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(71) Demandeur/Applicant:
JAPAN SCIENCE AND TECHNOLOGY AGENCY, JP

(72) Inventeurs/Inventors:
MORI, MASASHI, JP;
DOHI, KOJI, JP

(74) Agent: BLAKE, CASSELS & GRAYDON LLP

(54) Titre : VECTEUR VIRAL ET SON UTILISATION
(54) Title: VIRAL VECTOR AND USE THEREOF

(57) Abrégé/Abstract:
It is intended to provide a polynucleotide containing a viral nucleotide sequence, wherein the viral nucleotide sequence contains a first nucleotide sequence encoding a viral replication protein and a second nucleotide sequence encoding a viral movement protein, the second nucleotide sequence is located downstream of the first nucleotide sequence, it has a linking site for linking an exogenous nucleotide sequence encoding a polypeptide to be expressed downstream of the second nucleotide sequence, and the second nucleotide sequence is obtained by further inserting, substituting or adding a nucleotide sequence in a native sequence derived from a virus. By using this, a vector containing a viral nucleotide sequence is constructed, and a protein is efficiently produced without lowering the growth condition of a host cell containing the vector.

Abstract

It is intended to provide a polynucleotide comprising a viral base sequence, the viral base sequence containing: a first base sequence encoding a viral replication protein, and a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition. By using this, a vector containing a viral base sequence is constructed, and a protein is efficiently produced without worsening growth of a host cell containing the vector.

Description

Title of Invention

VIRAL VECTOR AND USE THEREOF

Technical Field

[0001]

The present invention relates to a technique for producing a protein from a polynucleotide containing a viral base sequence. More specifically, the present invention relates to (i) a polynucleotide containing a modified viral base sequence, (ii) a vector containing the polynucleotide, (iii) a plant or a transformant into which the vector is introduced, and (iv) a protein producing method and a protein producing kit, each of which utilizes the polynucleotide, the vector, the plant, or the transformant.

Background Art

[0002]

Examples of a method for producing a useful protein in a plant includes a method of using a transformed plant in which a foreign gene is introduced into a cell, and a method of infecting a plant cell with a virus vector. The method using a virus vector is advantageous since it provides higher expression efficiency than the method using a transformed plant.

[0003]

Non Patent Document 1 discloses a method for expressing a foreign gene in a plant cell by infecting the plant cell with at least two agrobacteria into which virus vectors are introduced respectively. This method eliminates the need for creating a construct for each of plural genes when combinations of various genes are tested to find a combination for encoding a useful protein. Therefore, this method is useful in analyzing functions or the like of a large number of proteins. Further, a virus vector disclosed in Non Patent Literature 1 can realize high expression speed, can be

constructed at a low cost, and can eliminate steps of a conventional gene recombination process.

[0004]

It is desired that a useful protein produced in a plant can be produced efficiently and in mass scale since it is used not only for food, but also for medical products. In view of this, the inventors of the present invention have constructed a system for producing a protein by using a virus vector (see Patent Literatures 1 through 3).

[0005]

As another system for producing a protein by using a virus vector, Patent Literature 4 discloses a method for increasing a production amount of a useful protein by improving efficiency of producing a transcription product. Patent Literature 4 discloses a method for expressing a target protein by inserting an intron sequence into a replication sequence of a virus vector, and introducing the virus vector thus obtained into a host cell. According to this method, in which an intron region containing a lot of adenosine (A), and thymidine (T) or uracil (U) is removed from the replication sequence of the virus vector or is substituted with an intron derived from a plant cell so that (i) decomposition of the transcription product in the plant cell can be suppressed and (ii) efficiency of producing the transcription product can be improved, it is possible to increase a production amount of a target protein.

[0006]

Meanwhile, each of Non Patent Literatures 2 and 3 discloses a method for improving replication efficiency of a vector which is introduced in a host cell and which contains a base sequence of potyvirus. According to the method disclosed in Non Patent Literatures 2 and 3, an intron sequence is inserted into the base sequence of potyvirus so that a transformed sequence is obtained, and a vector

containing the transformed sequence is introduced into *Escherichia coli*. In the *E. coli* to which the vector is introduced, introduction of intron suppresses expression of a virus protein encoded by the base sequence of potyvirus. This controls toxic influence of the virus on the *E. coli*, and attains better growth of the *E. coli*, thereby improving replication efficiency of the vector in the *E. coli*.

Citation List

Patent Literature 1

Japanese Patent Application Publication, Tokukai, No. 2005-102652 A (Publication Date: April 21, 2005)

Patent Literature 2

Japanese Patent Application Publication, Tokukai, No. 2005-245228 A (Publication Date: September 15, 2005)

Patent Literature 3

Japanese Patent Application Publication, Tokukai, No. 2005-110594 A (Publication Date: April 28, 2005)

Patent Literature 4

WO2005/049839 (Publication Date: February 6, 2005)

Non Patent Literature 1

S. Marillonnet et al., PNAS, 101 (18): 6852-6857 (2004)

Non Patent Literature 2

I. E. Johansen, PNAS. USA, 93: 12400-12405 (1996)

Non Patent Literature 3

S. J. Yang et al., Arch Virol, 143: 2443-2451 (1998)

Summary of Invention

[0007]

Introduction of a vector containing a virus DNA sequence into a host cell such as *E. coli* or agrobacterium worsens growth of the host cell, thereby completely inhibiting the growth of the host cell, or even if the host cell can grow, the host cell grows with a poorer growth rate. This causes a

reduction in yield of the vector, thereby undesirably preventing a target useful protein from being efficiently produced using the vector.

[0008]

According to the conventional protein producing methods described above, it is possible to increase a production amount of a target useful protein by improving a transcriptional activity in a host cell or increasing a production amount of a transcription product. However, these methods do not take into consideration growth of a host cell into which a vector containing a virus base sequence is introduced. As such, the growth of the host cell is inhibited, and this causes a reduction in yield of the vector containing the virus base sequence. This makes it difficult to efficiently carry out genetic recombination using the vector. That is, in a case where the vector is used for protein production, a reduction in yield of the vector causes a reduction in production amount of a useful protein using the vector.

[0009]

The method disclosed in Non Patent Literatures 2 and 3 allows an improvement in growth of a host cell. However, expression of a virus protein is suppressed by inserting an intron into a viral sequence. This necessitates extracting an intron sequence from each molecule of a transcription product. This causes a reduction in growth rate of virus contained in a vector, thereby making it impossible to use the vector in efficiently producing a useful protein.

[0010]

The present invention was attained in view of the above problems, and an object of the present invention is to provide a technique in which growth of a host cell, into which a vector containing a polynucleotide is introduced, is improved by using the polynucleotide containing a viral base sequence so that (i) replication efficiency of the vector in the host cell can

be improved and (ii) efficiency of producing a protein using the vector can be improved.

[0011]

In order to construct a virus vector, which contains a viral base sequence and does not causes deterioration in growth of a host cell, and loss of replication capability of the virus vector, the inventors of the present invention studied conditions required to construct such a virus vector. As a result of the study, the inventors of the present invention found that, in a case where a specific region of a base sequence of a tomato mosaic virus is modified, growth of a host cell, into which a vector containing the base sequence of the tomato mosaic virus is introduced, is not worsened, and that a yield of the vector in the host cell is increased accordingly. Based on this finding, the inventors of the present invention attained the present invention.

[0012]

A polynucleotide of the present invention includes a viral base sequence, the viral base sequence containing: a first base sequence encoding a viral replication protein; and a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, and the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition.

[0013]

The virus preferably belongs to a tobomovirus. Further, the virus is preferably a tobacco mosaic virus or a tomato mosaic virus.

[0014]

It is preferable that the viral replication protein is: (i) polypeptides having amino acid sequences shown in SEQ ID NO: 1 and 2, respectively, or (ii) polypeptides having amino acid sequences which are mutants of the amino acid sequences shown in SEQ ID NO: 1 and 2, respectively, or which are one of the amino acid sequences shown in SEQ ID NO: 1 and 2 and a mutant of the other, wherein mutation of the mutants is deletion, substitution, or addition of one or several amino acids therein.

[0015]

It is preferable that the viral movement protein is: (i) a polypeptide having an amino acid sequence shown in SEQ ID NO: 3, or (ii) polypeptide having an amino acid sequence in which one or several amino acids are deleted, substituted, or added in the amino acid sequence shown in SEQ ID NO: 3.

[0016]

It is preferable that a polynucleotide having the second base sequence is: (i) a polynucleotide having the base sequence shown in any one of SEQ ID NO: 4 through 17, (ii) a polynucleotide having a base sequence in which one or several amino acids are deleted, substituted, or added in the base sequence shown in any one of SEQ ID NO: 4 through 17, (iii) a polynucleotide which hybridizes with a polynucleotide having a base sequence that is complementary to the base sequence shown in any one of SEQ ID NO: 4 through 17 under a stringent condition, and (iv) a polynucleotide having a base sequence which has at least 80% identity with the base sequence shown in any one of SEQ ID NO: 4 through 17.

[0017]

It is preferable that the base sequence with which the second base sequence is modified by the insertion, substitution, or addition has a base length of 100 or more. Further, it is preferable that the second base sequence is

obtained by adding the base sequence at any position from 17th base to 795th base of the base sequence shown in SEQ ID NO: 20.

[0018]

A vector of the present invention contains any one of the polynucleotides.

[0019]

A plant of the present invention contains any one of the polynucleotides.

[0020]

A plant of the present invention contains the vector.

[0021]

A transformant of the present invention contains any one of the polynucleotides.

[0022]

A transformant of the present invention contains the vector.

[0023]

A method of the present invention for producing a polypeptide, includes: transforming or transfecting a plant with the polynucleotide.

[0024]

A method of the present invention for producing a polypeptide, includes: transforming a cell with the polynucleotide.

[0025]

A kit of the present invention for producing a polypeptide, includes the polynucleotide.

[0026]

A method of the present invention for producing a polypeptide, includes: transforming or transfecting a plant with the vector.

[0027]

A method of the present invention for producing a

polypeptide, includes: transforming a cell with the vector.

[0028]

A kit of the present invention for producing a polypeptide, includes the vector.

[0029]

A method of the present invention for producing a polypeptide, includes the step of: using the plant.

[0030]

A method of the present invention for producing a polypeptide, includes the step of: using the transformant.

[0031]

A kit of the present invention for producing a polypeptide, includes the plant.

[0032]

A kit of the present invention for producing a polypeptide, includes the transformant.

[0033]

For a fuller understanding of the nature and advantages of the invention, reference should be made to the ensuing detailed description taken in conjunction with the accompanying drawings.

Brief Description of Drawings

[0034]

Fig. 1

Fig. 1 is a view schematically illustrating a structure of a polynucleotide, according to the present invention, containing a viral base sequence.

Fig. 2A

Fig. 2A is a view schematically illustrating structures of plasmid constructs constructed in an Example of the present invention.

Fig. 2B

Fig. 2B is a view schematically illustrating structures of

plasmid constructs constructed in the Example of the present invention.

Fig. 3

Fig. 3 is a graph showing the diameter of *Escherichia coli* colonies having respective plasmid constructs in an Example of the present invention.

Fig. 4

Fig. 4 is a view schematically illustrating structures of plasmid constructs constructed in an Example of the present invention.

Description of Embodiments

[0035]

Introduction of a vector containing a viral sequence into a host cell causes deterioration in growth of the host cell, and thereby causes a reduction in growth rate of the vector. This causes a reduction in amount of a protein that is produced using the vector and that is encoded by a foreign gene.

[0036]

The inventors of the present invention aimed to construct an efficient protein producing system by constructing a vector which contains a viral sequence and which does not deteriorate growth of a host cell, and studied conditions required to construct such a vector.

[1. Polynucleotide Containing Viral Base Sequence, And Vector Containing The Polynucleotide]

The present invention provides a polynucleotide containing a viral base sequence which contains a first base sequence encoding a viral replication protein and a second base sequence encoding a viral movement protein.

[0037]

The polynucleotide of the present invention containing the viral base sequences is a polynucleotide that is capable of functioning as a virus vector. The term "virus vector" used

herein refers to a polynucleotide which contains a sequence derived from a viral genome and contains a foreign gene expressively, and preferably refers to (i) an RNA containing an RNA sequence derived from a virus, (ii) a DNA containing a cDNA sequence of an RNA derived from a virus, each of which contains a foreign gene expressively, or (ii) an RNA transcribed from this.

[0038]

According to the polynucleotide of the present invention, the second base sequence is located downstream of the first base sequence. The polynucleotide of the present invention contains a part of or all of a native base sequence derived from a virus, and can be used to produce any protein in a cell.

[0039]

The term "viral base sequence" used herein refers to a genome base sequence of a wild-type virus, and preferably refers to a genome RNA of an RNA virus or a cDNA obtained from the genome RNA.

[0040]

The term "viral replication protein" used herein refers to a protein which is derived from a virus and which is involved in replication of a virus, and may be referred to simply as "replication protein". The protein which is involved in replication of a virus refers to a protein which replicates a virus in a cell infected with the virus. Such a protein may be a conventional replication protein, and examples of such a protein include an RNA dependent RNA polymerase (RdRp), an RNA replication enzyme, a tobacco mosaic virus 130K protein, a tobacco mosaic virus 180K protein, a tomato mosaic virus 130K protein, a tomato mosaic virus 180K protein, and the like.

[0041]

A base sequence encoding the viral replication protein is preferably a native base sequence derived from a virus, but

can be a base sequence which is transformed from a native base sequence derived from a virus and which encodes a protein having a replication functional activity. The term "replication functional activity" used herein refers to a functional activity of replicating a virus in a cell infected with the virus.

[0042]

The term "viral movement protein" used herein refers to a protein which is derived from a virus and which is involved in intercellular movement of a virus, and may be referred to simply as "movement protein". The protein which is involved in intercellular movement of a virus refers to a protein which contributes to spread of infection of the virus by causing the virus to move from a cell infected with the virus to a neighboring cell. Such a protein may be a conventionally known movement protein, and examples of such a protein include a tobacco mosaic virus 30K protein, a tomato mosaic virus 30K protein, and the like.

[0043]

A base sequence encoding the viral movement protein is preferably a native base sequence derived from a virus, but can be a base sequence which is transformed from a native base sequence derived from a virus and which encodes a protein having a movement functional activity or a base sequence which is transformed from a native base sequence derived from a virus and which encodes a protein that has lost the movement functional activity due to the transformation. The term "movement functional activity" used herein refers to a functional activity of causing a virus to move from a cell infected with the virus to a neighboring cell.

[0044]

The virus is preferably a virus belonging to a tobamovirus, but is not limited to this. Examples of the virus include a tobacco mosaic virus (TMV), a tobacco mosaic

virus-OM (TMV-OM), a tobacco mosaic virus-Cg (TMV-Cg), a tomato mosaic virus (ToMV), and a Sunn-hemp mosaic virus (SHMV). It should be noted that the virus is not limited to these.

[0045]

The following description deals with the viral replication protein and the viral movement protein by taking a tomato mosaic virus as an example. Note that the tomato mosaic virus is a virus belonging to a tobamovirus.

[0046]

Polypeptides constituting a replication protein of the tomato mosaic virus are provided as the amino acid sequences shown in SEQ ID NOs: 1 and 2, and base sequences of polynucleotide encoding the polypeptides are provided as the base sequences shown in SEQ ID NOs: 18 and 19.

[0047]

In one aspect, the replication protein of the tomato mosaic virus can be (i) polypeptides respectively having the amino acid sequences shown in SEQ ID NO: 1 and 2 or (ii) polypeptides having amino acid sequences which are mutants of the amino acid sequences shown in SEQ ID NO: 1 and 2, or which are one of them and a mutant of the other one of them, each mutant polypeptide having a functional activity of replicating a virus genome.

[0048]

In another aspect, the replication protein of the tomato mosaic virus can be (i) polypeptide encoded by polynucleotides respectively having the base sequences shown in SEQ ID NO: 18 and 19 or (ii) polypeptides encoded by base sequences which are mutants of the base sequences shown in SEQ ID NO: 18 and 19, or which are one of them and a mutant of the other one of them, each mutant polypeptide having a functional activity of replicating a virus genome.

[0049]

That is, the replication protein of the tomato mosaic virus is constituted by two proteins, i.e., a 130K protein (referred to also as a 126K protein) having the amino acid sequence shown in SEQ ID NO: 1, and a 180K protein (referred to also as a 183K protein) having the amino acid sequence shown in SEQ ID NO: 2. The 130K protein is a direct translation product of the genome sequence of the tomato mosaic virus which is shown in SEQ ID NO: 35, and is encoded by the polynucleotide having the base sequence shown SEQ ID NO: 18. The 180K protein is a read-through translation product of the genome sequence of the tomato mosaic virus which is shown in SEQ ID NO: 35, and is encoded by a polynucleotide having the base sequence shown in SEQ ID NO: 19.

[0050]

A polypeptide constituting a movement protein of the tomato mosaic virus is provided as an amino acid sequence shown in SEQ ID NO: 3, and a base sequence constituting a polynucleotide encoding the polypeptide is provided as a base sequence shown in SEQ ID NO: 20.

[0051]

In one aspect, the movement protein of the tomato mosaic virus can be (i) a polypeptide having an amino acid sequence shown in SEQ ID NO: 3, (ii) a polypeptide which is a mutant of the polypeptide having an amino acid sequence shown in SEQ ID NO: 3 and which has a functional activity of causing a virus genome to move between cells, or (iii) a polypeptide which is a mutant of the polypeptide having an amino acid sequence shown in SEQ ID NO: 3 and which has lost the movement functional activity due to the mutation.

[0052]

In another aspect, the movement protein of the tomato mosaic virus can be (i) a polypeptide which is encoded by a

polynucleotide having a base sequence shown in SEQ ID NO: 20, (ii) a polypeptide which is encoded by a mutant of the polynucleotide having a base sequence shown in SEQ ID NO: 20 and which has a functional activity of causing a virus genome to move between cells, or (iii) a polypeptide which is encoded by a mutant of the polynucleotide having a base sequence shown in SEQ ID NO: 20 and which has lost the movement functional activity due to the mutation.

[0053]

As long as it is used in association with a protein or a polypeptide, the term "mutant" used herein refers to a polypeptide which is different in amino acid sequence, but preserves an activity of a wild-type polypeptide. That is, in this specification, a mutant of a polypeptide can be a mutant having an amino acid sequence in which one or several amino acids are deleted, substituted, or added in a specific amino acid sequence.

[0054]

It is known in the art that several amino acids in an amino acid sequence of a polypeptide can be easily modified without causing a significant influence on a structure or a function of the polypeptide. Further, it is also known that mutation occurs not only in an artificially modified protein, but also in a naturally existing protein without causing a significant change in structure and function of the protein. A person skilled in the art can easily modify one or several amino acids in an amino acid sequence of a polypeptide by utilizing a known art.

[0055]

The above description has discussed the viral replication protein and the viral movement protein by taking the tomato mosaic virus as an example. However, a person skilled in the art will readily understand that the virus is not limited to the tomato mosaic virus.

[0056]

Note that the term "protein" is exchangeable with "peptide" or "polypeptide". Further, the term "base sequence" is exchangeable with "nucleic acid sequence" or "nucleotide sequence", and is expressed as a sequence of bases, i.e., adenine (A), guanine (G), cytosine (C), and thymine (T) in deoxyribonucleotide, or adenine (A), guanine (G), cytosine (C), and uracil (U) in ribonucleotide.

[0057]

The polynucleotide of the present invention has a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence to a native sequence derived from a virus by insertion, substitution, or addition. That is, a base sequence is added by insertion, substitution, or addition in the second base sequence which encodes the viral movement protein and which is located downstream of the first base sequence and upstream of the linking site for linking with the exogenous base sequence.

[0058]

According to the polynucleotide of the present invention, the second base sequence is provided as a polynucleotide shown in SEQ ID NO: 4 through 17 or as a mutant of the polynucleotide.

[0059]

As long as it is used in association with gene or polynucleotide, the term "mutant" used herein refers to a polynucleotide encoding a polypeptide which is different in base sequence but which preserves an activity inherent in polypeptide encoded by a wild-type polynucleotide. That is, in this specification, a mutant of a polynucleotide refers to (i) a polynucleotide having a base sequence in which one or

several bases are deleted, substituted, or added in a specific base sequence, (ii) a polynucleotide which hybridizes with a polynucleotide having a specific base sequence or a base sequence that is complementary to the specific base sequence under a stringent condition, or (iii) a polynucleotide having a base sequence which has at least 80% identity with a specific base sequence.

[0060]

The hybridization can be carried out by a known method such as a method described in "Molecular Cloning: A Laboratory Manual 3rd Edition, J. Sambrook and D. W. Russll, Cold Spring Harbor Laboratory, NY (2001)" (the contents of which are hereby incorporated by reference).

[0061]

The term "stringent condition for hybridization" used herein refers to such a condition that (i) incubation is carried out overnight at 42°C in a hybridization solution (50% formamide, 5×SSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 μg/ml of denatured and fragmented salmon sperm DNA); and then (ii) a filter is washed with 0.1 × SSC at approximately 65°C.

[0062]

The polynucleotide of the present invention is such that the linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed is located downstream of the second base sequence which is located downstream of the first base sequence. The exogenous base sequence is linked with the linking site and a cell is transformed using the polynucleotide containing the exogenous base sequence so that a polypeptide encoded by the exogenous base sequence can be expressed in the cell. Note that the exogenous base sequence linked with the polynucleotide of the present invention does not need to be located adjacently to the second

base sequence.

[0063]

The linking site, of the polynucleotide of the present invention, for linking with the exogenous base sequence does not need to exist as a cassette in the base sequence of the polynucleotide of the present invention, provided that the exogenous base sequence can be inserted into or linked with the base sequence of the polynucleotide of the present invention. The polynucleotide of the present invention makes it possible to amplify a gene having the exogenous base sequence linked with the linking site, and thereby makes it possible to produce a product of the gene.

[0064]

According to the polynucleotide of the present invention, the second base sequence is a mutant of a native sequence derived from a virus, wherein the mutation adds a base sequence to the native sequence by insertion, substitution, or addition. The term "native sequence derived from a virus" used herein refers to a sequence indigenous in a wild-type virus. That is, such a native sequence can be a natural sequence which is obtained from a wild-type virus and which is not mutated.

[0065]

The second base sequence of the present invention may be obtained by modifying with a base sequence in a mutant base sequence of a native base sequence derived from a virus which mutant base sequence encodes a polypeptide having the movement functional activity wherein the modification modifies with a base sequence in the native base sequence by insertion, substitution, or addition. Further, the second base sequence of the present invention may be obtained by modifying with a base sequence in a mutant base sequence of a native base sequence derived from a virus which mutant base sequence encodes a polypeptide having no movement

functional activity due to the mutation wherein the modification modifies with a base sequence in the native base sequence by insertion, substation, or addition.

[0066]

In the polynucleotide of the present invention, the base sequence which is included in the second base sequence by insertion, substitution, or addition can be any base sequence having a certain base length, and therefore can have any sequence and can be derived from anything. In this specification, such a base sequence which is added to the second base sequence by insertion, substitution, or addition may be also referred to simply as "insertion sequence". In later described Examples of the present invention, a sequence derived from *Escherichia coli* transposon IS2 and a sequence derived from reverse complement of a GUS gene were used as the insertion sequences. These sequences were successfully used as the insertion sequences in the Examples.

[0067]

In one embodiment, an insertion sequence used in the polynucleotide of the present invention may have 100 bases or more, preferably has 100-1609 bases, and more preferably has 300-1609 bases. The inclusion of such an insertion sequence having not less than 100 base length in the second base sequence of the polynucleotide of the present invention by insertion, substitution, or addition causes a further improvement in growth of a host cell into which the polynucleotide is introduced (see the Example described later). This improves efficiency of replicating a vector in the cell, thereby allowing a further increase in yield of the vector.

[0068]

The insertion sequence can be inserted in any position in a native sequence derived from a virus in order to obtain the second base sequence used in the polynucleotide of the present invention. The insertion sequence is preferably

inserted in a region of the second base sequence which exists between a C-terminal region of the first base sequence and a start codon region of the exogenous base sequence linked with the linking site, and is more preferably inserted between a stop codon of the first base sequence and a subgenome promoter of a base sequence encoding a coat protein. However, the position where the insertion sequence is inserted is not limited to these. Further, the second base sequence can be obtained by adding the insertion sequence to the native sequence derived from a virus or can be obtained by substituting a part of the native sequence derived from a virus with the insertion sequence. Further, the second base sequence can be obtained by deleting a part of the native sequence derived from a virus and inserting the insertion sequence in a section where the part of the native sequence was deleted.

[0069]

That is, the second base sequence of the present invention is a sequence obtained by mutating, as described above, a sequence encoding a protein which preserves a function of causing a viral genome to move between cells.

[0070]

In one embodiment, the insertion sequence can be inserted in any position of the second base sequence used in the polynucleotide of the present invention. The position where the insertion sequence is inserted is not limited to a specific one, but the insertion sequence is preferably inserted in any position from 17th base to 795th base of the base sequence shown in SEQ ID NO: 20, and more preferably inserted in any position from 17th base to 620th base of the base sequence shown in SEQ ID NO: 20.

[0071]

Further, the second base sequence used in the polynucleotide of the present invention may be obtained by adding the

insertion sequence to 5' or 3' terminal of the base sequence shown in SEQ ID NO: 20 or may be obtained by substituting a part of the base sequence shown in SEQ ID NO: 20 with the insertion sequence.

[0072]

The present invention also provides a vector for producing a polypeptide as desired. The vector of the present invention can be such a vector that contains a polynucleotide containing a viral base sequence and that is capable of expressing the polynucleotide in a host cell into which the vector is incorporated, the viral base sequence containing a first base sequence encoding a viral replication protein and a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition.

[0073]

A vector containing a viral base sequence can be easily mutated, and therefore construction of such a vector is very difficult, or impossible in some cases depending on the type of a foreign gene to be expressed. However, the use of the polynucleotide of the present invention made it possible to construct such a vector that could not be constructed before. It can be estimated from this that a vector constructed using the polynucleotide of the present invention is a stable vector in which occurrence of mutation is suppressed.

[0074]

Further, the vector of the present invention allows an improvement in growth of the host cell into which the vector

is introduced, thereby improving replication efficiency in the host cell. Because of this, a useful protein, which is encoded by a foreign gene, can be efficiently produced by using a replicated vector.

[0075]

The vector containing the polynucleotide of the present invention may be, for example, an expression vector (e.g. phage vector or plasmid vector), which can express the polynucleotide, such as a pBR type or a pUC type. A vector which can express the polynucleotide in a host cell into which the vector of the present invention is introduced can be appropriately selected as such a vector. Further, a vector which has a property of being incorporated into a genome of a plant cell can be a vector such as a pBI type or a pCAMBIA type, and can be a Ti plasmid vector, for example.

[0076]

How to construct the polynucleotide of the present invention and the vector of the present invention is not limited particularly, and they may be constructed by a known genetic engineering method.

[0077]

The vector constructed using the polynucleotide of the present invention can be suitably used in production of a protein encoded by a foreign gene. That is, transformation of a host cell by using a vector containing the polynucleotide of the present invention can efficiently replicate the vector without worsening growth of the host cell, thereby making it possible to efficiently produce, by using the replicated vector, the protein encoded by the foreign gene.

[0078]

The foreign gene linked with the linking site contained in the polynucleotide of the present invention or the vector of the present invention is not limited to a specific one, and therefore can be a GFP gene, a human gamma interferon gene,

an alpha interferon gene, a calmodulin gene, a myosin phosphatase inhibitor protein (CPI-17) functional domain gene (amino acid residue: 22-120), or a single chain antibody gene, for example. The use of the polynucleotide of the present invention or the vector of the present invention allows easy preparation of a vector carrying such a gene, efficient replication of such a vector and efficient production of a protein from the gene by using the replicated vector.

[0079]

[2. Plant Containing Viral Base Sequence]

The present invention also provides a plant containing a viral base sequence. The term "plant" used herein refers to a plant cell or a plant individual, and examples of the plant include plants such as Arabidopsis, tobacco, or benthamiana, and plant cells such as a tobacco BY2 cell or an Arabidopsis mm2d cell.

[0080]

The plant of the present invention contains a first base sequence encoding a viral replication protein and a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition. With this, the polypeptide can be efficiently expressed.

[0081]

In one embodiment, the plant of the present invention is obtained by introducing, into an organism, a polynucleotide containing a viral base sequence or a vector containing the polynucleotide, the viral base sequence containing a first

base sequence encoding a viral replication protein and a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition.

[0082]

In one aspect, the plant of the present invention may be obtained by transforming a plant or a plant cell using the polynucleotide of the present invention or a vector containing the polynucleotide of the present invention. The plant of the present invention can be obtained, for example, by introducing the polynucleotide of the present invention into a plant cell by a method such as electroporation.

[0083]

In another aspect, the plant of the present invention can be obtained by transfecting a plant or a plant cell with the polynucleotide of the present invention or a vector containing the polynucleotide of the present invention. The plant of the present invention can be obtained, for example, by infecting a plant or a plant cell with the polynucleotide of the present invention. Further, the plant of the present invention can also be obtained by transfecting a plant cell with a plasmid into which cDNA obtained by adding a promoter to the polynucleotide of the present invention has been introduced and transcribing the cDNA in the cell. Further, the plant of the present invention can also be obtained by transfecting a plant cell with cDNA of the polynucleotide of the present invention and transcribing the cDNA in the cell.

[0084]

Further, the plant of the present invention can also be obtained by infecting a plant cell with agrobacterium into which a plasmid vector containing the polynucleotide of the present invention is introduced, for example. Further, the plant of the present invention can also be obtained by agroinfiltration utilizing agrobacterium. Specifically, the polynucleotide of the present invention is locally introduced into a plant body by infiltrating a culture solution, in which agrobacterium containing the polynucleotide of the present invention is incubated, into intercellular space of the plant body.

[0085]

That is, the plant of the present invention may be a transformed plant which has been transformed using the polynucleotide of the present invention or the vector of the present invention, or can be an infected plant which is infected with the polynucleotide of the present invention or the vector of the present invention. In a case where the plant of the present invention is a transformed plant, it can be a transient transformant in which the polynucleotide of the present invention which is introduced into a plant does not integrate with the genome of the plant and is transiently expressed, or can be a stable transformant in which the polynucleotide of the present invention which is introduced in a plant integrates with the genome of the plant and is stably and continuously expressed. Further, in the transformed plant, polynucleotide of the present invention which is introduced into the plant may be constantly expressed or may be inducibly expressed using steroid hormone or the like. In a case where the plant of the present invention is an infected plant, the plant may be entirely infected with the polynucleotide of the present invention or may be locally infected with the polynucleotide of the present invention.

[0086]

Since the plant of the present invention contains the polynucleotide of the present invention, the use of the plant of the present invention allows efficient production of a protein encoded by a foreign gene which is incorporated in the polynucleotide of the present invention or the vector of the present invention.

[0087]

[3. Transformant Containing Viral Base Sequence]

The present invention also provides a transformant containing a viral base sequence. The term "transformant" includes not only cell, tissue, and organ, but also individual organism, but the transformant is preferably a cell (especially prokaryotic cell, fungus, or the like). A transformant of the present invention can be *Escherichia coli*, agrobacterium, or yeast, for example.

[0088]

The transformant of the present invention contains a polynucleotide containing a first base sequence encoding a viral replication protein and a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition. As such, the transformant of the present invention can be used in efficient expression of the polypeptide.

[0089]

In one embodiment, the transformant of the present invention is obtained by introducing, into an organism, a polynucleotide containing a viral base sequence or a vector

containing the polynucleotide, the viral base sequence containing a first base sequence encoding a viral replication protein and a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, the linking site being located downstream of the second base sequence, the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition.

[0090]

In one aspect, the transformant of the present invention can be obtained by transforming an organism using the polynucleotide of the present invention or the vector containing the polynucleotide of the present invention. For example, the transformant of the present invention can be obtained by introducing a plasmid, into which the polynucleotide of the present invention is incorporated, into *Escherichia coli* by a method such as a calcium chlorite method.

[0091]

The use of the transformant of the present invention allows an increase in yield of the vector of the present invention which is introduced into a host cell, for example. That is, the use of the transformant of the present invention makes it possible to easily and efficiently produce a vector for producing a protein encoded by a foreign gene incorporated into the vector. Further, since growth of the transformant can be improved, the use of the transformant allows efficient production of a protein encoded by a foreign gene incorporated into the vector of the present invention.

[0092]

[4. Method and Kit for Producing Polypeptide in a Cell as

desired]

The present invention also provides (i) a method for producing a polypeptide using the polynucleotide, the vector, the plant, or the transformant, and (ii) a kit for producing a polypeptide, the kit including the polynucleotide, the vector, the plant, or the transformant.

[0093]

A method of the present invention for producing a polypeptide uses the polynucleotide, the vector, the plant, or the transformant. A kit of the present invention for producing a polypeptide includes the polynucleotide, the vector, the plant, or the transformant. Note that the term "kit" used herein means that at least one of the components is contained in another material (e.g. container).

[0094]

The present invention provides a method and a kit for efficiently producing any polypeptide. The use of the method of the present invention for producing any polypeptide in a cell does not cause deterioration in growth of the cell even if a viral base sequence is introduced into the cell, thereby allowing efficient production of the polypeptide.

[0095]

In one embodiment, the method of the present invention for producing a polypeptide uses the polynucleotide of the present invention or the vector containing the polynucleotide, the method including the step of transforming or transfecting aliving specimen with the polynucleotide of the present invention or the vector of the present invention, wherein the living specimen may or may not be a plant body or a plant cell. A polypeptide encoded by an exogenous base sequence contained in the polynucleotide of the present invention or the vector of the present invention is expressed in the organism thus transformed or transfected in the step.

[0096]

In another embodiment, the method of the present invention for producing a polypeptide uses the plant of the present invention or the transformant of the present invention, the method including the step of growing or incubating the plant of the present invention or the transformant of the present invention under a condition that a polypeptide can be expressed. A polypeptide encoded by an exogenous base sequence contained in the plant or the transformant is expressed in the plant or the transformant in the step.

[0097]

As described above, the use of the method of the present invention for producing a polypeptide does not cause deterioration in growth of an organism in which a predetermined polypeptide is produced, thereby making it possible to efficiently produce the polypeptide.

[0098]

A method of the present invention for introducing a polypeptide or a vector into a host is not limited to a specific one, and a conventionally known method such as an agrobacterium method, electroporation, a calcium phosphate method, a liposome method, or a DEAE dextran method can be suitably used as such a method. Further, an organism which is transformed or transfected with the vector of the present invention is not limited to a specific one, and therefore can be a cell derived from an animal or a cell derived from a plant. Further, a microorganism such as *Bacillus subtilis*, *Escherichia coli*, fungus, or yeast can be used as the host.

[0099]

A method of the present invention for introducing the polynucleotide of the present invention or the vector of the present invention into a plant body or a cell derived from a

plant is not limited to a specific one, and a method such as the agrobacterium method, the agroinfiltration, a polyethylene glycol method, the electroporation, or a particle gun method can be suitably used as such a method.

[0100]

The kit of the present invention for producing a polypeptide includes the polynucleotide of the present invention, the vector of the present invention, the plant of the present invention, or the transformant of the present invention. In a preferable embodiment, the kit of the present invention for producing a polypeptide, including the polynucleotide of the present invention or the vector of the present invention preferably further includes a plant body or an organism to be transformed or transfected. With this arrangement, a cell is transformed or transfected using the polynucleotide of the present invention or the vector of the present invention so that a polynucleotide encoded by an exogenous base sequence contained in the polynucleotide of the present invention or the vector of the present invention can be expressed in the plant body or the organism into which the cell has been introduced.

[0101]

Note that the method and the kit for producing any polypeptide in a cell is not limited to those explained above, and a person skilled in the art who read this specification can easily understand other aspects of the method and the kit for producing a polypeptide.

[0102]

The following description deals with more detailed explanation of the present invention with reference to the Examples, but the present invention is not limited to these Examples, but may be altered by a skilled person within the scope of the claims and the embodiment. An embodiment based on a proper combination of technical means disclosed

in different embodiments is encompassed in the technical scope of the present invention.

Examples

[0103]

[Example 1: Construction of Plasmid for Producing GFP]

cDNAs of a tomato mosaic virus were synthesized by inserting various base sequences (SEQ ID NO: 21 through 34) into a base sequence (SEQ ID NO: 20) of a gene encoding a movement protein. The base sequence is located between a base sequence of a gene encoding a tomato mosaic virus replication protein and a base sequence of a gene encoding a target foreign protein. The cDNAs thus synthesized were used to construct plasmid constructs, respectively (see Fig. 1).

[0104]

Figs. 2 A and 2B show positions of respective insertion base sequences with which the base sequence of the gene encoding the movement protein was modified by insertion, substitution, or addition. In Figs. 2A and 2B, A through F indicate respective positions where the respective insertion base sequences were inserted, and numbers below the alphabets indicate respective positions of the respective insertion base sequences in a native sequence of the tomato mosaic virus. Note that a construct indicated by No. 16 (piLrcG11erG3SRz) was obtained by substituting a base sequence between B and D with the base sequence shown in SEQ ID NO: 33, and a construct indicated by No. 17 (piLrcG12erG3SRz) was obtained by substituting a base sequence between B and D with the base sequence shown in SEQ ID NO: 34.

[0105]

In this Example, an insertion sequence added into the constructs indicated by No. 4 and No. 5 (piLIS2erG3SRz and piLIS2(-SpeI)erG3SRz) in Table 1 was a base sequence derived

from *Escherichia coli* transposon IS2.Meanwhile, an insertion sequence added into each of the other constructs was a sequence derived from reverse complement of a GUS gene. Further, in this Example, a gene encoding a GFP protein was used as a gene encoding a target foreign protein.

[0106]

The plasmid constructs constructed as above were used to transform *Escherichia coli*. One of colonies obtained from the transformed *E. coli* was inoculated into a 3ml LB culture medium containing antibiotics for selection, and then was incubated at 37°C for 20 hours with shaking. A plasmid was purified from a 1.5ml incubation solution by an alkali SDS method. The plasmid thus purified was quantified using a DNA assay kit (Quant-it dsDNA Assay Kit (invitrogen)).

[0107]

Yields of the plasmid constructs obtained in a cell was compared with a yield of a plasmid construct into which no insertion sequence was inserted, and obtained relative values are shown in Tables 1 and 2.

[0108]

Table 1

	Plasmid Name	Inserted Position	SEQ ID NO	Number of Inserted Bases (bp)	Yield of Plasmid (relative value)	SE
1	piLerG3SRz	-	-	-	1.0	0.1
2	piLerG3(SF3)SRz	-	-	-	0.6	0.0
3	piLΔMPerG3SRz	-	-	-	0.6	0.0
4	piLIS2erG3SRz	C	21	1336	17.2	0.6
5	piLIS2(-SpeI)erG3SRz	C	22	1258	16.3	0.6
6	piLrcG1erG3SRz	A	23	1333	14.1	0.3
7	piLrcG2erG3SRz	B	24	1333	13.1	0.4
8	piLrcG3erG3SRz	C	25	1338	13.4	0.4
9	piLrcG8erG3SRz	D	26	1333	12.3	0.3
10	piLrcG9erG3SRz	E	27	1333	12.5	0.4
11	piLrcG10erG3SRz	F	28	1333	14.4	0.2
14	piLrcG6erG3SRz	C	31	100	2.2	0.1
15	piLrcG7.5erG3SRz	C	32	1604	13.6	0.7
16	piLrcG11erG3SRz	B/D	33	480	9.4	0.5
17	piLrcG12erG3SRz	B/D	34	1333	14.0	0.5

[0109]

Table 2

	Plasmid Name	Inserted Position	SEQ ID NO	Number of Inserted Bases (bp)	Yield of Plasmid (relative value)	SE
1	piLerG3SRz	-	-	-	1.0	0.1
12	piLrcG4erG3SRz	C	29	600	5.0	0.1
13	piLrcG5erG3SRz	C	30	300	4.3	0.3

[0110]

As shown in Tables 1 and 2, yields of the plasmid constructs (indicated by No. 4 through No. 17, respectively) into which the base sequences respectively shown in SED ID

NO: 21 through 34 were inserted increased by 2.2 to 17.2 times compared with the plasmid construct (indicated by No. 1) containing a native base sequence into which no insertion sequence was inserted. Note that a plasmid construct in which a gene encoding the movement protein was frameshifted (plasmid construct indicated by No. 2), and a plasmid construct in which a gene encoding the movement protein was deleted (plasmid construct indicated by No. 3) did not increase in yield.

[0111]

[Example 2: Improvement in Growth Condition of Host Microorganism Cell]

The plasmid constructs constructed in the Example 1 were used to transform *Escherichia coli* JM109 (TOYOBO). The *Escherichia coli* JM109 thus transformed was placed on an LB agar medium containing 100μg/ml carbenicillin and was incubated at 37°C for 18 hours. Five colonies whose growth was not affected by other colonies were randomly selected from obtained colonies, and each of the five colonies was measured in major axis.

[0112]

A colony having a plasmid construct containing an insertion sequence and a colony having a plasmid construct containing no insertion sequence among the plasmid constructs constructed in the Example 1 were compared in major axis. Table 1 and Fig. 3 show obtained relative values of the major axis.

[0113]

Table 3

	Plasmid Name	Major Axis (relative value)
1	piLerG3SRz	1.0
3	piLΔMPerG3SRz	0.91
4	piLIS2erG3SRz	1.84
8	piLrcG3erG3SRz	1.70
12	piLrcG4erG3SRz	1.53
13	piLrcG5erG3SRz	1.46
14	piLrcG6erG3SRz	1.18
15	piLrcG7.5erG3SRz	1.89
16	piLrcG11erG3SRz	1.14

[0114]

As shown in Table 3, the major axis of an *Escherichia coli* colony having a plasmid construct containing an insertion sequence (plasmid construct indicated by 4, 8, 12, 13, 14, 15, or 16) was 1.14 to 1.89 times larger than the major axis of an *Escherichia coli* colony having a plasmid construct containing no insertion sequence (plasmid construct indicated by 1 or 3). This demonstrates that a growth condition of Escherichia coli into which a plasmid construct containing a viral base sequence was introduced was improved (see Fig. 3).

[0115]

[Example 3: Construction of Plasmid for Production of Foreign Protein]

In the Example 3, plasmid constructs were constructed by using a human gamma interferon (hIFNγ) gene as a gene encoding a foreign protein.

[0116]

The hIFNγ gene was amplified by the PCR method by using an AatII recognition site at the 5'-terminal side and a

BstEII site at the 3'-terminal side of a GFP gene of each of the plasmid constructs constructed in the Example 1 (No. 1 (piLerG3SRz), No. 3 (piLΔMPerG3SRz), No. 4 (piLIS2erG3SRz), No. 6 (piLrcG1erG3SRz), and No. 8 (piLrcG3erG3SRz) (see Table 1)). The hIFNγ gene was then accurately substituted, so that plasmid constructs (No. 1' (piLhIFNγSRz), No. 3' (piLΔMPhIFNγSRz), No. 4' (piLIS2hIFNγSRz), No. 6' (piLrG1hIFNγSRz), and No. 8' (piLrG3hIFNγSRz)) were constructed.

[0117]

Yields of the plasmid constructs in respective cells was quantitatively analyzed in the same manner as in the Example 1. The result demonstrated that a yield of a plasmid construct into which an insertion base sequence was inserted (No. 4', 6', or 8') was much larger than that of a plasmid construct in which no insertion base sequence was inserted (No. 1' or 3').

[0118]

It was also possible to easily construct a plasmid construct, into which a cDNA of a virus genome RNA mutated as shown in No. 4 of Fig. 2A was introduced, the virus genome RNA being mutated by using, as a gene encoding a foreign protein, an alpha interferon gene, a myosin phosphatase inhibitor protein (CPI-17) functional domain gene (amino acid residue: 22-120), a single chain antibody gene, or a calmodulin gene in a similar manner to the above Example. The plasmid construct was obtained in good yield with good stability.

[0119]

[Example 4: Construction of Binary Plasmid]

Further, each of the plasmid constructs constructed as above was cleaved with SpeI and AvrII, and was linked with a SpeI recognition site of pBICER8-ToMV5'-Spe (Dohi et al, 2006, Archives of Virlogy, 151: 1075-1084) in order to

introduce a base sequence of a virus containing the hIFNγ gene into a binary plasmid that was to be used for inducing expression of the viral sequence therein. Although a binary plasmid into which a gene fragment derived from the plasmid construct indicated by 1' or 3' was inserted could not be obtained, a binary plasmid into which a gene fragment derived from the plasmid construct indicated by 4', 6', or 3' was inserted could be easily obtained. This revealed that a plasmid construct which contains a foreign gene and whose construction is difficult can be constructed by inserting, substituting or adding an insertion sequence in a base sequence encoding a viral movement protein.

[0120]

It was also possible to easily construct a binary plasmid, into which a cDNA of a virus genome RNA mutated as shown in No. 4 of Fig. 2A was introduced, the virus genome RNA being mutated by using, as a gene encoding a foreign protein, an alpha interferon gene, a CPI-17 protein functional domain gene, a single chain antibody gene, or a calmodulin gene in a similar manner to the above Example. The binary plasmid was obtained in good yield with good stability.

[0121]

[Example 5: Expression of Protein in Protoplast]

As shown in No. 4 through No. 15 of Figs. 2A and 2B, an insertion sequence was inserted, substituted, or added in a virus genome RNA that was synthesized in a test tube with the use of T7RNA polymerase. Thus, a mutant of the virus genome RNA was created. The virus genome RNA thus created was inoculated into a protoplast, which was prepared from a tobacco BY2 cell, by electroporation (as for an experimental method, see Watanabe et al, FEBS Letters, 219:65-69). A transformant of the protoplast thus obtained was incubated at 26°C for 24 hours, and then was sampled.

[0122]

In a protoplast which contains a virus genome RNA into which a GFP gene was introduced as a foreign gene, proliferation of the virus genome RNA was confirmed by northern blotting. In protoplasts which respectively contain virus genome RNAs shown in No. 4 through No. 15, respectively, proliferation of the virus genome RNAs was confirmed. Further, proliferation of a sub genome GFP messenger RNA was confirmed in each of protoplasts respectively containing virus genome RNAs having respective insertion sequences shown in No. 4 through No. 9 and No. 12 through No. 15, respectively. Meanwhile, accumulation of the sub genome GFP messenger RNA could not be detected in each of protoplasts respectively containing virus genome RNAs shown in No. 10 and No. 11, respectively.

[0123]

In the protoplasts, expression of a GFP gene was confirmed with the use of a fluorescent microscope. Note that expression of a GFP gene was confirmed in each of the protoplasts respectively containing the virus genome RNAs shown in No. 4 through No. 9 and No. 12 through No. 15, but expression of a GFP gene was not confirmed in each of the protoplasts respectively containing the virus genome RNAs shown in No. 10 and No. 11.

[0124]

It can be estimated that the reason why the sub genome GFP messenger RNA was not accumulated in each of the protoplasts respectively containing the virus genome RNAs having insertion sequences shown in No. 10 and No. 11 lies in that a viral sub genome RNA promoter region was modified due to insertion or addition of the insertion sequences. This follows that the GFP gene can be expressed also in these virus genome RNAs by further adding a native sub genome RNA promoter sequence.

[0125]

Further, in a protoplast which contains a virus genome RNA into which the hIFNγ gene was introduced as a foreign gene, proliferation capability of the virus genome RNA was confirmed by northern blotting, and expression of the hIFNγ gene was confirmed by western blotting. In protoplasts which respectively contain the virus genome RNAs having insertion sequences shown in No. 6, No. 7, and No. 12 through No. 14, proliferation of the virus genome RNAs and proliferation of a sub genome hIFNγ messenger RNA was confirmed, and expression of the hIFNγ gene was confirmed since a hIFNγ protein was detected.

[0126]

Similarly, proliferation of a genome RNA and a sub genome messenger RNA was confirmed in a protoplast containing a mutant of a virus genome into which a cDNA of a virus genome RNA mutated as shown in No. 4 of Fig. 2A was introduced, the virus genome RNA being mutated by using, as a gene encoding a foreign protein, an alpha interferon gene, a CPI-17 protein functional domain gene, a single chain antibody gene, or a calmodulin gene in a similar manner to the above Example.

[0127]

[Example 6: Expression of Protein in Tobacco BY2 Cell]

A cDNA of a virus genome RNA into which a GFP gene or a hIFNγ gene was introduced as a foreign gene (see No. 4 of Fig. 2A) was used to transform a tobacco BY2 cell (Dohi et al., Archives of Virology, 151, 1075-1084) in which a transcription factor XVE that was activated by estrogen was expressed with the use of the agrobacterium method. Estrogen was added to a culture medium containing the tobacco BY2 cell thus transformed, and three days later, a sample was taken (as for an experimental method, see Dohi et al., Archives of Virology, 151, 1075-1084).

[0128]

In a transformed tobacco BY2 cell containing the GFP gene, proliferation of a virus genome RNA and a sub genome GFP messenger RNA was confirmed (northern blotting), and expression of the GFP gene was confirmed (fluorescence microscope observation and SDS-PAGE).

[0129]

Also in a transformed tobacco BY2 cell containing the hIFNγ gene, proliferation of a virus genome RNA and a sub genome hIFNγ messenger RNA was confirmed (northern blotting), and accumulation of a hIFNγ protein was confirmed (western blotting).

[0130]

Further, proliferation of a virus genome RNA and a sub genome messenger RNA was confirmed, and accumulation of a protein was confirmed in a transformed tobacco BY2 cell into which a cDNA of a virus genome RNA mutated as shown in No. 4 of Fig. 2A was introduced, the virus genome RNA being mutated by using, as a gene encoding a foreign protein, a CPI-17 protein functional domain gene or a single chain antibody gene in a similar manner to the above Example.

[0131]

[Example 7: Study on Base Length of Insertion Sequence]

cDNAs of a modified tomato mosaic virus were synthesized by inserting base sequences having base length of 300 base pairs, 100 base pairs, 50 base pairs, and 20 base pairs (SEQ ID NO: 36 through 39) at a position of 5166 bases from the 5' terminal of a base sequence (SEQ ID NO: 20) of a gene encoding a movement protein of a tomato mosaic virus. A cDNA of a modified tomato mosaic virus encoded by a plasmid vector piL.erG3SRz(Avr) was substituted with the cDNAs thus synthesized so that plasmid constructs were constructed (see Fig. 4). In Fig. 4, a plasmid construct into which no insertion

sequence was inserted is indicated by piL.erG3SRz(Avr), and plasmid constructs into which insertion sequences of 300 base length, 100 base length, 50 base length, and 20 base length were inserted are indicated by piL.erG3(C0.3)SRz(Avr), piL.erG3(C0.1)SRz(Avr), piL.erG3(C0.05)SRz(Avr), piL.erG3(C0.02)SRz(Avr), respectively.

[0132]

The plasmid constructs thus constructed were used to transform *Escherichia coli* JM109 (TOYOBO). The *Escherichia coli* JM109 thus transformed was placed on an LB agar medium containing 100μg/ml carbenicillin and was incubated at 37°C for 26 hours. Five colonies whose growth was not affected by other colonies were randomly selected from obtained colonies, and the diameter of each of the five colonies was measured.

[0133]

The diameter of a colony of *Escherichia coli* having a plasmid containing an insertion sequence was compared with the diameter of a colony of *Escherichia coli* having a plasmid containing no insertion sequence. Table 4 shows obtained relative values and standard errors (n=5). In Table 4, "*" indicates that the t-test revealed that there is a significant difference in colony diameter between a plasmid containing an insertion sequence and piL.erG3SRz(Avr) containing no insertion sequence.

[0134]

Table 4

Plasmid Name	Number of Inserted Bases (bp)	Colony Diameter (relative value±S.E.)	Yield of Plasmid (relative value±S.E.)
piL.erG3SRz(Avr)	-	1.00±0.05	1.00±0.06
piL.erG3(C0.3)SRz(Avr)	300	1.59±0.05*	3.87±0.36*
piL.erG3(C0.1)SRz(Avr)	100	1.17±0.03*	1.53±0.10*
piL.erG3(C0.05)SRz(Avr)	50	1.10±0.02	1.27±0.08
piL.erG3(C0.02)SRz(Avr)	20	1.10±0.04	1.33±0.15

[0135]

As shown in Table 4, the colony diameter of *Escherichia coli* having piL.erG3(C0.3)SRz(Avr) into which an insertion sequence of 300 base length was inserted or piL.erG3(C0.1)SRz(Avr) into which an insertion sequence of 100 base length was inserted is significantly larger than that of *Escherichia coli* having piL.erG3SRz(Avr) into which no insertion sequence was inserted. That is, an improvement could be observed in growth of *Escherichia coli* having piL.erG3(C0.3)SRz(Avr) and piL.erG3(C0.1)SRz(Avr). Meanwhile, the colony diameter of *Escherichia coli* having piL.erG3(C0.05)SRz(Avr) into which an insertion sequence of 50 base length was inserted or piL.erG3(C0.02)SRz(Avr) into which an insertion sequence of 10 base length was inserted is larger than that of *Escherichia coli* having piL.erG3SRz(Avr) into which no insertion sequence was inserted, but the difference was not significant.

[0136]

Further, the plasmid constructs were used to transform *Escherichia coli* JM109. One of colonies obtained from the transformed *E. coli* was inoculated into a 3ml LB culture medium containing antibiotics for selection, and then was

incubated at 37°C for 24 hours with shaking. A plasmid was purified from a 1.5ml incubation solution by an alkali SDS method. The plasmid thus purified was quantified using a DNA assay kit (Quant-it dsDNA Assay Kit (invitrogen)). A yield of each of the plasmid constructs into which an insertion sequence was inserted was compared to that of a plasmid construct into which no insertion sequence was inserted. Table 4 shows obtained relative values and standard errors (n=3).

[0137]

As shown in Table 4, a yield of *Escherichia coli* having piL.erG3(C0.3)SRz(Avr) into which an insertion sequence of 300 base length was inserted or piL.erG3(C0.1)SRz(Avr) into which an insertion sequence of 100 base length was inserted was significantly larger than that of *Escherichia coli* having piL.erG3SRz(Avr) into which no insertion sequence was inserted. This means that these plasmids showed good stability. Meanwhile, a yield of *Escherichia coli* having piL.erG3(C0.05)SRz(Avr) into which an insertion sequence of 50 base length was inserted or piL.erG3(C0.02)SRz(Avr) into which an insertion sequence of 10 base length was inserted was larger than that of *Escherichia coli* having piL.erG3SRz(Avr) into which no insertion sequence was inserted, but the difference was not significant.

[0138]

The use of the present invention allows an improvement in growth of a host cell into which a vector containing a polynucleotide containing a viral base sequence is introduced, thereby improving efficiency of replicating the vector in the cell. As a result, it becomes possible to efficiently produce a useful protein with the use of a vector that is efficiently replicated.

[0139]

The embodiments and concrete examples of

implementation discussed in the foregoing detailed explanation serve solely to illustrate the technical details of the present invention, which should not be narrowly interpreted within the limits of such embodiments and concrete examples, but rather may be applied in many variations within the spirit of the present invention, provided such variations do not exceed the scope of the patent claims set forth below.

Industrial Applicability

[0140]

The use of the present invention makes it possible to efficiently produce any protein, and a protein produced with the use of the present invention can be effectively applied to various fields such as plant biotechnology industry, pharmaceutical industry, and food industry.

Claims

Claim 1

A polynucleotide comprising a viral base sequence,

the viral base sequence containing:

a first base sequence encoding a viral replication protein; and

a second base sequence encoding a viral movement protein, the second base sequence being located downstream of the first base sequence and having a linking site for linking with an exogenous base sequence encoding a polypeptide to be expressed, and the linking site being located downstream of the second base sequence,

the second base sequence being obtained by modifying with a base sequence in a native sequence derived from a virus by insertion, substitution, or addition.

Claim 2

The polynucleotide according to claim 1, wherein

the base sequence with which the second base sequence is modified by the insertion, substitution, or addition has a base length of 100 or more.

Claim 3

The polynucleotide according to claim 1 or 2, wherein

the second base sequence is obtained by inserting the base sequence at any position from 17th base to 795th base of the base sequence shown in SEQ ID NO: 20.

Claim 4

The polynucleotide according to any one of claims 1 through 3, wherein

the viral replication protein is:

(i) polypeptides having amino acid sequences shown in SEQ ID NO: 1 and 2, respectively, or

(ii) polypeptides having amino acid sequences which are mutants of the amino acid sequences shown in SEQ ID NO: 1 and 2, respectively, or which are one of the amino acid sequences shown in SEQ ID NO: 1 and 2 and a mutant of the other, wherein mutation of the mutants is deletion, substitution, or addition of one or several amino acids therein.

Claim 5

The polynucleotide according to any one of claims 1 through 4, wherein

the viral movement protein is:

(i) a polypeptide having an amino acid sequence shown in SEQ ID NO: 3, or

(ii) polypeptide having an amino acid sequence in which one or several amino acids are deleted , substituted, or added in the amino acid sequence shown in SEQ ID NO: 3.

Claim 6

The polynucleotide according to any one of claims 1 through 5, wherein

a polynucleotide having the second base sequence is:

(i) a polynucleotide having the base sequence shown in any one of SEQ ID NO: 4 through 17,

(ii) a polynucleotide having a base sequence in which one or several amino acids are deleted, substituted, or added in the base sequence shown in any one of SEQ ID NO: 4 through 17,

(iii) a polynucleotide which hybridizes with a polynucleotide having a base sequence that is complementary to the base sequence shown in any one of SEQ ID NO: 4 through 17 under a stringent condition, and

(iv) a polynucleotide having a base sequence which has at least 80% identity with the base sequence shown in any one

of SEQ ID NO: 4 through 17.

Claim 7

The polynucleotide according to any one of claims 1 through 6, wherein

the virus belongs to a tobamovirus.

Claim 8

The polynucleotide according to any one of claims 1 through 7, wherein

the virus is a tobacco mosaic virus or a tomato mosaic virus.

Claim 9

A vector comprising a polynucleotide recited in any one of claims 1 through 8.

Claim 10

A plant comprising a polynucleotide recited in any one of claims 1 through 8.

Claim 11

A plant comprising a vector recited in claim 9.

Claim 12

A transformant comprising a polynucleotide recited in any one of claims 1 through 8.

Claim 13

A transformant comprising a vector recited in claim 9.

Claim 14

A method for producing a polypeptide, comprising:

transforming or transfecting a plant with a

polynucleotide recited in any one of claims 1 through 8.

Claim 15

A method for producing a polypeptide, comprising the step of:

transforming a cell with a polynucleotide recited in any one of claims 1 through 8.

Claim 16

A kit for producing a polypeptide, comprising a polynucleotide recited in any one of claims 1 through 8.

Claim 17

A method for producing a polypeptide, comprising the step of:

transforming or transfecting a plant with a vector recited in claim 9.

Claim 18

A method for producing a polypeptide, comprising the step of:

transforming a cell with a vector recited in claim 9.

Claim 19

A kit for producing a polypeptide, comprising a vector recited in claim 9.

Claim 20

A method for producing a polypeptide, comprising the step of:

using a plant recited in claim 10 or claim 11.

Claim 21

A method for producing a polypeptide, comprising the

step of:

using a transformant recited in claim 12 or claim 13.

Claim 22

A kit for producing a polypeptide, comprising a plant recited in claim 10 or 11.

Claim 23

A kit for producing a polypeptide, comprising a transformant recited in claim 12 or 13.

FIG. 1

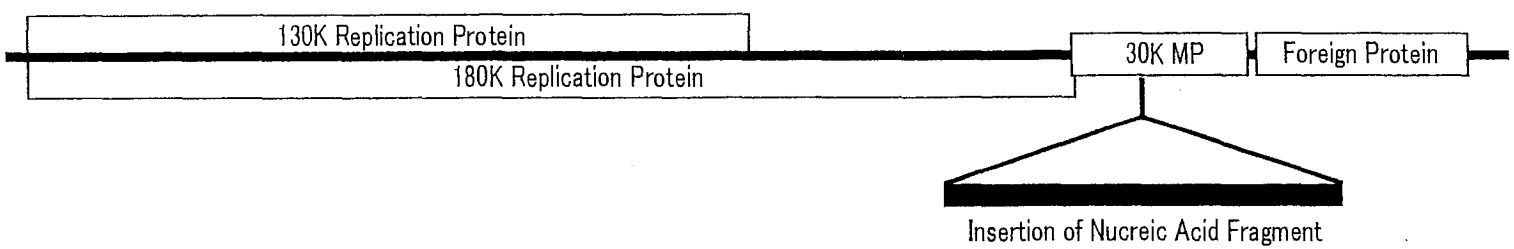

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FIG. 2A

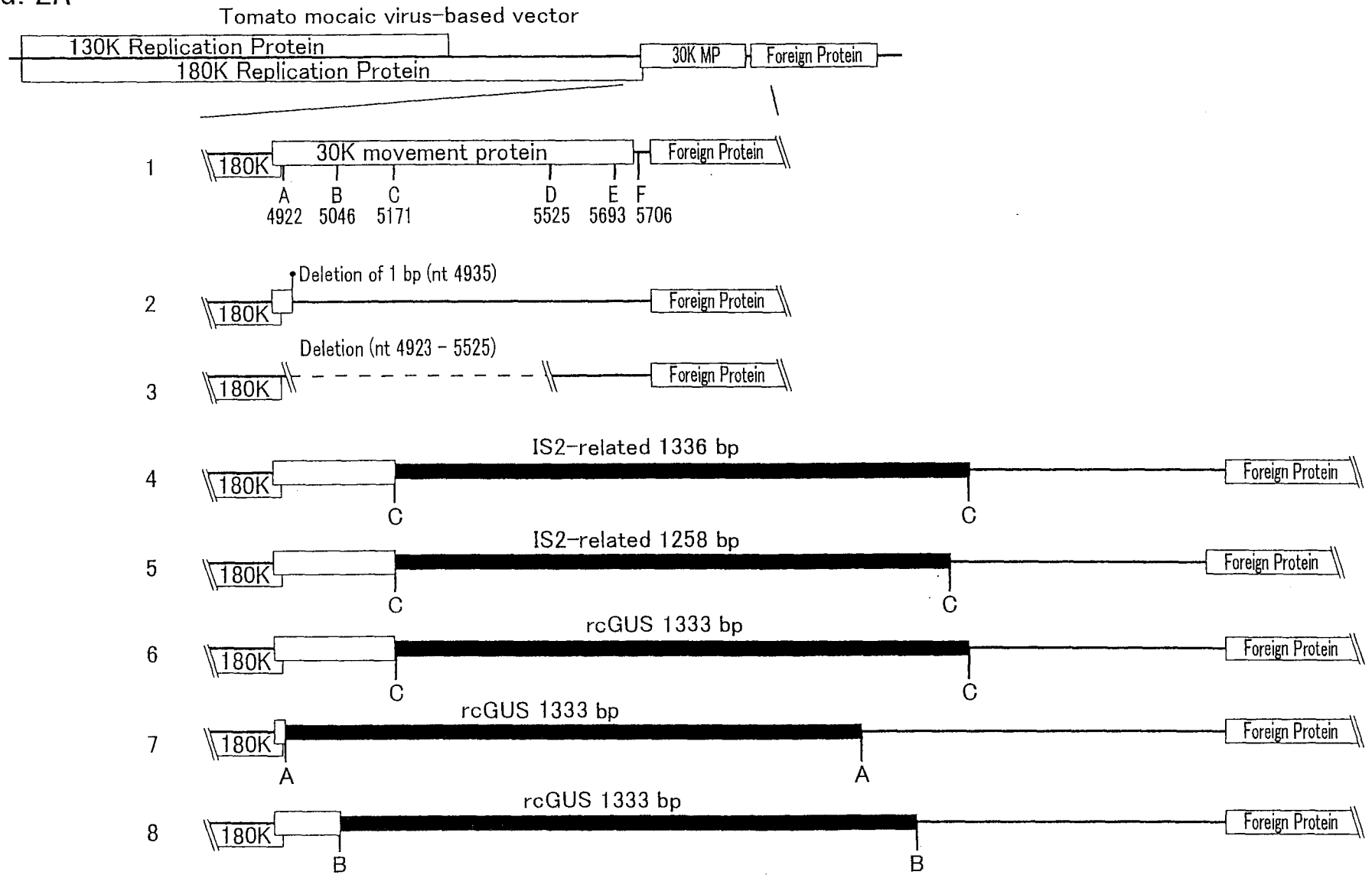
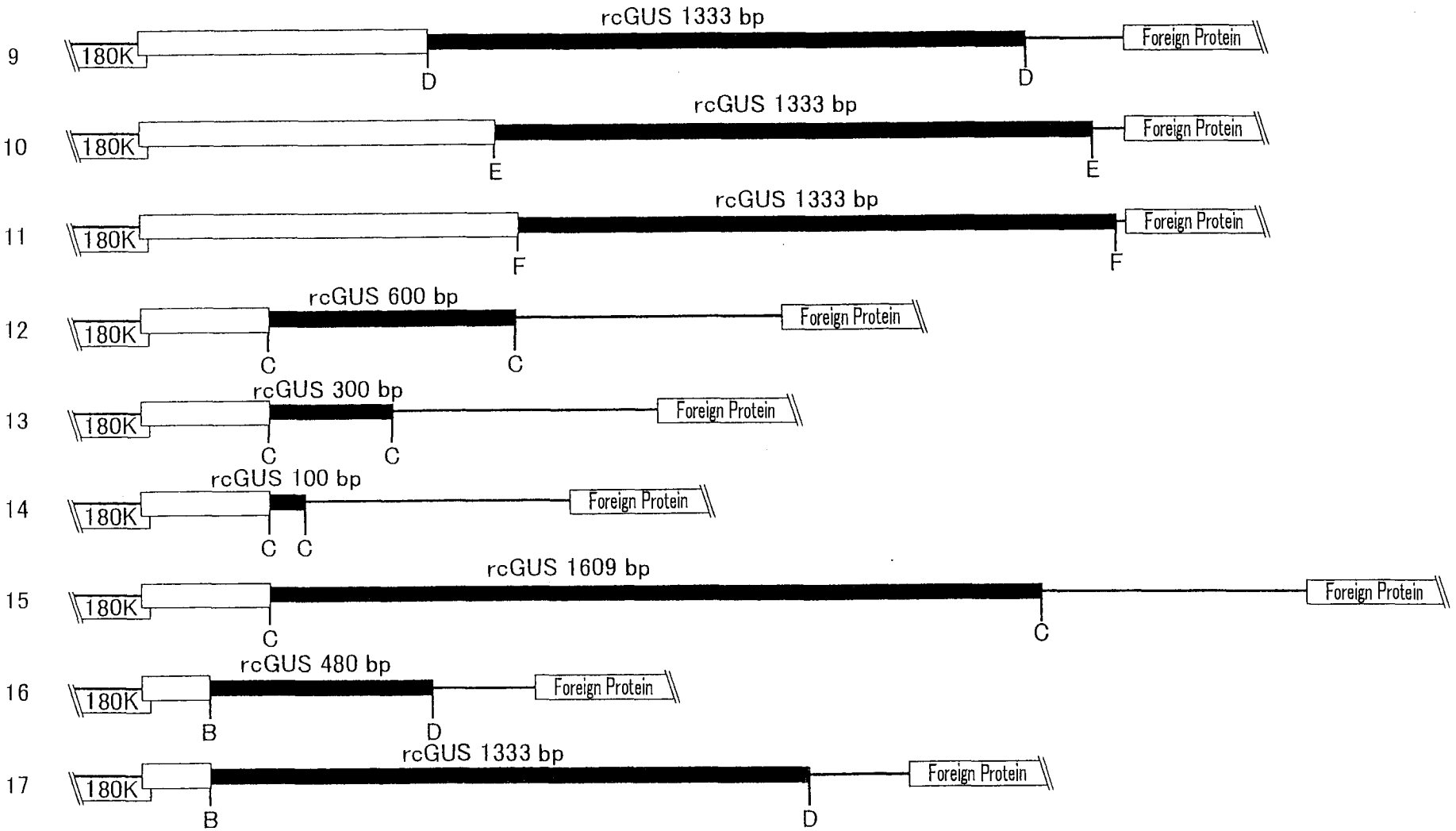


FIG. 2B



Number indicate the position in the wild-type ToMV sequence

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FIG. 3

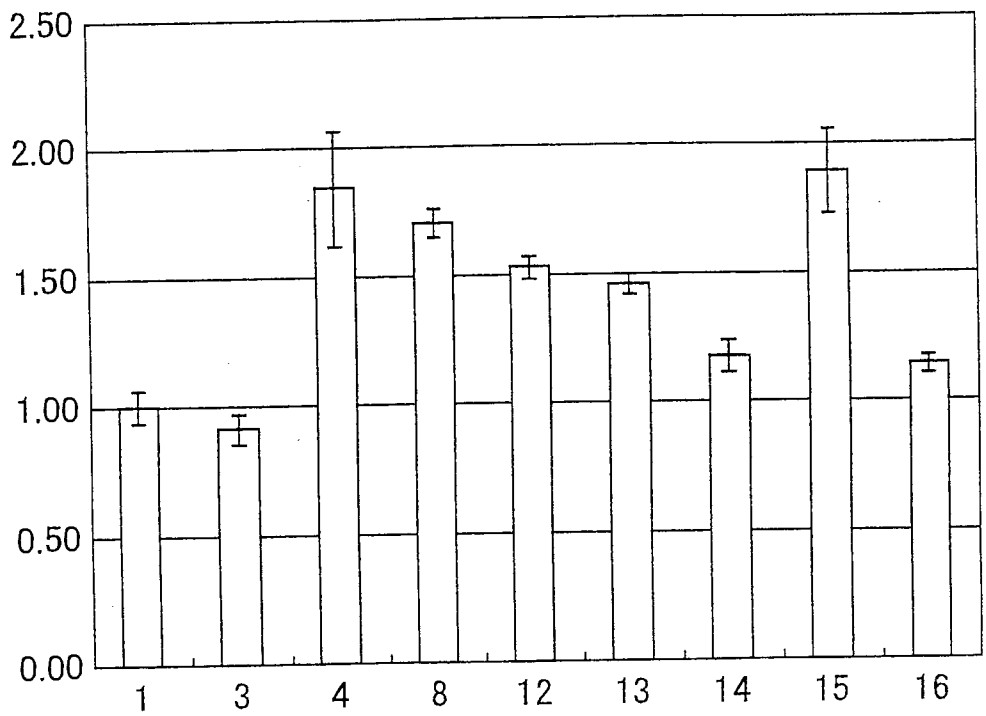
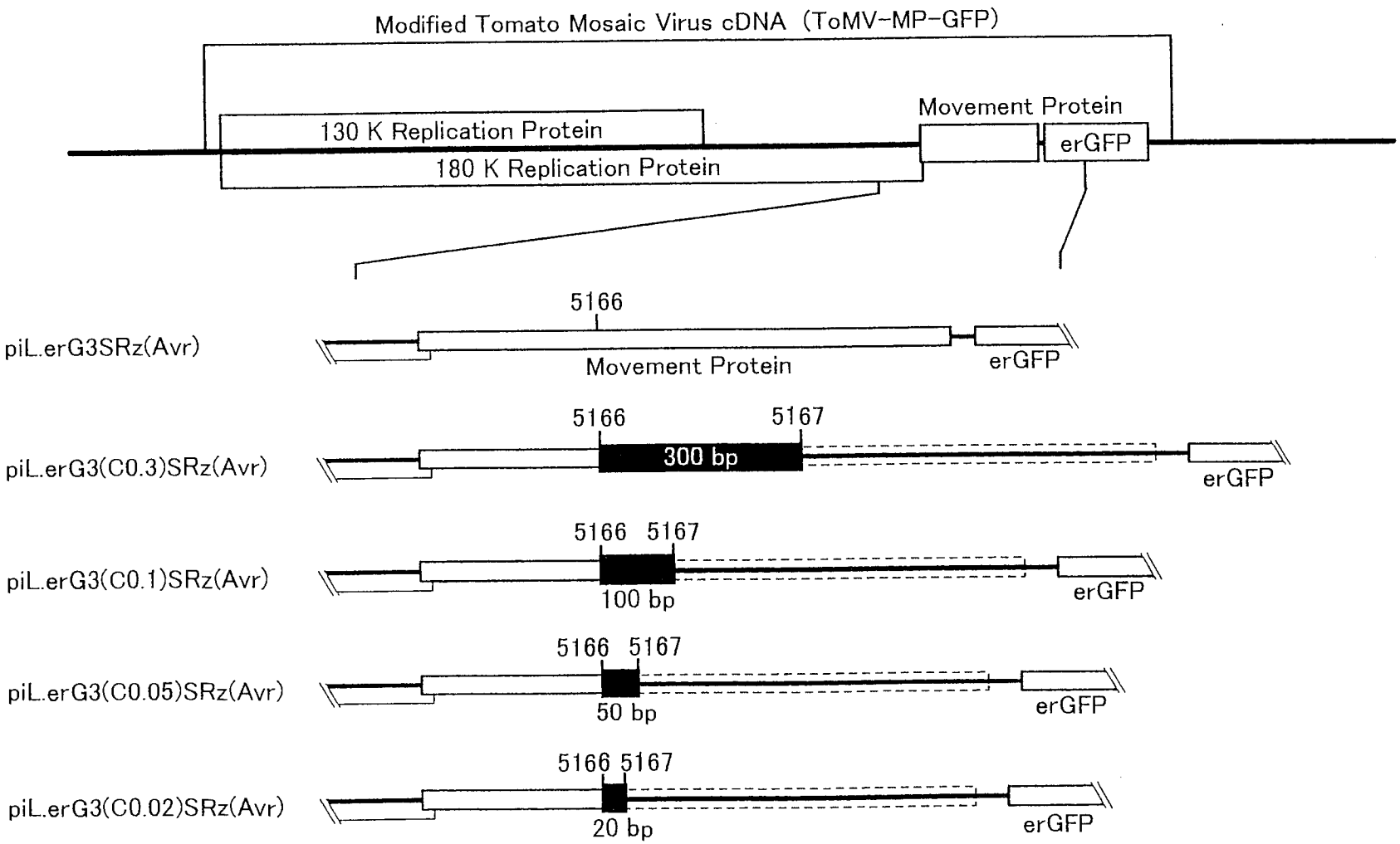


FIG. 4



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