggccaaagt cgagaagctc    600
tgcgtctgga acaacaagac cccccacgcc atcgccgcca tcagcatggc caacagatcc    660
ctgggttctg gtcttggttc tcctagatcc acccccaga acatcacga cctctgcgcc    720
gagagccaca acacccaaat ctacaccctc aacgacaaga ttttcagcta caccgagagc    780
ctgcgccgga agaggagat ggccatcatc accttaaga acggcgccat cticcaggtc    840
gaggtccccg gcagccagca catcgacagc cagaagaagg ccatcgagag gatgaaggac    900
accttcagga tcgcctacct caccgaggcc aaggtcgaga agctctgcgt ctggaacaac    960
aagaccccc acgccatgcg cgccatcagc atggccaact ga                                1002

```

<210> 94

<211> 333

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 94

```

Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His Asn Thr Gln
1      5      10      15
Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser Tyr Thr Glu Ser Leu Ala
20     25     30
Gly Lys Arg Glu Met Ala Ile Ile Thr Phe Lys Asn Gly Ala Ile Phe
35     40     45
Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln Lys Lys Ala
50     55     60
Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Ala Tyr Leu Thr Glu Ala
65     70     75     80
Lys Val Glu Lys Leu Cys Val Trp Asn Asn Lys Thr Pro His Ala Ile
85     90     95
Ala Ala Ile Ser Met Ala Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser
100    105    110
Pro Arg Ser Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His
115    120    125
Asn Thr Gln Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser Tyr Thr Glu
130    135    140
Ser Leu Ala Gly Lys Arg Glu Met Ala Ile Ile Thr Phe Lys Asn Gly
145    150    155    160
Ala Ile Phe Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln
165    170    175
Lys Lys Ala Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Ala Thr Leu
180    185    190
Thr Glu Ala Lys Val Glu Lys Leu Cys Val Trp Asn Asn Lys Thr Pro
195    200    205
His Ala Ile Ala Ala Ile Ser Met Ala Asn Arg Ser Pro Gly Ser Gly
210    215    220
Pro Gly Ser Pro Arg Ser Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala
225    230    235    240
Glu Ser His Asn Thr Gln Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser
245    250    255
Tyr Thr Glu Ser Leu Ala Gly Lys Arg Glu Met Ala Ile Ile Thr Phe
260    265    270
Lys Asn Gly Ala Ile Phe Gln Val Glu Val Pro Gly Ser Gln His Ile
275    280    285
Asp Ser Gln Lys Lys Ala Ile Glu Arg Met Lys Asp Thr Leu Arg Ile
290    295    300
Ala Tyr Leu Thr Glu Ala Lys Val Glu Lys Leu Cys Val Trp Asn Asn
305    310    315    320
Lys Thr Pro His Ala Ile Ala Ala Ile Ser Met Ala Asn
325    330

```

<210> 95

<211> 1038

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 95

```

acccccaga acatcacga cctctggcc gagagccaca acacccaaat ctacaccctc      60
aacgacaaga ttttcagcta caccgagagc ctgccggcca agagggagat ggccatcatc      120
accttaaga acggcgccat ctccaggic gaggtccccg gcagccagca catcgacagc      180
cagaagaagg ccatcgagag gatgaaggac accctcagga tcgcctacct caccgaggcc      240
aaggtcgaga agctctgcgt ctgggaacaac aagaccccc acgccatcgc cgccatcagc      300
atggccaaca gatccccgg ttctggtcct ggttctccta gatccacccc ccagaacatc      360
accgacctct gcgcccagag ccacaacacc caaatctaca ccctcaacga caagattttc      420
agctacaccg agagccctgc cggcaagagg gagatggcca tcaicacctt caagaacggc      480
gccatcttcc aggtcgaggt ccccggcagc cagcacatcg acagccagaa gaaggccatc      540
gagaggatga aggacacctt caggatcgcc tacttcaccg aggccaaaggi cgagaagctc      600
tgcctctgga acaacaagac ccccccagcc atcgccgcca tcagcaiggc caacagatcc      660
cctgggtctg gtctctggtt tcctagatct acccccaga acatcaccca cctctgcgcc      720
gagagccaca acacccaaat ctacaccctc aacgacaaga ttttcagcta caccgagagc      780
ctgccggca agaggcgagat ggccatcatc accttaaga acggcgccat ctccaggctc      840
gaggtccccg gcagccagca catcgacagc cagaagaagg ccatcgagag gatgaaggac      900
accctcagga tcgcctacct caccgaggcc aaggtcgaga agctctgcgt ctggaacaac      960
aagaccccc acgccatcgc cgccatcagc atggccaaca gatccccgg tictggtcct      1020
gttctccta gatcttga

```

<210> 96

<211> 345

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 96

```

Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His Asn Thr Gln      1
1
Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser Tyr Thr Glu Ser Leu Ala      5
20
Gly Lys Arg Glu Met Ala Ile Ile Thr Phe Lys Asn Gly Ala Ile Phe      35
40
Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln Lys Lys Ala      50
55
Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Ala Tyr Leu Thr Glu Ala      65
70
Lys Val Glu Lys Leu Cys Val Trp Asn Asn Lys Thr Pro His Ala Ile      85
90
Ala Ala Ile Ser Met Ala Asn Arg Ser Pro Gly Ser Gly Pro Gly Ser      100
105
Pro Arg Ser Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala Glu Ser His      115
120
Asn Thr Gln Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser Tyr Thr Glu      130
135
Ser Leu Ala Gly Lys Arg Glu Met Ala Ile Ile Thr Phe Lys Asn Gly      145
150
Ala Ile Phe Gln Val Glu Val Pro Gly Ser Gln His Ile Asp Ser Gln      165
170
Lys Lys Ala Ile Glu Arg Met Lys Asp Thr Leu Arg Ile Ala Tyr Leu      180
185
Thr Glu Ala Lys Val Glu Lys Leu Cys Val Trp Asn Asn Lys Thr Pro      195
200
His Ala Ile Ala Ala Ile Ser Met Ala Asn Arg Ser Pro Gly Ser Gly      210
215
Pro Gly Ser Pro Arg Ser Thr Pro Gln Asn Ile Thr Asp Leu Cys Ala      225
230
Glu Ser His Asn Thr Gln Ile Tyr Thr Leu Asn Asp Lys Ile Phe Ser      245
250
Tyr Thr Glu Ser Leu Ala Gly Lys Arg Glu Met Ala Ile Ile Thr Phe      260
265
Lys Asn Gly Ala Ile Phe Gln Val Glu Val Pro Gly Ser Gln His Ile      275
280

```

```

Asp Ser Gln Lys Lys Ala Ile Glu Arg Met Lys Asp Thr Leu Arg Ile
290          295          300
Ala Tyr Leu Thr Glu Ala Lys Val Glu Lys Leu Cys Val Trp Asn Asn
305          310          315
Lys Thr Pro His Ala Ile Ala Ala Ile Ser Met Ala Asn Arg Ser Pro
          325          330          335
Gly Ser Gly Pro Gly Ser Pro Arg Ser
          340          345

```

<210> 97

<211> 708

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 97

```

acccccaga acatcacga cctctgcgc gagagccaca acacccaaat ctacacccctc      60
aacgacaaga ttttcagcta caccgagagc ctgcgccgca agagggagat ggccatcatc      120
accttaaga acggcgccat cticcaggtc gaggiccccg gcagccagca catcgacagc      180
cagaagaagg ccacgcgagag gatgaaggac accctcagga tcgcctacct caccgaggcc      240
aaggtcgaga agctctgcgt ctggaacaac aagacccccc acgccatcgc cgccatcagc      300
atggccaaca gatccctcgg ttctggtcct ggttctccta gatccctcgg ttccagatct      360
acccccaga acatcacga cctctgcgc gagagccaca acacccaaat ctacacccctc      420
aacgacaaga ttttcagcta caccgagagc ctgcgccgca agagggagat ggccatcatc      480
accttaaga acggcgccat cticcaggtc gaggiccccg gcagccagca catcgacagc      540
cagaagaagg ccacgcgagag gatgaaggac accctcagga tcgcctacct caccgaggcc      600
aaggtcgaga agctctgcgt ctggaacaac aagacccccc acgccatcgc cgccatcagc      660
atggccaaca gatccctcgg ttctggtcct ggttctccta gatcttga      708

```

<210> 18

<211> 235

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 98

```

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

```

<210> 99

<211> 723

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 99

```

acccccaga acatcacga cctctgcgc gagagccaca acacccaaat ctacaccctc      60
aacgacaaga ttttcagcta caccgagagc ctgcgcgca agaggagat ggccatcatc      120

```

```

accttcaaga acggcgccat ctccaggtc gaggtcccg gcagccagca catcgacagc      180
cagaagaagg ccatcgagag gatgaaggac accctcagga tcgcctacct caccgagcc      240
aaggtcgaga agctctgcgt ctggaacaac aagacccccc acgcatcgc cgccatcagc      300
atggccaaca gatccctgg ttcgggtcct ggctctccta gatccctgg tctgggtcct      360
ggctctccta gatctacccc ccagaacatc accgacctct gcgcgagag ccacaacacc      420
caaatctaca ccttcaacga caagatttc agctacacgc agagcctcgc cggcaagagg      480
gagatggcca tcatcacctt caagaacggc gccatcttcc aggtcgaggt cccggcagc      540
cagcacatcg acagccagaa gaaggccatc gagaggatga aggacacctt caggatcgcc      600
tacctcaccg aggccaaggt cgagaagctc tgcgtctgga acaacaagac cccccacgcc      660
atgcgcgcca tcagcatggc caacagatcc cctggttctg gtcctggttc tcctagatct      720
tga                                                                    723

```

<210> 100

<211> 240

<212> PRT

<213> Artificial

<220>

<223> an artificially synthesized protein

<400> 100

```

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

```

<210> 101

<211> 583

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 101

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaatt	ttcctatttt	60
agtgatcagt	gaaggaatc	aagaaaaata	aatggcggcg	gattgtgcta	aaggtaaaat	120
tgaattttcc	aagtataatg	aggataatac	ctttacigtg	aaggigtcag	gaagagaata	180
cggacgaac	agatggaatt	tgacgccatt	gttacaaagt	gcicagctga	cagggatgac	240
tgtacaatc	atacttaata	cttgcagttc	aggctcaggc	tttgcccagg	tgaagttaa	300
cagatcccc	ggttctggtc	ctggttctcc	tagatccgcg	gcggaattgt	ctaaaggtaa	360
aattgagttt	tccaagtata	atgaggataa	tacctttact	gtgaagggtg	caggaagaga	420
atactggacg	aacagatgga	atttgcagcc	attgttacaa	agtcctcagc	tgacagggat	480
gactgttaac	atcatactta	atacctgcag	ttcaggctca	ggctttgcc	aggtgaagtt	540
taacagatcc	cciggttctg	gtcctgtgtc	tcciaagatcc	tga		583

<210> 102

<211> 682

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 102

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaatt	ttcctatttt	60
agtgatcagt	gaaggaatc	aagaaaaata	aatggggaga	atgtcaatac	ccatgatggg	120
ttttgtggtg	ttagtctat	gggcagtggt	agcagaagga	tccggggcgg	attgtgctaa	180
aggtaaaatt	gagttttcca	agtataatga	ggataatacc	tttactgtga	aggtgtcagg	240
aagagaatac	tggaacgaac	gatggaattt	gcagccattg	ttacaagtg	ctcagctgac	300
agggatgact	gtacaatatc	tatctaatac	ctgcagttca	ggctcaggct	tgtcccagg	360
gaagtttaac	agatccccctg	gttctgggtc	tgtttctcct	agatccccctg	gttccagatc	420
tgcggcggaat	tgtgctaaag	gttaaatatga	gttttccaag	tataatgagg	ataatacctt	480
tactgtgaag	gtgtcaggaa	gagaatactg	gacgaacaga	tggaaatttg	agccattggt	540
acaaagtgtc	cagttgacag	ggatgactgt	aacaatcata	tctaatacct	gcagttcagg	600
ctcaggcttt	gccagggtga	agtttaacag	atccccctgt	tctgggtcctg	gttctcctag	660
atctgaacat	gatgaattgt	ga				682

<210> 103

<211> 697

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 103

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaatt	ttcctatttt	60
agtgatcagt	gaaggaatc	aagaaaaata	aatggggaga	atgtcaatac	ccatgatggg	120
ttttgtggtg	ttagtctat	gggcagtggt	agcagaagga	tccggggcgg	attgtgctaa	180
aggtaaaatt	gagttttcca	agtataatga	ggataatacc	tttactgtga	aggtgtcagg	240
aagagaatac	tggaacgaac	gatggaattt	gcagccattg	ttacaagtg	ctcagctgac	300
agggatgact	gtacaatatc	tatctaatac	ctgcagttca	ggctcaggct	tgtcccagg	360
gaagtttaac	agatccccctg	gttctgggtc	tgtttctcct	agatccccctg	gttctgggtc	420
tgtttctcct	agatctgcgg	cggattgtgc	taaaggtaaa	attgagtttt	ccaagtataa	480
tgaaggataat	acctttactg	tgaagggtgc	aggaagagaa	tactggacga	acagatggaa	540
tttgcagcca	ttgttacaaa	gtgtctagct	gacagggaig	actgtaacaa	tcatacttaa	600
tacctgcagt	tcagggtcag	gcctttgccca	ggtgaagttt	aacagatccc	ctggttctgg	660
tccgtgttct	cctagatctg	aacaigatga	attgtga			697

<210> 104

<211> 805

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 104

iatitaaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaaatt	ttcctatitit	60
agtgaicagt	gaaggaaatc	aagaaaaata	aatggccctcc	atctccctcc	cagccatcgc	120
caccgtcaac	cggaccacct	ccaccaaacg	tagcttgcca	gctccattca	cggccctcaa	180
gtctaacgta	gtcttccag	ttaccaagaa	ggctaacat	gacttttcat	ccctaccag	240
caacgggtga	agagtacaat	gcattgaagg	gtggccacca	attgggttga	agaagtacga	300
gactctttca	tacctagcgg	cggattgtgc	taaaggtaaa	attgagttt	ccaagtataa	360
tgaggataat	acctttactg	tgaagggtgc	aggaagagaa	tactggacga	acagatggaa	420
tttgcagcca	ttgttacaaa	gtgtcagct	gacagggatg	actgtacaaa	tcataatctaa	480
tacctgcagt	tcagggtcag	gttttgccca	ggtagaagtt	aacagatccc	ctggttctgg	540
tcctgtgtct	cctagatccg	cggcggattg	tgctaaaggt	aaaattgagt	tttccaagta	600
taatgaggat	aataccttta	ctgtgaagg	gtcaggaaga	gaalacttga	cgaacagatg	660
gaatttgcag	ccattgtttac	aaagtgtctca	gctgacaggg	atgactgtaa	caatcatatc	720
taatacctgc	agttcaggct	caggctttgc	ccaggtgaag	tttaacagat	cccctgttcc	780
tggtcctggt	ttccttagat	cttga				805

<210> 105

<211> 826

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 105

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaaatt	ttcctatitit	60
agtgaicagt	gaaggaaatc	aagaaaaata	aatggcggcg	gattgtgcta	aaggtaaaaat	120
tgagttttcc	aagtataatg	aggataatac	ctttactgtg	aagggtgcag	gaagagataa	180
ctggacgaac	agatggaaat	tgacgccatt	gttacaaagt	gctcagctga	cagggatgac	240
tgtaacaatc	atacttaata	ctgtcagttc	aggctcaggc	tttggccagg	tgaagittaa	300
cagatcccc	ggttctgttc	ctggttctcc	tagatccggc	gaggattgtg	ctaaaggtaa	360
aatgtgattt	lccaagtata	atgaggataa	tacctttact	gtgaagggtg	caggaagaga	420
atactggacg	aacagatgga	atttgcagcc	attgttacaa	agtgctcagc	tgacagggat	480
gactgttaaca	atcatatcta	atacctgcag	ttcaggtcca	ggctttggcc	aggtagaatt	540
taacagaicc	cctgggttctg	gttctgttcc	tcttagatcc	gcggcggatt	gtgctaaagg	600
taaaaattgag	ttttccaagt	ataatgagga	taataccttt	actgtgaagg	tgtaggaag	660
agaatactgg	acgaacagat	ggaaatttga	gccattgtta	caaagtgtct	agctgacagg	720
gatgactgtg	acaatcatat	ctaataccig	cagttcaggc	tcaggctttg	cccaggtaga	780
gtttaacaga	tcccctggtt	ctgggtcctg	ttctcctaga	tctga		826

<210> 106

<211> 910

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 106

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaaatt	ttcctatitit	60
agtgaicagt	gaaggaaatc	aagaaaaata	aatggggaga	atgtcaaatc	ccaigtatggg	120
ttttgtgggt	ttatgtctat	gggcagtggt	agcagaagga	lccggggcgg	atttgtctaa	180
aggtaaaaat	gagttttcca	agtataatga	ggataatacc	ttactgttga	agggtgtcagg	240
aagagaatac	tggacgaaca	gatggaattt	gcagccattg	ttacaaagt	ctcagctgac	300
agggatgact	gtacaaatca	tatctaatac	ctgcagttca	ggctcaggct	ttgcccagg	360
gaagttaaac	agatccccctg	gttctgggtcc	tggttctcct	agatccgcgg	cggattgtgc	420
taaaggtaaa	attgagtttt	ccaagtataa	tgaggataat	acctttactg	tgaagggtgc	480
aggaagagaa	tactggacga	acagatggaa	tttgcagcca	ttgttacaaa	gtgtcagct	540
gacagggatg	actgtacaaa	tcataatctaa	tacctgcagt	tcaggctcag	gctttgccc	600
ggtgaagttt	aacagatccc	ctggttctgg	tctgtgttct	cctagatccg	cggcggattg	660
tgctaaaggt	aaaattgagt	tttccaagta	taatgaggat	aataccttta	ctgtgaagg	720
gtcaggaaga	gaatfactgga	cgaacagatg	gaatttgcag	ccattgtttac	aaagtgtctca	780
gctgacaggg	atgactgtaa	caatcatatc	taatacctgc	agttcaggct	caggctttgc	840
ccagggtgaag	tttaacagat	cccctgtgtc	tggtccctgt	ttctcctagat	cgaacatga	900
tgaattgtga						910

<210> 107

<211> 1048

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 107

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaaatt	ttcctatitt	60
agtgatcagt	gaaggaaatc	aagaaaaata	aatggcctcc	atctcctcct	cagccatcgc	120
caccgtaaac	gggaccacct	ccacccaagc	tagcttggca	gctccattca	ccggcctcaa	180
gtctaacgta	gctttcccag	ttaccaagaa	ggctaacaat	gacttttcat	ccctaccacg	240
caacgggtga	agagtacaat	gcatgaagg	gtggccacca	attgggttga	agaagtacga	300
gactctttca	tacctagcgg	cggattgtgc	taaaggtaaa	attgagtttt	ccaagtataa	360
tgaggataat	acctttactg	tgaagggtgc	aggaagagaa	tactggacga	acagatggaa	420
tttgcagcca	ttgttacaaa	gtgtcagct	gacagggatg	acigtaacaa	tcataatctaa	480
tacctgcagt	tcaggctcag	gcittgcccc	ggtaagttt	aacagatccc	ctggttctgg	540
tcttggttct	cctagatccg	cggcggatig	tgttaaaggt	aaaattgagt	tttccaagta	600
taatgaggat	aatacccttta	ctgtgaagg	gtcaggaaga	gaatactlga	cgaacagatg	660
gaatttgcag	ccattgtttc	aaagtgtcca	gctgacaggg	atgactgtaa	caatcatatc	720
taataactgc	agttcaggct	caggctttgc	ccagggtgaag	tttaacagat	ccccgtgttc	780
tgttcctggt	tctcctagat	ccgcccggga	ttgtgctaaa	ggtaaaattg	agttttccaa	840
gtataatgag	gataaatcct	ttactgtgaa	ggtgtcagga	agagaatact	ggacgaacag	900
aiggaatttg	cagccatigt	tacaaagltc	tcagctgaca	gggaigactg	taacaatcat	960
atctaatacc	tgcagttcag	gtcagggctt	tgcccagggtg	aagittaaaca	gatccccctgg	1020
ttctggtcct	ggttctccta	gacctctga				1048

<210> 108

<211> 787

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 108

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaaatt	ttcctatitt	60
agtgatcagt	gaaggaaatc	aagaaaaata	aatgaccccc	cagaacatca	ccgacctctg	120
cgccgagagc	cacaacaccc	aaatctacac	cttcaacgac	aagattttca	gctacaccga	180
gagccttcgc	ggcaagagg	agatggccat	catcaccttc	aagaacggcg	ccatcttcca	240
ggtcagggtc	cccggcagcc	agcacatcga	cagccagaag	aagccatcgc	agaggatgaa	300
ggacacccic	aggatcgcct	acctcaccca	ggccaagggtc	gagaagctct	gcgtctggaa	360
caacaagacc	ccccacggca	tgcgcggcat	cagcatggcc	aacagatccc	ctggttctgg	420
tcttggttct	cctagatcca	ccccccagaa	catcacccgac	ctctgcggcg	agagccacaa	480
cacccaatc	tacacccitca	acgacaagat	tttcagctac	accgagagcc	tgcggcgcaa	540
gaggagagat	gccatcatca	ctttcaagaa	cggcgccatc	ttccaggtcg	aggtccccgg	600
cagccagcac	atcgatcgcc	agaagaaggc	catcgagagg	atgaaggaca	ccctcaggat	660
cgcctacctc	accgagggcca	aggtcgagaa	gctctgcgtc	tggaaacaaca	agacccccca	720
cgccatcgcc	gccatcagca	tggccaacag	atccccctggt	tcgtgtcttg	gttctcctag	780
atcctga						787

<210> 109

<211> 886

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 109

tatttaactc	agtattcaga	aacaacaaaa	gttcttctct	acataaaaatt	ttcctatitt	60
agtgatcagt	gaaggaaatc	aagaaaaata	aatggggaga	atgtcaatac	ccatgatggg	120
tttgtgtgtg	ttatgtctat	gggcagtggt	agcagaagga	tcaccccccc	agaacatcac	180
cgacctctgc	gccgagagcc	acaacaccca	aatctacacc	ctcaacgaca	agattttcag	240

```

ctacaccgag agcctcgcgc gcaagaggga gatggccatc atcaccttca agaacggcgc 300
catcttccag gtcgagatcc cgggcagcca gcacatcgac agccagaaga aggccatcga 360
gaggatgaag gacacccctca ggatcgcccta cctcaccgag gccaaaggtcg agaagctctg 420
cgcttggaaac aacaagaccc cccacgccat cgcgcgccatc agcatggcca acagatcccc 480
tggttctggt cctggtttctc ctatgacccc tggttccaga tctaccccc agaacatcac 540
cgacctctgc gccgagagcc acaacaccca aatctacacc ctcaacgaca agattttcag 600
ctacaccgag agcctcgcgc gcaagaggga gatggccatc atcaccttca agaacggcgc 660
catcttccag gtcgagatcc cgggcagcca gcacatcgac agccagaaga aggccatcga 720
gaggatgaag gacacccctca ggatcgcccta cctcaccgag gccaaaggtcg agaagctctg 780
cgcttggaaac aacaagaccc cccacgccat cgcgcgccatc agcatggcca acagatcccc 840
tggttctggt cctggtttctc ctatgactga acaatgaaga ttgtga 886

```

<210> 110

<211> 901

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 110

```

tatttaactc agtattcaga aacaacaaaa gtctctctct acataaaait ttcttatitt 60
agtatcagt gaaggaaatc aagaaaaata aatggggaga atgtcaatac ccatgatggg 120
ttttgtggig ttatgtctat gggcagtggt agcagaagga tccaccccc agaacatcac 180
cgacctctgc gccgagagcc acaacaccca aatctacacc ctcaacgaca agattttcag 240
ctacaccgag agcctcgcgc gcaagaggga gatggccatc atcaccttca agaacggcgc 300
catcttccag gtcgagatcc cgggcagcca gcacatcgac agccagaaga aggccatcga 360
gaggatgaag gacacccctca ggatcgcccta cctcaccgag gccaaaggtcg agaagctctg 420
cgcttggaaac aacaagaccc cccacgccat cgcgcgccatc agcatggcca acagatcccc 480
tggttctggt cctggtttctc ctatgacccc tggttctggt cctggtttctc ctatgactac 540
ccccagaaac atcacccgac tctgcgccga gagccacaac acccaaatct acacccctca 600
cgacaagatt ttacgctata ccgagagcct cgcgcgcaag agggagatgg ccatcatcac 660
cttcaagaaac ggcgccatct tccaggtcga ggtccccggc agccagcaca tcgacagcca 720
gaagaaggcc atcgagagga tgaaggacac cctcaggatc gcctacctca ccgaggccaa 780
ggtcgagaag ctctcgctct ggaacaacaa gacccccac gccatcgccg ccattagcat 840
ggccaacaga tccccgggtt ctggctctgg ttctctctga tctgaacaig atgaattgtg 900
a 901

```

<210> 111

<211> 1009

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized DNA construct

<400> 111

```

tatttaactc agtattcaga aacaacaaaa gtctctctct acataaaait ttcttatitt 60
agtatcagt gaaggaaatc aagaaaaata aatggccctc atctctctct cagccatcgc 120
caccgtcaac cggaccacct ccacccaagc tagcttggca gctccaltca ccggcctcaa 180
gtctaacgta gctttccag ttaccaagaa ggcctaacaat gacttttcat ccttaccag 240
caacggtgga agagtacaat gcattgaagg tggccacca attgggttga agaagtacga 300
gactctttca taccctaacc ccagaaacat caccgacctc tgcgcccaga gccacaacac 360
ccaaatctac accctcaacg acaagatttt cagctacacc gagagcctcg ccggcaagag 420
ggagatggcc atcatcacct tcaagaacgg cgcctcttc caggtcgagg tccccggcag 480
ccagcacatc gacagccaga agaaggccat cgagaggatg aaggacaccc tcaggatcgc 540
ctacctacc gaggccaagg tcgagaagct ctgcgtctgg aacaacaaga cccccacgc 600
catcgccgcc atcagcatgg ccaacagatc ccttggtct ggtcctgggt ctcttagatc 660
caccctccag aacatcacgc acctctgcgc cgagagccac aacacccaaa tctacacct 720
caacgacaag attttcagct acaccgagag cctcgcgggc aagagggaga tggccatcat 780
caccitcaag aacggcgcca tcttcagggt cgaggtcccc ggccagccagc acatcgacag 840
ccagaagaag gccatcgaga ggatgaagga caccctcagg atcgccctacc tcaccgaggc 900
caaggctcag aagctctctg tctggaacaa caagaccccc caccgcatcg ccgcatcag 960
catggccaac agatccccgt gtctctggtc tggttctct agatcctga 1009

```

<210> 112

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 112

tataggatcc cattatttt cttgatttc 30

<210> 113

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 113

aaatgcatgg ccctccatctc ctctcagcc 30

<210> 114

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer

<400> 114

tttgatcct aggtatgaaa gagtctcgta 30

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [WO2005011733A \[0008\]](#)
- [WO200189456A \[0008\]](#)

- [JP10507916A \[0010\]](#)
- [JP2000166411A \[0010\]](#)
- [JP2002533068A \[0010\]](#)
- [WO2009004842A1 \[0010\]](#)
- [JP2003079372A \[0057\]](#)
- [JP8120573A \[0209\]](#)

Non-patent literature cited in the description

- **RAN** Veterinary Microbiology, 2007, vol. 127, 1-2209-215 [\[0008\]](#)
- **MAKINO et al.** Microbial Pathogenesis, 2001, vol. 31, 11-8 [\[0010\]](#)
- **KIM et al.** Protein Expression and Purification, 2006, vol. 51, 122-27 [\[0010\]](#)
- **BIRNBOIM, H. C. DOLY, J.** Nucleic acid Res, 1979, vol. 7, 1513- [\[0075\]](#)
- **DONSON J. KERNEY CM. HILF ME. DAWSON WO** Systemic expression of a bacterial gene by a tobacco mosaic virus-based vector Proc. Natl. Acad. Sci., 1991, vol. 88, 7204-7208 [\[0075\]](#)
- **ODELL et al.** Nature, 1985, vol. 313, 810- [\[0076\]](#)
- **ZHANG et al.** Plant Cell, 1991, vol. 3, 1155- [\[0076\]](#)
- **CORNEJO et al.** Plant Mol. Biol., 1993, vol. 23, 567- [\[0076\]](#)
- **HOOD et al.** Transgenic, Res., 1993, vol. 2, 218- [\[0082\]](#)
- **HIEI et al.** Plant J., 1994, vol. 6, 271- [\[0082\]](#)
- **TADA et al.** Theor. Appl. Genet, 1990, vol. 80, 475- [\[0082\]](#)
- **LAZZERI et al.** Theor. Appl. Genet., 1991, vol. 81, 437- [\[0082\]](#)
- **SANFORD et al.** J. Part. Sci. tech., 1987, vol. 5, 27- [\[0082\]](#)
- **VISSER et al.** Theor. Appl. Genet, 1989, vol. 78, 594- [\[0084\]](#)
- **NAGATATAKE BE** Planta, 1971, vol. 99, 12- [\[0084\]](#)
- **SATOH et al.** The 5'-untranslated region of the tobacco alcohol dehydrogenase gene functions as an effective translational enhancer in plant J. Biosci. Bioeng., 2004, vol. 98, 1-8 [\[0096\]](#)
- **CAMPBELL R. E. et al.** A monomeric red fluorescent protein Proc. Nat. Acad. Sci., 2002, vol. 99, 7877-7882 [\[0126\]](#)
- Plant J., 1998, vol. 15, 449-457 [\[0133\]](#)

PATENTKRAV**1. Hybridprotein omfattende**

- 5 (a) to toksinproteiner valgt fra gruppen bestående af Shigatoksin (Stx)-protein, choleratoksin (CT)-protein- eller *Escherichia coli* varme-labilt toksin (LT)-protein, hvor toksinproteinerne er serieforbundet via et peptid med aminosyresekvensen repræsenteret ved SEQ ID NO: 2, 82 eller 84, og
- (b) et peptid med aminosyresekvensen repræsenteret ved SEQ ID NO: 2, 82 eller
- 10 84 forbundet med C-terminus på de serieforbundne toksinproteiner ifølge (a), hvor Stx-proteinet er et STx2eB-protein, hvor CT-proteinet er en CT-protein B subenhed og hvor LT proteinet er en LT protein B subenhed.

- 2. Hybridprotein ifølge krav 1, hvor toksinproteinerne er serieforbundet via et peptid**
- 15 med aminosyresekvensen repræsenteret ved SEQ ID NO:2.

- 3. Hybridprotein ifølge krav 1 eller 2, omfattende en aminosyresekvens repræsenteret ved SEQ ID NO: 14 eller 16.**

- 20 **4. Hybridprotein ifølge krav 1, omfattende en aminosyresekvens repræsenteret ved SEQ ID NO: 90, 92, 98 eller 100.**

- 5. Hybridprotein ifølge ethvert af kravene 1 til 4, hvor et sekretorisk signalpeptid afledt fra en plante er tilføjet til dettes aminotermus.**

25

- 6. Hybridprotein ifølge ethvert af kravene 1 til 5, hvor et endoplasmisk reticulum-tilbageholdelsessignalpeptid er tilføjet til dettes carboxylterminus.**

- 7. Et DNA-konstrukt, som omfatter DNA, som koder for hybridproteinet ifølge ethvert af**
- 30 **kravene 1 til 6.**

- 8. DNA-konstrukt ifølge krav 7, omfattende DNA med en basissekvens repræsenteret ved SEQ ID NO: 13, 15, 89, 91, 97 eller 99.**

- 9.** DNA-konstrukt ifølge krav 7 eller 8, hvor DNA'et, som koder for et hybridprotein, er funktionsmæssigt forbundet med en 5'-ikke-translateret region af et alkoholdehydrogenasegen afledt fra en plante.
- 5 **10.** DNA-konstrukt ifølge krav 9, hvor den 5'-ikke-translaterede region af et alkoholdehydrogenasegen afledt fra en plante er afledt fra *Nicotiana tabacum*.
- 10 **11.** DNA-konstrukt ifølge krav 10, omfattende en basissekvens repræsenteret ved enhver af SEQ ID NOS: 28, 29, 101, 102, 103, 104, 108, 109, 110 eller 111.
- 10 **12.** Rekombinant vektor omfattende DNA-konstruktet ifølge ethvert af kravene 7 til 11.
- 10 **13.** Værtscelle transformeret med den rekombinante vektor ifølge krav 12.
- 15 **14.** Værtscelle ifølge krav 13, hvor transformanten er en transformeret plantecelle eller en transformeret plante.
- 15 **15.** Frø, som er opnået fra transformanten ifølge krav 13 eller 14.
- 20 **16.** Peptid med en aminosyresekvens repræsenteret ved én af SEQ ID NO: 2, 82 eller 84.

DRAWINGS

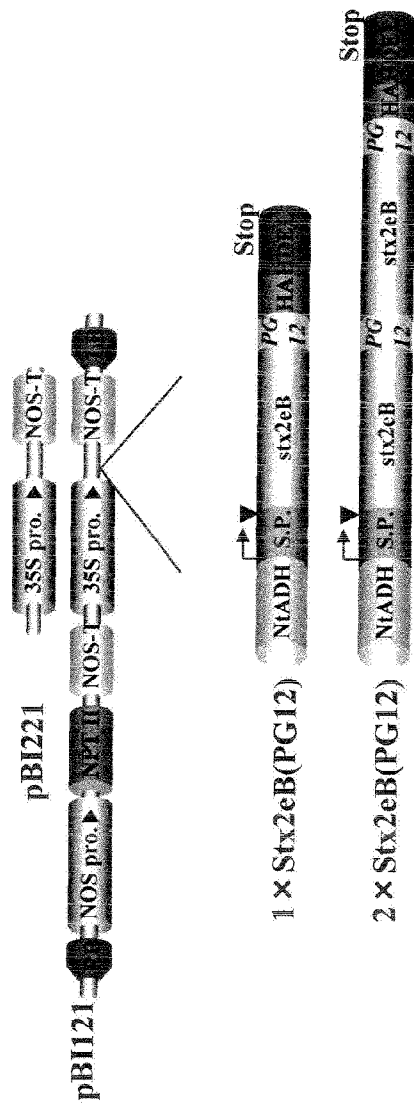


FIG. 1

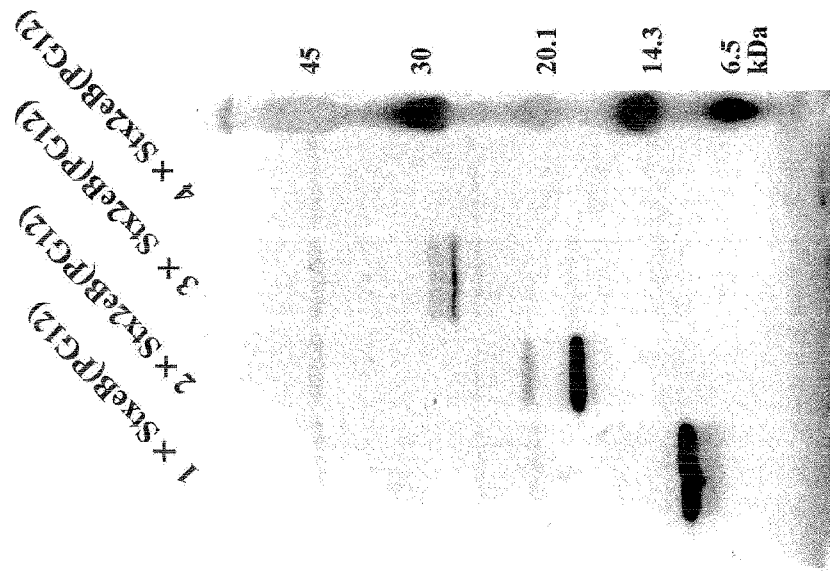


FIG. 2

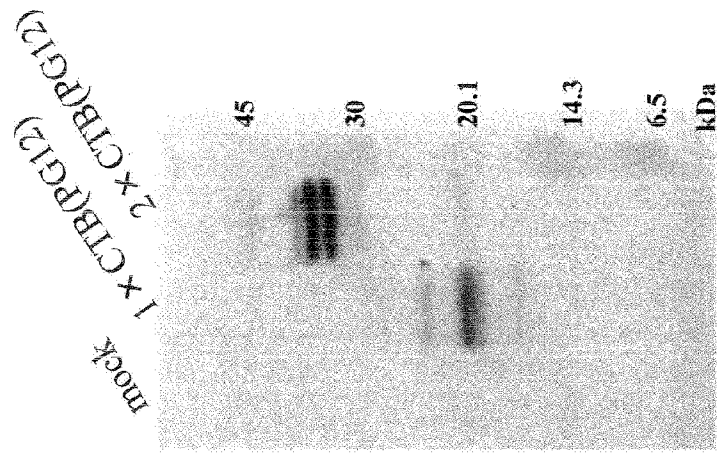


FIG. 3

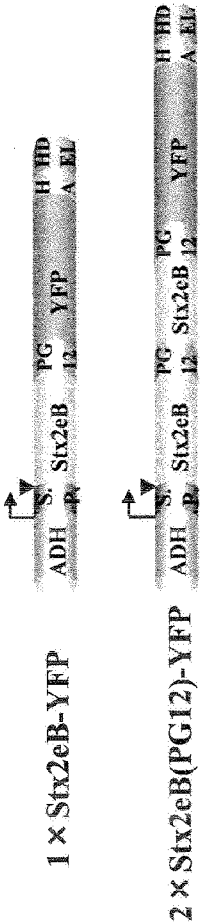
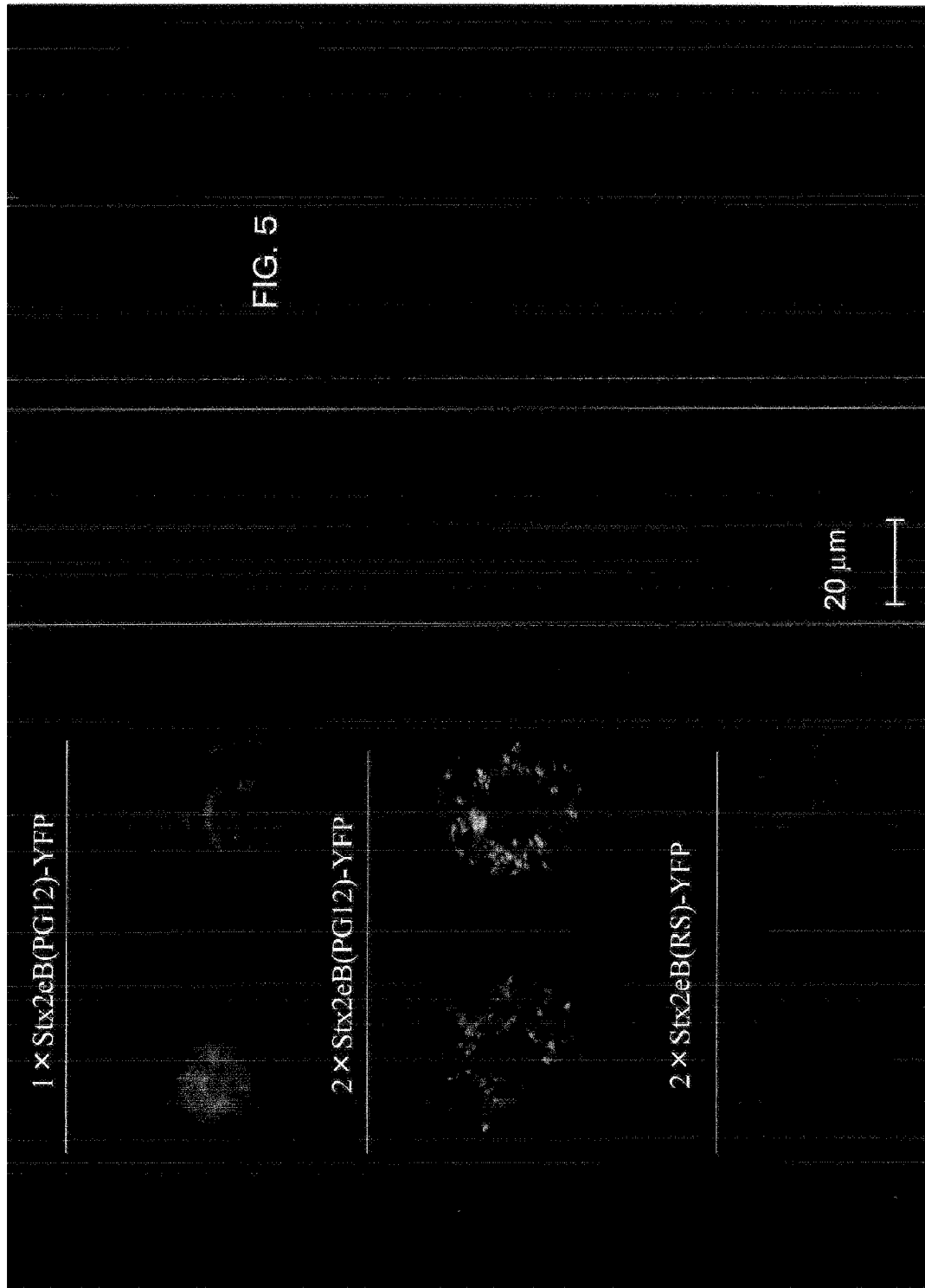
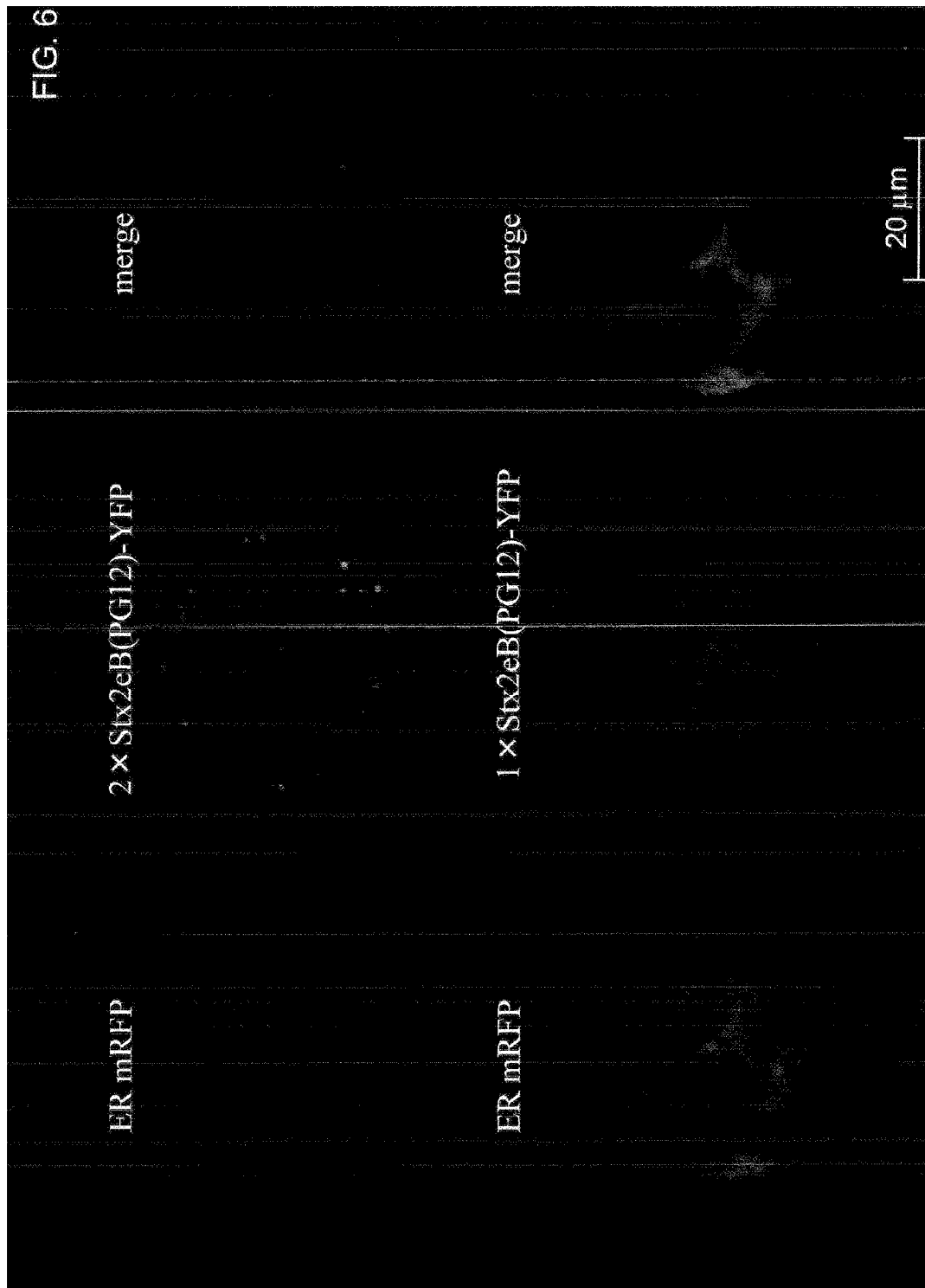


FIG. 4





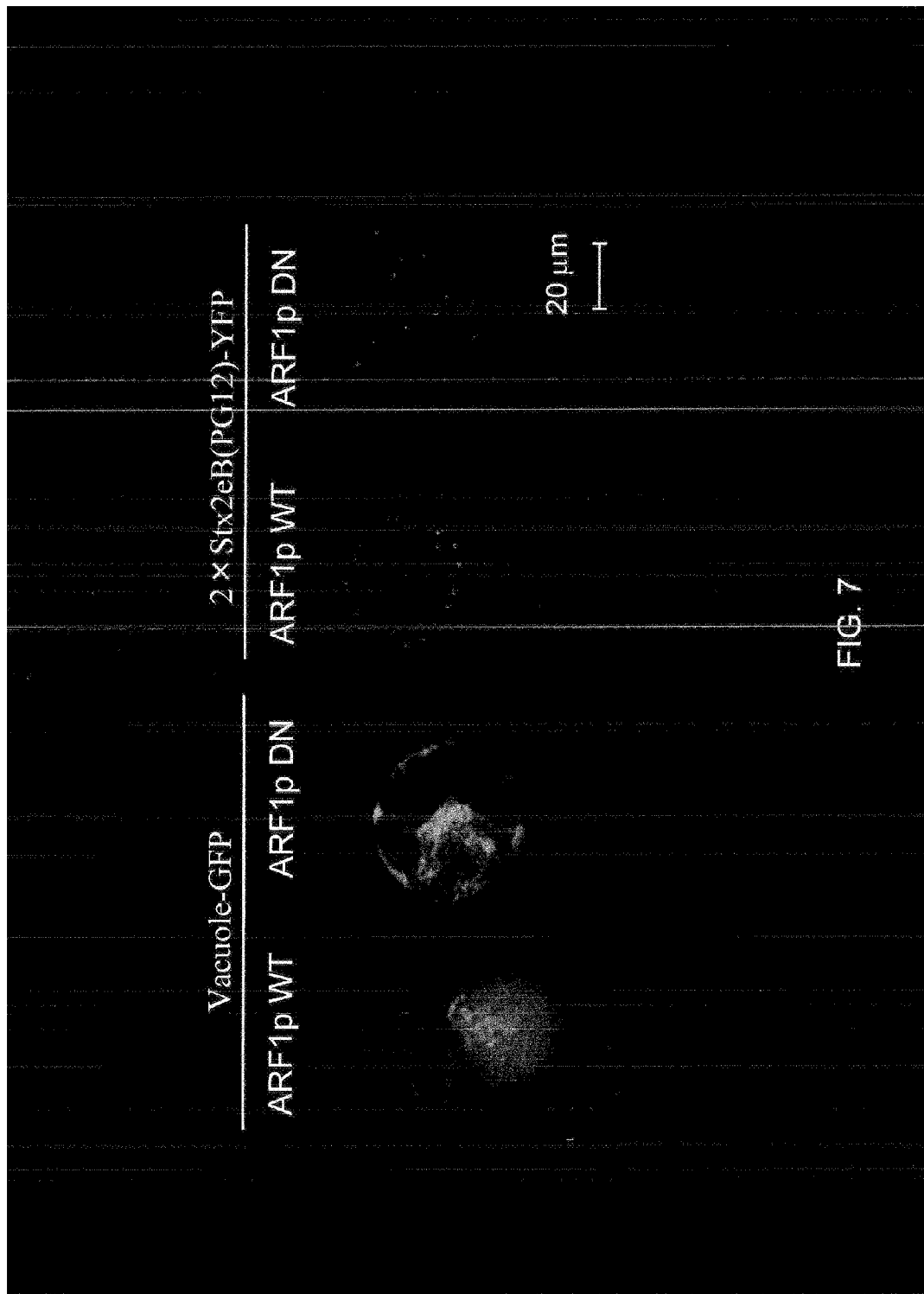


FIG. 8

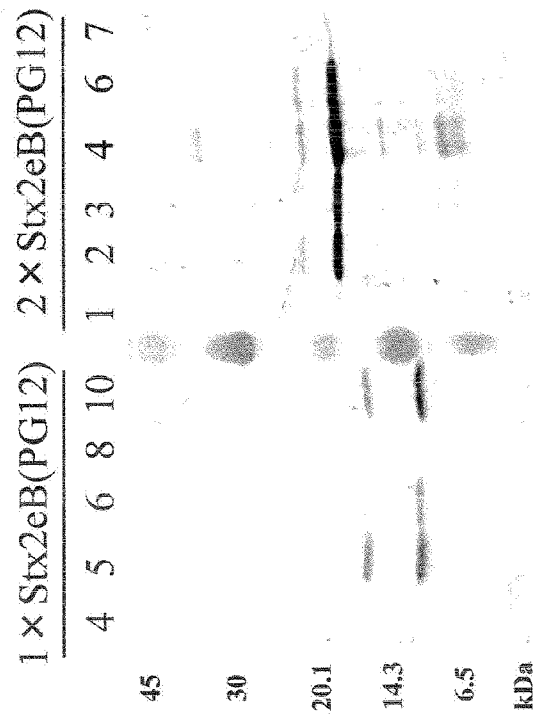
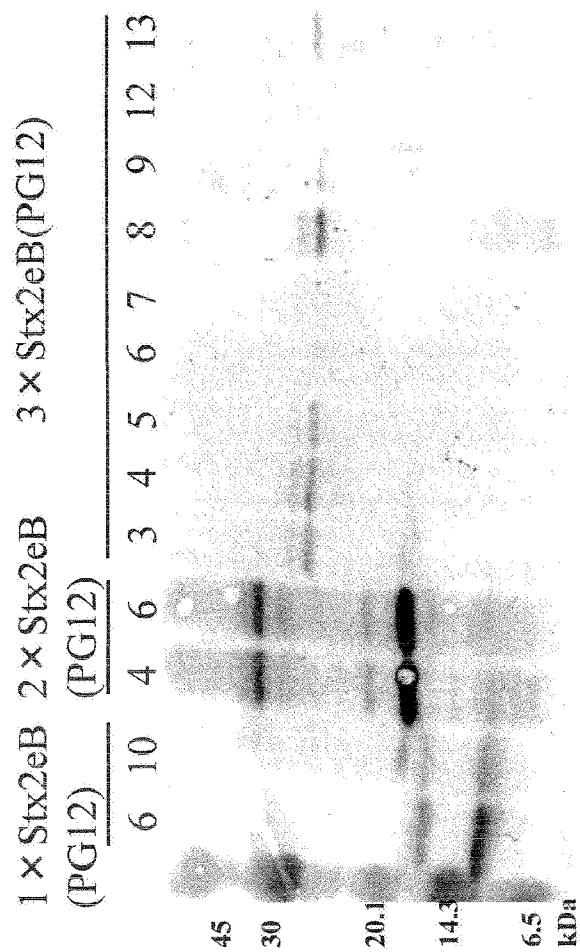


FIG. 9



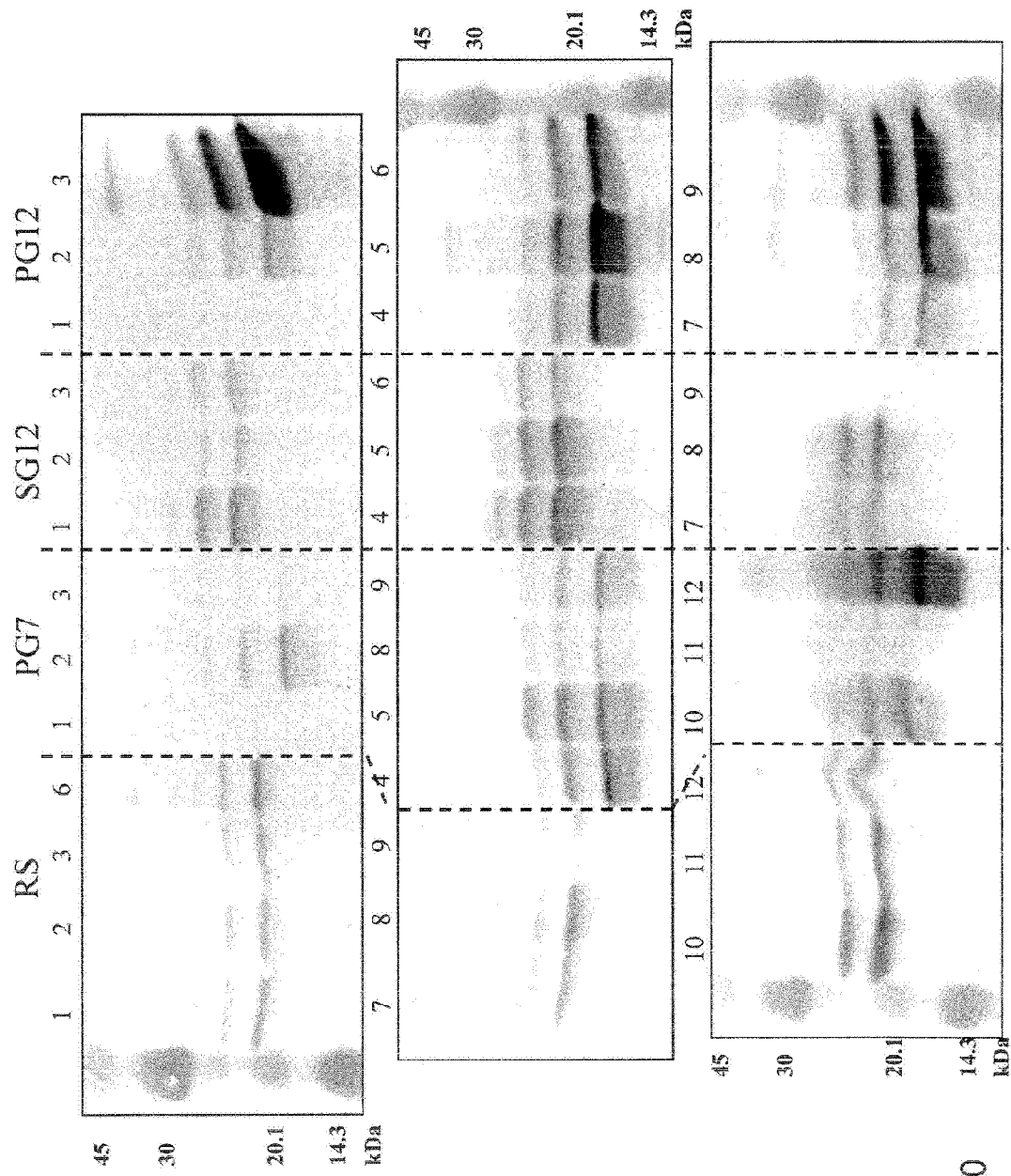


FIG. 10

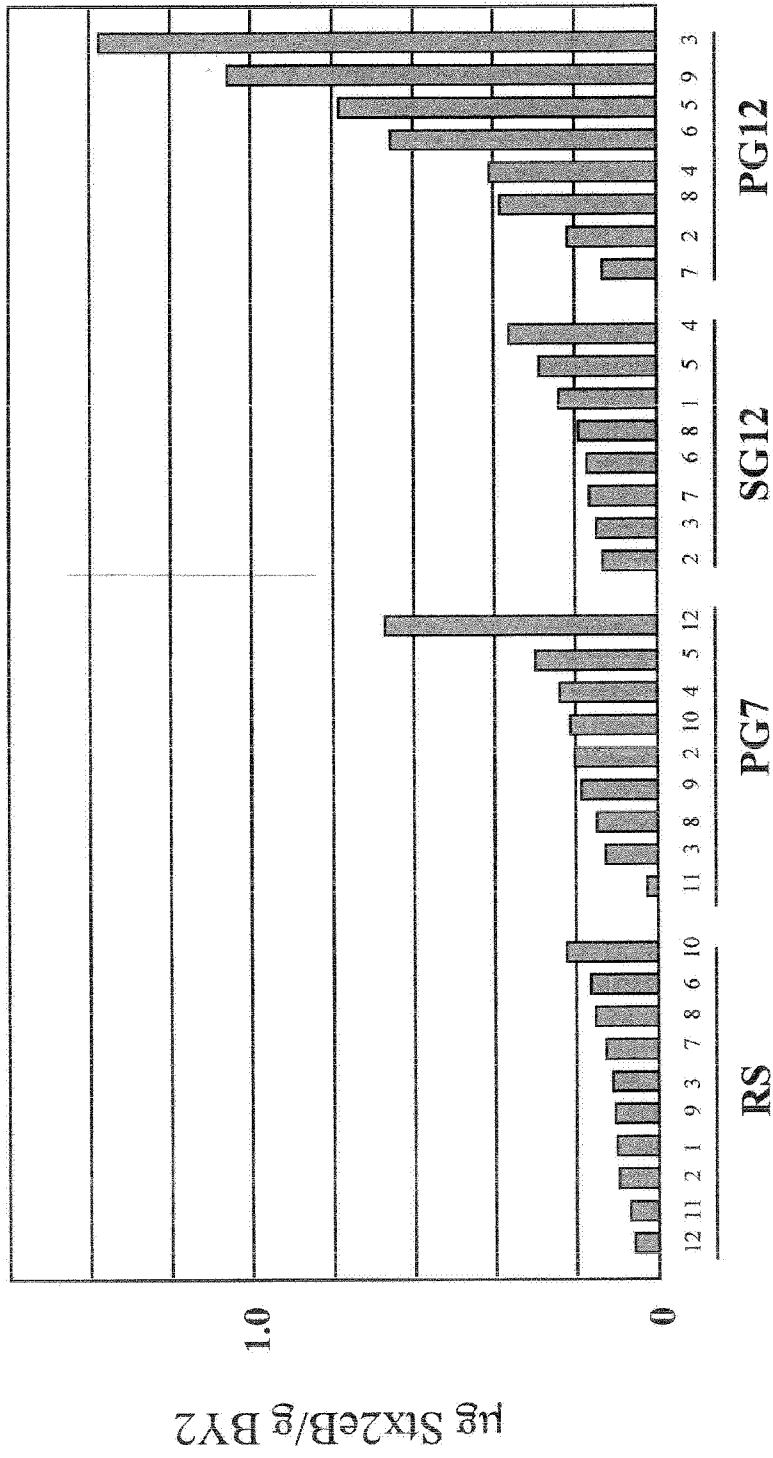


FIG. 11

FIG. 12

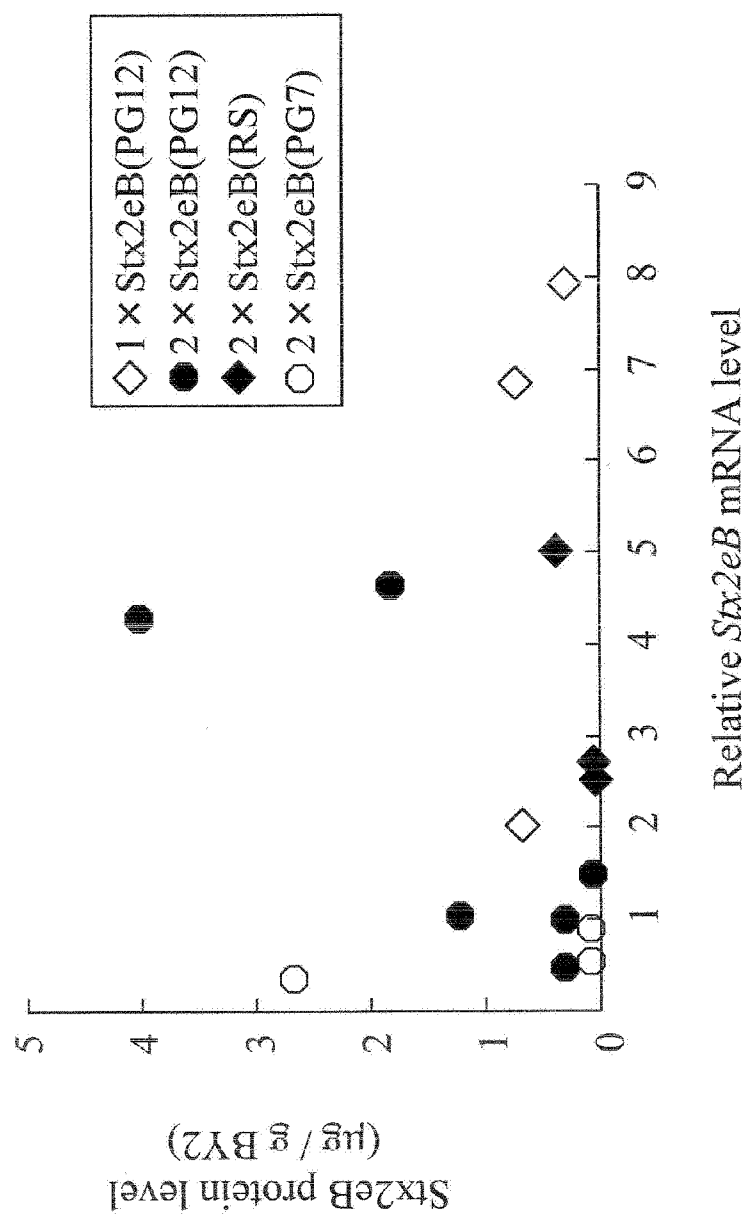


FIG. 13

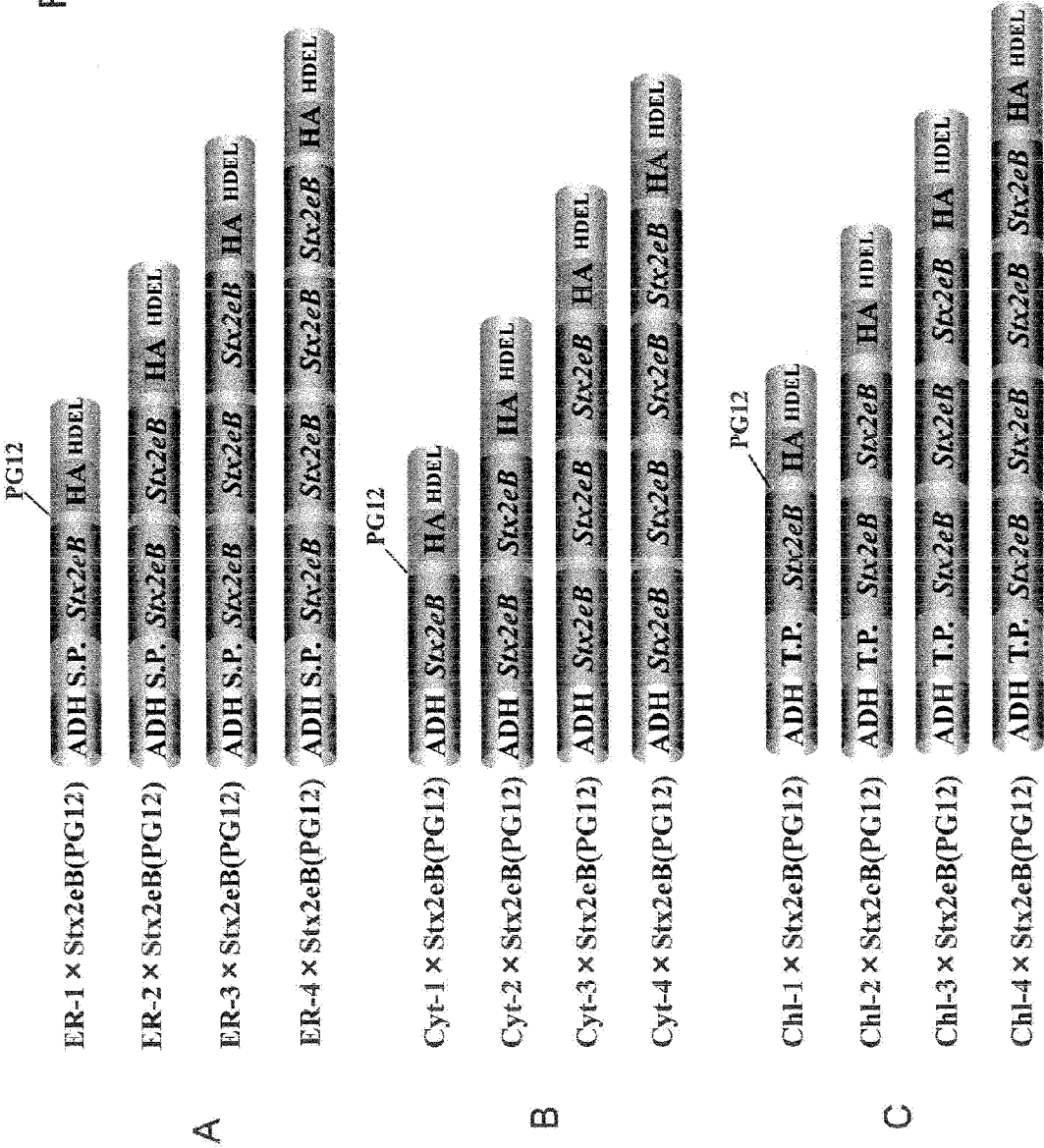


FIG. 14

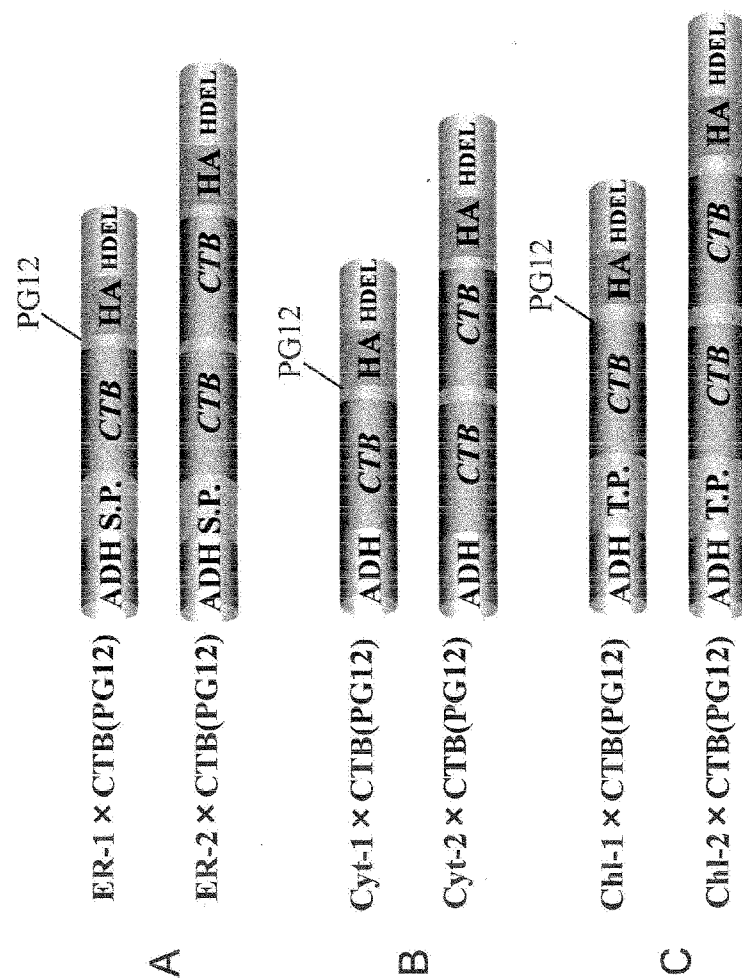


FIG. 15

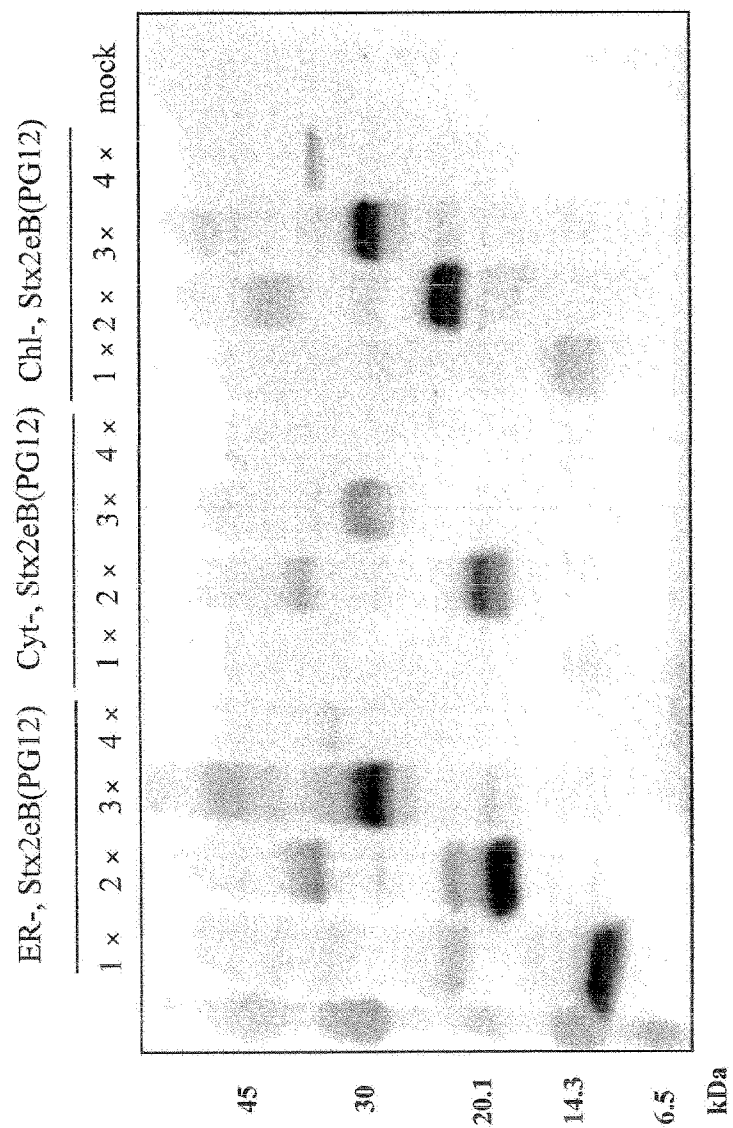


FIG. 16

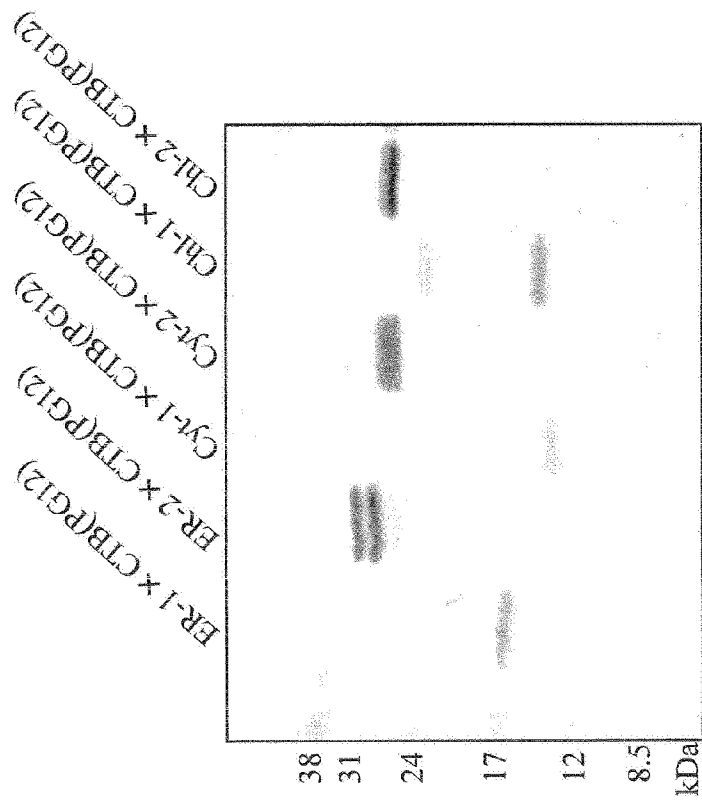
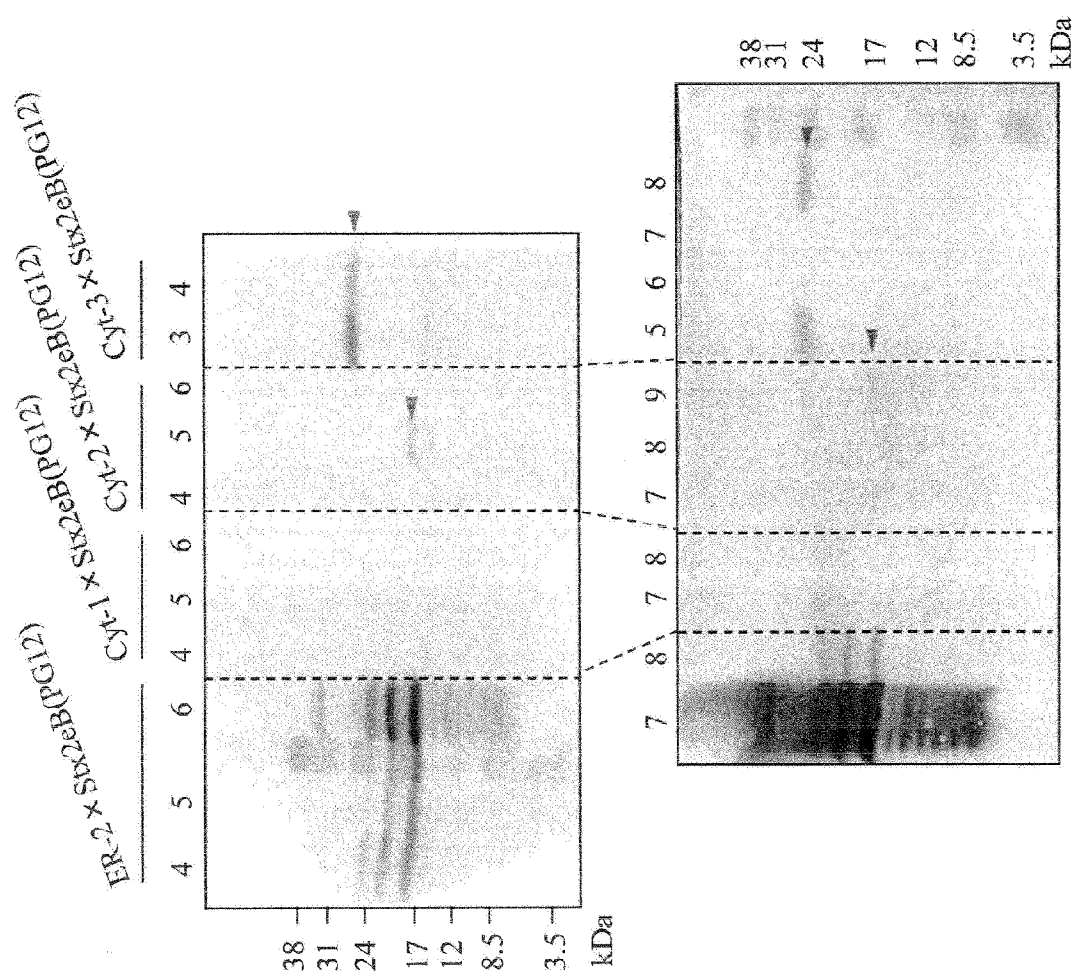


FIG. 17



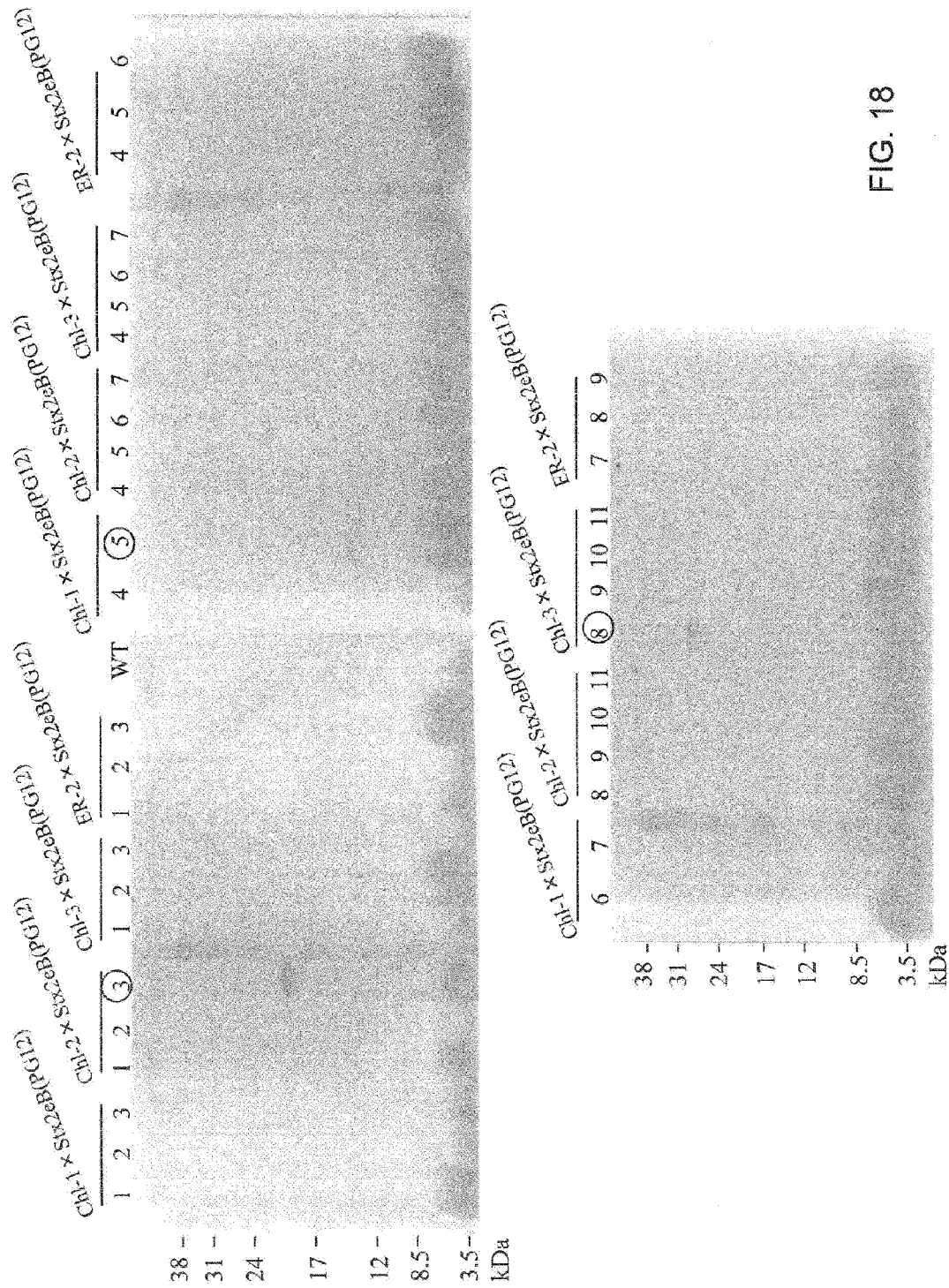


FIG. 18

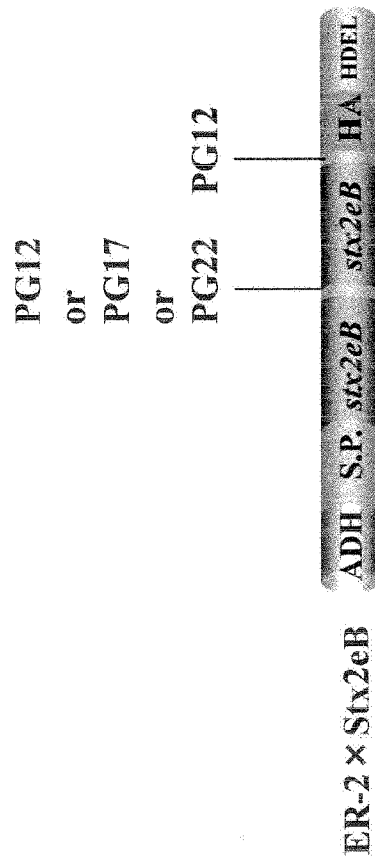


FIG. 20

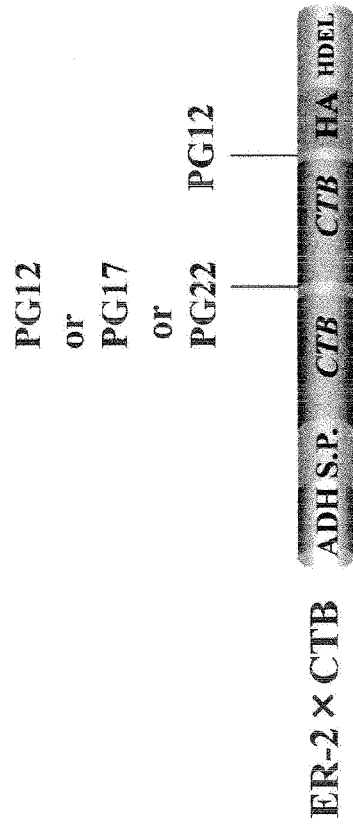


FIG. 21

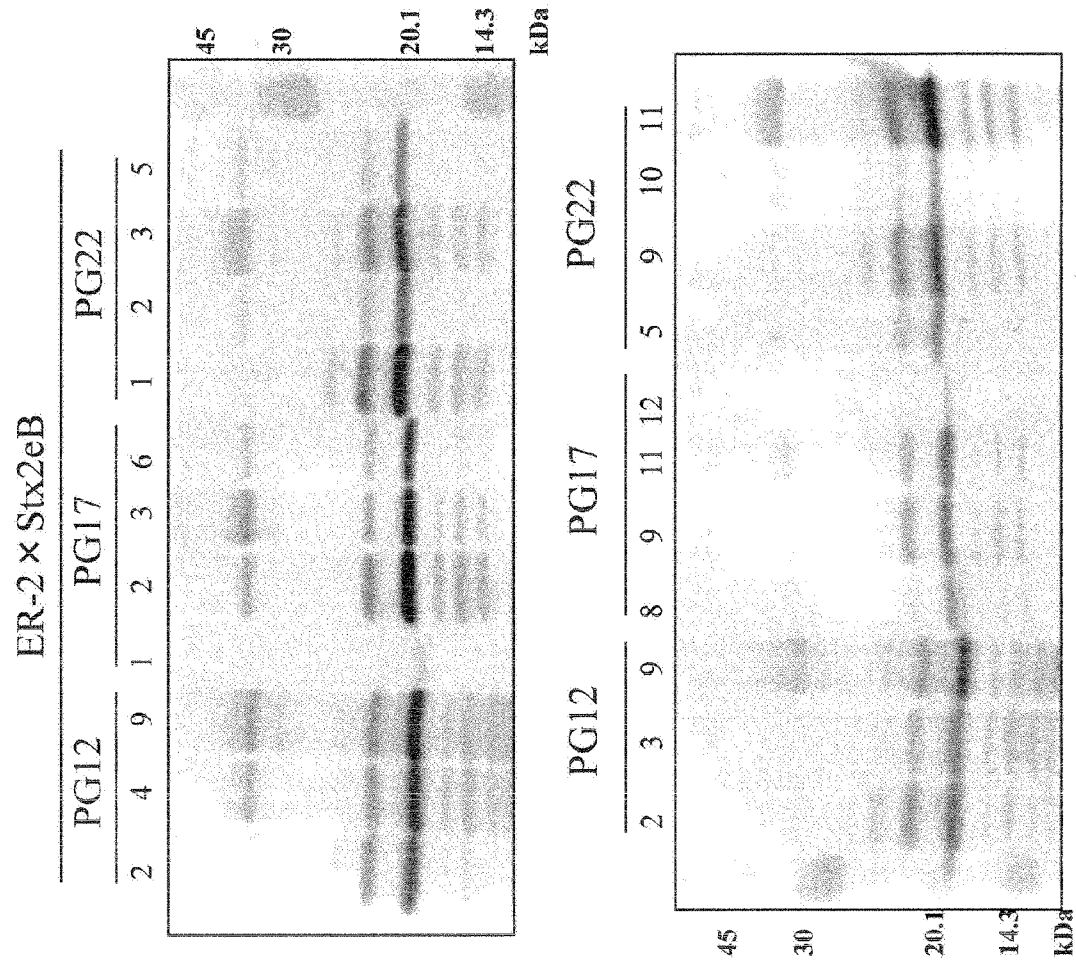


FIG. 22

FIG. 23

