DESCRIPTION

Title of Invention

DNA ELEMENT HAVING THE ACTIVITY OF ENHANCING FOREIGN GENE EXPRESSION

Technical Field

The present invention relates to a transformed mammalian host cell whose ability to secrete a foreign protein has been enhanced by using a foreign gene expression vector having a DNA element and a method for producing the foreign protein using the host cell.

Background Art

Due to the development of genetic recombination techniques, the market for protein pharmaceutical products such as therapeutic proteins and antibody drugs has rapidly expanded. In particular, antibody drugs have high specificity and do not cause an adverse immunoreaction even if they are administered to the human body, and therefore, the development thereof has been actively performed.

As a host cell in which a protein pharmaceutical typified by an antibody drug is produced, a microorganism, a yeast, an insect, an animal or plant cell, a transgenic animal or plant cell, or the like can be used. In order for the protein

pharmaceutical to have biological activity or immunogenicity, post-translational modification such as folding or glycosylation is essential, and therefore a microorganism with which complicated post-translational modification cannot be performed or a plant having a different glycan structure is not suitable as a host cell operating as a bioreactor. The use of a cultured mammalian cell such as a CHO cell which is from a species closely related to humans is currently standard considering that such a cell has a glycan structure similar to that of humans and is safe, and post-translational modification can be performed using such a cell.

In cases where a cultured mammalian cell is used as a host cell, there are the problems that the growth rate is low, the productivity is low, the cost is high, etc., as compared with a microorganism or the like (see Non-Patent Document 1). In addition, in order to use a protein pharmaceutical product in a clinical trial, it is necessary to administer a large amount of the product. Therefore, the lack of production ability thereof is also a worldwide problem. Accordingly, in order to improve the productivity of a foreign gene in a cultured mammalian cell, a lot of studies of promoters, enhancers, drug selection markers, gene amplification and culturing engineering techniques, and the like have been performed so far. However, the current situation is that a system capable of uniformly increasing gene expression has not

yet been established. As one of the causes of the low productivity of a foreign protein, a "position effect" is considered (see Non-Patent Document 2). When a foreign gene is introduced into a host cell, it is randomly integrated into the host chromosomal genome, and the transcription of the foreign gene is greatly affected by DNA around the region where the foreign gene has been integrated. A position effect is affected by factors such as the insertion site, copy number, structure, etc. of the foreign gene, however, it is very difficult to control the insertion site in the chromosome.

In order to solve the problem, regulatory polynucleotide sequences (also known as DNA elements) such as a locus control region (LCR), a scaffold/matrix attachment region (S/MAR), an insulator, a ubiquitous chromatin opening element (UCOE), and an anti-repressor (STAR element) have recently been identified (see Non-Patent Documents 3 to 6). LCR is not required to open the chromatin structure at an endogenous gene locus. However, LCR is a transcription regulatory element having an ability to open the chromatin structure around the DNA where the foreign gene has been integrated and to remodel a wide range of chromatin when it is used along with a foreign gene expression unit, and is said to require an AT-rich region (see Non-Patent Document 7).

The above-mentioned DNA element typified by LCR is often used in combination with a promoter, and it is known that in

cases where a DNA element is used in combination with a promoter, the expression level of a foreign gene is increased as compared with cases where only the promoter is used. However, very few types of DNA elements have been reported so far, and the various mechanisms contributing to the enhancement of foreign gene expression are different from one another. Further, even if a DNA element and a promoter are used in combination, sufficient amounts of a therapeutic protein under the control of the DNA element and the promoter are not produced. Therefore, it cannot be said that sufficient knowledge of a DNA element capable of increasing the productivity of a foreign protein has been obtained.

Accordingly, an object of the invention is to provide a method for increasing the production of a foreign protein to be used in a protein pharmaceutical product using a DNA element having high activity in enhancing foreign gene expression in a host cell such as a cultured mammalian cell.

Citation List

Non Patent Literature

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NPL 2: Ted H. J. Kwaks and Arie P. Otte. (2006) Employing epigenetics to augment the expression of therapeutic proteins

in mammalian cells. TRENDS in Biotechnol. 24(3): 137-142

NPL 3: Pierre-Alain Girod, Duc-Quang Nguyen. et al. (2007) Genome-wide prediction of matrix attachment regions that increase gene expression in mammalian cells. Nat. Methods. 4(9): 747-753

NPL 4: Adam C. Bell, Adam G. West, Gary Felsenfeld (2001) Insulators and Boundaries: Versatile Regulatory Elements in the Eukaryotic Genome, Science 291: 447-450

NPL 5: Steven Williams, Tracey Mustoe. et al. (2005) CpG-island fragments from the HNRPA2B1/CBX3 genomic locus reduce silencing and enhance transgene expression from the hCMV promoter/enhancer in mammalian cells. BMC Biotechnol. 5(17): 1-9

NPL 6: Arie P. Otte, Ted H. J. Kwaks. et al. (2007) Various Expression-Augmenting DNA Elements Benefit from STAR-Select, a Novel High Stringency Selection System for Protein Expression. Biotechnol. Prog. 23: 801-807

NPL 7: Qiliang Li, Kenneth R. Peterson, Xiangdong Fang, and George Stamatoyannopoulos, (2002) Locus control regions, Blood 100(9): 3077-3086

Summary of Invention

Technical Problems

As described above, there are still not many types of DNA elements which are regulatory polynucleotide sequences,

and, further, there are very few DNA elements among them that are highly effective in enhancing foreign gene expression. An object of the invention is to provide a method for stably achieving high expression in a mammalian cell using a DNA element which enhances the activation of transcription by being accompanied by a change in chromatin structure around a gene locus into which a foreign gene expression unit has been introduced, etc.

Solution to Problem

The present inventors made intensive studies in order to solve the above problems, and as a result, they found that the productivity and secretion of a foreign protein which is to be expressed can be improved by using one or more specific types of DNA elements in a cultured mammalian cell, and thus, completed the invention.

That is, the invention includes the following inventions.

(1) A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 1 in the Sequence Listing.

(2) A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 2 in the Sequence Listing.

(3) A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 3 in the Sequence Listing.

(4) A polynucleotide consisting of a polynucleotide

sequence represented by SEQ ID NO: 4 in the Sequence Listing.

(5) A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 5 in the Sequence Listing.

(6) A polynucleotide comprising at least 3000 consecutive nucleotides of a polynucleotide sequence represented by any one of SEQ ID NOS: 1 to 5 in the Sequence Listing.

(7) A polynucleotide comprising at least 2000 consecutive nucleotides of a polynucleotide sequence represented by any one of SEQ ID NOS: 1 to 5 in the Sequence Listing.

(8) A polynucleotide comprising at least 1500 consecutive nucleotides of a polynucleotide sequence represented by any one of SEQ ID NOS: 1 to 5 in the Sequence Listing.

(9) A polynucleotide consisting of a polynucleotide sequence having a homology of 95% or more to the polynucleotide sequence of the polynucleotide according to any one of (1) to (8).

(10) A polynucleotide consisting of a polynucleotide sequence having a homology of 99% or more to the polynucleotide sequence of the polynucleotide according to any one of (1) to (8).

(11) A polynucleotide consisting of a polynucleotide sequence containing two or more sequences of the polynucleotide

sequence of the polynucleotide according to any one of (1) to (10).

(12) A polynucleotide consisting of two or more types of polynucleotides selected from the polynucleotides according to any one of (1) to (10).

(13) A foreign gene expression vector comprising the polynucleotide sequence of a polynucleotide according to any one of (1) to (12).

(14) The foreign gene expression vector according to (13), wherein the protein encoded by the foreign gene is a multimeric protein.

(15) The foreign gene expression vector according to (14),

(16) The foreign gene expression vector according to (15), wherein the protein encoded by the foreign gene is an antibody or a functional fragment thereof.

(17) A transformed cell into which the foreign gene expression vector according to any one of (13) to (16) has been introduced.

(18) The transformed cell according to (17), wherein the cell is a cultured cell derived from a mammal.

(19) The transformed cell according to (18), wherein the cultured cell derived from a mammal is a cell selected from the group consisting of COS-1 cells, 293 cells, and CHO cells.

(20) The transformed cell according to any one of (17) to (18), wherein the protein encoded by the foreign gene is a multimeric protein.

(21) The transformed cell according to (20), wherein the protein encoded by the foreign gene is a hetero-multimeric protein.

(22) The transformed cell according to (21), wherein the protein encoded by the foreign gene is an antibody or a functional fragment thereof.

(23) A method for producing a protein characterized by comprising culturing the transformed cell according to any one of (17) to (22) and obtaining the protein encoded by the foreign gene from the resulting culture product.

(24) A method for enhancing foreign gene expression in a transformed cell into which a foreign gene or a foreign gene expression vector has been introduced, characterized by using a polynucleotide according to any one of (1) to (12) or a foreign gene expression vector according to any one of (13) to (16).

(25) Use of the polynucleotide according to any one of (1) to (12) for enhancing foreign gene expression in a transformed cell.

Advantageous Effects of Invention

According to the invention, by introducing a foreign gene expression vector using a DNA element into a mammalian host

cell, the expression of a foreign gene for a therapeutic protein, an antibody, or the like can be significantly enhanced.

The present invention also provides any one of the embodiments disclosed below and indicated with the numbers 1 to 25.

1. A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 1 in the Sequence Listing.

2. A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 2 in the Sequence Listing.

3. A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 3 in the Sequence Listing.

4. A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 4 in the Sequence Listing.

5. A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 5 in the Sequence Listing.

6. A polynucleotide comprising at least 3000 consecutive nucleotides of a polynucleotide sequence represented by any one of SEQ ID NOS: 1 to 5 in the Sequence Listing.

7. A polynucleotide comprising at least 2000 consecutive nucleotides of a polynucleotide sequence represented by any one of SEQ ID NOS: 1 to 5 in the Sequence Listing.

8. A polynucleotide comprising at least 1500 consecutive

nucleotides of a polynucleotide sequence represented by any one of SEQ ID NOS: 1 to 5 in the Sequence Listing.

9. A polynucleotide consisting of a polynucleotide sequence having a homology of 95% or more to the polynucleotide sequence of the polynucleotide according to any one of claims 1 to 8.

10. A polynucleotide consisting of a polynucleotide sequence having a homology of 99% or more to the polynucleotide sequence of the polynucleotide according to any one of claims 1 to 8.

11. A polynucleotide consisting of a polynucleotide sequence containing two or more sequences of the polynucleotide sequence of the polynucleotide according to any one of claims 1 to 10.

12. A polynucleotide consisting of two or more types of polynucleotides selected from the polynucleotides according to claims 1 to 10.

13. A foreign gene expression vector comprising the polynucleotide sequence of a polynucleotide according to any one of claims 1 to 12.

14. The foreign gene expression vector according to claim 13, wherein the protein encoded by the foreign gene is a multimeric protein.

15. The foreign gene expression vector according to claim 13, wherein the protein encoded by the foreign gene is a hetero-multimeric protein.

16. The foreign gene expression vector according to claim 15, wherein the protein encoded by the foreign gene is an antibody or a functional fragment thereof.

17. A transformed cell into which the foreign gene expression vector according to any one of claims 13 to 16 has been introduced.

18. The transformed cell according to claim 17, wherein the cell is a cultured cell derived from a mammal.

19. The transformed cell according to claim 18, wherein the cultured cell derived from a mammal is a cell selected from the group consisting of COS-1 cells, 293 cells, and CHO cells.

20. The transformed cell according to any one of claims 17

to 19, wherein the protein encoded by the foreign gene is a multimeric protein.

21. The transformed cell according to claim 20, wherein the protein encoded by the foreign gene is a hetero-multimeric protein.

22. The transformed cell according to claim 21, wherein the protein encoded by the foreign gene is an antibody or a functional fragment thereof.

23. A method for producing a protein characterized by comprising culturing the transformed cell according to any one of claims 17 to 22 and obtaining the protein encoded by the foreign gene from the resulting culture product.

24. A method for enhancing foreign gene expression in a transformed cell into which a foreign gene or a foreign gene expression vector has been introduced, characterized by using a polynucleotide according to any one of claims 1 to 12 or a foreign gene expression vector according to any one of claims 13 to 16.

25. Use of the polynucleotide according to any one of claims 1 to 12 for enhancing foreign gene expression in a transformed

cell.

Brief Description of Drawings

[Fig. 1] Fig. 1 shows a graph in which it was confirmed by the amplification of a GAPDH region that a sample subjected to ChIP-on-chip was chromatin-immunoprecipitated specifically with an anti-acetylated histone H3 antibody.

[Fig. 2] Fig. 2 is a schematic view of an SEAP expression vector into which a DNA element has been inserted.

[Fig. 3] Fig. 3 is a graph showing the expression of SEAP under the control of a CMV promoter in a stably expressing CHO cell line either without a DNA element or with DNA element A2, A7, A18, B5, or C14. The effects of DNA elements A2, A7, A18, B5, and C14 on enhancement of expression were confirmed.

[Fig. 4] Fig. 4 comprises two graphs showing the expression of SEAP under the control of either an EF-1α or an SV40 promoter in a stably expressing CHO cell line either without a DNA element or with DNA element A2 or A7. The effects of DNA elements A2 and A7 on enhancement of expression were confirmed.

[Fig. 5] Fig. 5 is a schematic view of an antibody expression (antibody gene X heavy chain and light chain co-expression) vector into which a DNA element has been inserted.

[Fig. 6] Fig. 6 comprises two graphs showing levels of secretion (measured by an ELISA method) of an antibody under the control of either a CMV or an EF-1α promoter in a stably expressing CHO cell line either without a DNA element or with DNA element A7. The effect of DNA element A7 on enhancement of expression was confirmed.

[Fig. 7] Fig. 7 is a table showing the sequence lengths of DNA element A2 and related sequences.

[Fig. 8] Fig. 8 comprises three graphs showing the expression of SEAP in a stably expressing CHO cell line either without a DNA element or with DNA element A2 or a related sequence. The effects of DNA element A2 and related sequences on enhancement of expression were confirmed.

[Fig. 9] Fig. 9 is a table showing the sequence lengths of DNA element A7 and related sequences.

[Fig. 10] Fig. 10 comprises three graphs showing the expression of SEAP in a stably expressing CHO cell line either without a DNA element or with DNA element A7 or a related sequence. The effects of DNA element A7 and related sequences on enhancement of expression were confirmed.

[Fig. 11] Fig. 11 is a table showing the sequence lengths of DNA element A18 and related sequences.

[Fig. 12] Fig. 12 is a graph showing the expression of SEAP in a stably expressing CHO cell line either without a DNA element or with DNA element A18 or a related sequence. The

effects of DNA element A18 and related sequences on enhancement of expression were confirmed.

[Fig. 13] Fig. 13 is a table showing the sequence lengths of DNA element B5 and related sequences.

[Fig. 14] Fig. 14 is a graph showing the expression of SEAP in a stably expressing CHO cell line either without a DNA element or with DNA element B5 or a related sequence. The effects of DNA element B5 and related sequences on enhancement of expression were confirmed.

[Fig. 15] Fig. 15 is a table showing the sequence lengths of DNA element C14 and related sequences.

[Fig. 16] Fig. 16 comprises three graphs showing the expression of SEAP in a stably expressing CHO cell line either without a DNA element or with DNA element C14 or a related sequence. The effects of DNA element C14 and related sequences on enhancement of expression were confirmed.

[Fig. 17] Fig. 17 is a graph showing the expression of SEAP in a stably expressing HEK293 cell line either without a DNA element or with DNA element A2, A7, A18, B5, or C14. The effects of DNA elements A2, A7, A18, B5, and C14 on enhancement of expression in HEK293 cells were confirmed.

[Fig. 18] Fig. 18 is a view showing nucleotides at the starting and end points on the basis of the full-length sequence of a DNA element A2, A7, or A18.

[Fig. 19] Fig. 19 is a view showing nucleotides at the

starting and end points on the basis of the full-length sequence of a DNA element B5 or C14.

Description of Embodiments

Hereinafter, the invention will be specifically described with reference to the Examples. However, these Examples do not limit the technical scope of the invention. The plasmids, restriction enzymes, DNA modification enzymes, and the like to be used in the Examples in the invention are commercially available products and can be used according to common procedures. Further, procedures used for DNA cloning, polynucleotide sequence determination, transformation of a host cell, culturing of a transformed host cell, isolation of an antibody from an obtained culture solution, purification of an antibody, and the like are also well known to those skilled in the art or are available from the literature.

The term "gene" as used herein includes not only DNA, but also mRNA thereof, cDNA, and RNA thereof.

The term "polynucleotide" as used herein is used in the same meaning as a nucleic acid and also includes DNA, RNA, probes, oligonucleotides, and primers.

The terms "polypeptide" and "protein" as used herein are used without distinction.

The term "gene expression" as used herein refers to a phenomenon in which an mRNA is transcribed from a gene and/or

a phenomenon in which a protein is translated from the mRNA.

The term "foreign gene" as used herein refers to a gene which is artificially introduced into a host cell.

The term "foreign protein" as used herein refers to a protein encoded by a foreign gene.

The term "gene expression unit" as used herein refers to a polynucleotide having, in the direction of the reading frame of transcription, at least a promoter region, a foreign gene, and a transcription terminator region (poly(A) addition signal).

The term "activity of enhancing foreign gene expression" as used herein refers to the activity of enhancing the production of a foreign protein in a host cell by creating an environment advantageous to transcription and translation for DNA around a gene expression unit containing a foreign gene and significantly improving the transcription and translation efficiency.

The term "DNA element" as used herein refers to a polynucleotide having an activity of enhancing foreign gene expression in cases where the polynucleotide is located in the vicinity of a gene expression unit or in a foreign gene expression vector containing a gene expression unit.

The term "functional fragment of an antibody" as used herein refers to a partial fragment of an antibody having antigen-binding activity and includes Fab, $F(ab')_2$, and the

like. However, the term is not limited to these molecules as long as the fragment has a binding affinity for an antigen.

1. DNA element to be used for enhancing foreign gene expression

As shown in Example 1, a DNA element according to the invention can be obtained by using the interaction between acetylated histone H3 and genomic DNA. In general, it is said that the acetylation of histones (H3 and H4) is associated with the activation of transcription, and two main theories have been advocated. One theory is that the acetylation of histones is associated with a change in nucleosome conformation in such a manner that histone tails are acetylated, thereby being electrically neutralized, resulting in weakening of DNA-histone interactions (Mellor J. (2006) Dynamic nucleosomes and gene transcription. Trends Genet. 22(6): 320-329).The other theory is that the acetylation of histones is associated with the recruitment of various transcription factors (Nakatani Y. (2001) Histone acetylases - versatile players. Genes Cells. 6(2): 79-86). In either theory, there is a high possibility that the acetylation of histones is associated with the activation of transcription, and by performing chromatin immunoprecipitation (ChIP) using an anti-acetylated histone H3 antibody, it is possible to concentrate a DNA element interacting with acetylated histone H3.

In the present invention, A2 is an example of a DNA element to be used for enhancing foreign gene expression. A2 is located in the region from 80966429 to 80974878 of human chromosome 15 and is a polynucleotide sequence of 8450 bp, having an AT content of 62.2%. The polynucleotide sequence of A2 is represented by SEQ ID NO: 1 in the Sequence Listing.

A7, A18, B5, and C14 are examples of similar DNA elements. A7 is located in the region from 88992123 to 89000542 of human chromosome 11 and is a polynucleotide sequence of 8420 bp, having an AT content of 64.52%. The polynucleotide sequence of A7 is represented by SEQ ID NO: 2 in the Sequence Listing.

A18 is located in the region from 111275976 to 111284450 of human chromosome 4 and is a polynucleotide sequence of 8475 bp, having an AT content of 62.54%. The polynucleotide sequence of A18 is represented by SEQ ID NO: 3 in the Sequence Listing.

B5 is located in the region from 143034684 to 143043084 of human chromosome 1 and is a polynucleotide sequence of 8401 bp, having an AT content of 66.37%. The polynucleotide sequence of B5 is represented by SEQ ID NO: 4 in the Sequence Listing.

Finally, C14 is located in the region from 46089056 to 46097482 of human chromosome 11 and is a polynucleotide sequence of 8427 bp, having an AT content of 63.81%. The polynucleotide sequence of C14 is represented by SEQ ID NO:

5 in the Sequence Listing.

In the invention, the activity of enhancing foreign gene expression of the DNA element can be assayed by using the activity of a protein encoded by a reporter gene such as SEAP as an index. In cases where the activity of a reporter protein in the presence of the DNA element is increased, preferably by two times or more, more preferably four times or more, even more preferably five times or more as compared with the case where the DNA element is not present, the DNA element can be determined to have an activity of enhancing foreign gene expression. Even in cases where the activity is increased by two times or more, it is expected that this will reduce the cell culture scale and the cell culture time, and as a result, it is possible to increase the yield and reduce the cell culture cost. If the yield is increased, then it is possible to supply stably a foreign protein to be used as a pharmaceutical. In addition, if the cell culture cost is reduced, the cost for the foreign protein to be used as a pharmaceutical is reduced, and the financial burden on patients to whom the foreign protein is to be administered is also reduced.

In the invention, any one of the above DNA elements may be used alone, and two or more copies of one type of the DNA element may be used. Alternatively, two or more different types of the above DNA elements may be used in combination.

A2, A7, A18, B5, and C14 are preferred examples of the

DNA element to be used in the invention.

The DNA element to be used in the invention may be a polynucleotide sequence which comprises a polynucleotide sequence having a homology of 80% or more to any of the polynucleotide sequences represented by SEQ ID NOS: 1 to 5 and has an activity of enhancing foreign gene expression. The homology of 80% or more is preferably a homology of 90% or more, more preferably a homology of 95% or more, most preferably a homology of 99% or more. The polynucleotide sequence homology search can be performed in, for example, the DNA Databank of Japan or the like using a program such as FASTA or BLAST.

The DNA element to be used in the invention may be a DNA element which hybridizes to a polynucleotide consisting of a polynucleotide sequence complementary to a polynucleotide consisting of a polynucleotide sequence selected from the group consisting of the polynucleotide sequences represented by SEQ ID NOS: 1 to 5 under stringent conditions and has an activity of enhancing foreign gene expression.

The term "stringent conditions" as used herein refers to conditions in which a so-called specific hybrid is formed but a non-specific hybrid is not formed. For example, conditions in which a complementary strand of a nucleic acid consisting of a polynucleotide sequence having a high homology, i.e., a polynucleotide sequence having a homology of 80% or more, preferably 90% or more, more preferably 95% or more, most

preferably 99% or more to a polynucleotide sequence selected from the group consisting of the polynucleotide sequences represented by SEQ ID NOS: 1 to 5 hybridizes, and a complementary strand of a nucleic acid comprising a polynucleotide sequence having a lower homology does not hybridize are exemplary stringent conditions. To be more specific, conditions in which the concentration of sodium salt is from 15 to 750 mM, preferably from 50 to 750 mM, more preferably from 300 to 750 mM, the temperature is from 25 to 70°C, preferably from 50 to 70°C, more preferably from 55 to 65°C, and the concentration of formamide is from 0 to 50%, preferably from 20 to 50%, more preferably from 35 to 45% can be exemplified. Further, as the stringent conditions, conditions for washing a filter after hybridization in which the concentration of sodium salt is generally from 15 to 600 mM, preferably from 50 to 600 mM, more preferably from 300 to 600 mM, and the temperature is from 50 to 70°C, preferably from 55 to 70°C, more preferably from 60 to 65°C can be exemplified.

A person skilled in the art can easily obtain such a homologue gene with reference to Molecular Cloning (Sambrook, J. et al., Molecular Cloning: a Laboratory Manual 2nd ed., Cold Spring Harbor Laboratory Press, 10 Skyline Drive Plainview, NY (1989)) or the like. Further, the homology of the above-mentioned polynucleotide sequence can be determined by a FASTA search or BLAST search in the same manner.

Introduction of a mutation (deletion, substitution, and/or addition) into the above-mentioned polynucleotide sequence can be performed by a method known in this technical field such as a Kunkel method or a gapped duplex method, or based on this method. For example, a mutation introduction kit utilizing a site-directed mutagenesis method (for example, Mutant-K (manufactured by TaKaRa Bio, Inc.), Mutant-G (manufactured by TaKaRa Bio, Inc.), or a LA PCR in vitro Mutagenesis series kit (manufactured by TaKaRa Bio, Inc.)), or the like can be used. Such a mutated polynucleotide can also be used as the DNA element of the invention.

As the DNA element of the invention, a partial fragment comprising at least 3000 or at least 2000 consecutive nucleotides of a polynucleotide sequence represented by any one of SEQ ID NOS: 1 to 5 in the Sequence Listing can be used. Examples of such a partial fragment include: A2-1 to A2-17 which are partial fragments of A2; A7-1 to A7-18 which are partial fragments of A7; A18-1 to A18-4 which are partial fragments of A18; B5-1 to B5-6 which are partial fragments of B5; and C14-1 to C14-14 which are partial fragments of C14. However, the DNA element is not limited to these partial fragments as long as it has an activity of enhancing foreign gene expression.

In the invention, any one of the above partial fragments may be used alone, and also two or more copies of one type of the partial fragment may be used. Alternatively, two or more

different types of the partial fragments may be used in combination. Further, a full-length sequence and a partial fragment of any of the above-mentioned DNA elements may be used in combination. In the above combination, the full-length sequence and the partial fragment may be derived from the same DNA element or from different DNA elements.

As for the polynucleotide sequences of the respective fragments of A2, A2-1 corresponds to the polynucleotide sequence of nucleotides 1 to 3000 of SEQ ID NO: 1 in the Sequence Listing; A2-2 corresponds to the polynucleotide sequence of nucleotides 2801 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-3 corresponds to the polynucleotide sequence of nucleotides 5401 to 8450 of SEQ ID NO: 1 in the Sequence Listing; A2-4 corresponds to the polynucleotide sequence of nucleotides 701 to 2700 of SEQ ID NO: 1 in the Sequence Listing; A2-5 corresponds to the polynucleotide sequence of nucleotides 701 to 2200 of SEQ ID NO: 1 in the Sequence Listing; A2-6 corresponds to the polynucleotide sequence of nucleotides 701 to 3700 of SEQ ID NO: 1 in the Sequence Listing; A2-7 corresponds to the polynucleotide sequence of nucleotides 2001 to 5000 of SEQ ID NO: 1 in the Sequence Listing; A2-8 corresponds to the polynucleotide sequence of nucleotides 4001 to 7000 of SEQ ID NO: 1 in the Sequence Listing; A2-9 corresponds to the polynucleotide sequence of nucleotides 1 to 3700 of SEQ ID NO: 1 in the Sequence Listing; A2-10 corresponds to the

polynucleotide sequence of nucleotides 2001 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-11 corresponds to the polynucleotide sequence of nucleotides 2801 to 7000 of SEQ ID NO: 1 in the Sequence Listing; A2-12 corresponds to the polynucleotide sequence of nucleotides 701 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-13 corresponds to the polynucleotide sequence of nucleotides 2001 to 7000 of SEQ ID NO: 1 in the Sequence Listing; A2-14 corresponds to the polynucleotide sequence of nucleotides 2801 to 8450 of SEQ ID NO: 1 in the Sequence Listing; A2-15 corresponds to the polynucleotide sequence of nucleotides 1 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-16 corresponds to the polynucleotide sequence of nucleotides 701 to 7000 of SEQ ID NO: 1 in the Sequence Listing; and A2-17 corresponds to the polynucleotide sequence of nucleotides 2001 to 8450 of SEQ ID NO: 1 in the Sequence Listing.

As for the polynucleotide sequences of the respective fragments of A7, A7-1 corresponds to the polynucleotide sequence of nucleotides 601 to 3600 of SEQ ID NO: 2 in the Sequence Listing; A7-2 corresponds to the polynucleotide sequence of nucleotides 3601 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-3 corresponds to the polynucleotide sequence of nucleotides 5401 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-4 corresponds to the polynucleotide sequence of nucleotides 3401 to 6400 of SEQ ID NO: 2 in the

Sequence Listing; A7-5 corresponds to the polynucleotide sequence of nucleotides 1501 to 4500 of SEQ ID NO: 2 in the Sequence Listing; A7-6 corresponds to the polynucleotide sequence of nucleotides 4401 to 7400 of SEQ ID NO: 2 in the Sequence Listing; A7-7 corresponds to the polynucleotide sequence of nucleotides 2401 to 5400 of SEQ ID NO: 2 in the Sequence Listing; A7-8 corresponds to the polynucleotide sequence of nucleotides 1 to 3600 of SEQ ID NO: 2 in the Sequence Listing; A7-9 corresponds to the polynucleotide sequence of nucleotides 1501 to 5400 of SEQ ID NO: 2 in the Sequence Listing; A7-10 corresponds to the polynucleotide sequence of nucleotides 2401 to 6400 of SEQ ID NO: 2 in the Sequence Listing; A7-11 corresponds to the polynucleotide sequence of nucleotides 3401 to 7400 of SEQ ID NO: 2 in the Sequence Listing; A7-12 corresponds to the polynucleotide sequence of nucleotides 4401 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-13 corresponds to the polynucleotide sequence of nucleotides 1 to 5400 of SEQ ID NO: 2 in the Sequence Listing; A7-14 corresponds to the polynucleotide sequence of nucleotides 1501 to 6400 of SEQ ID NO: 2 in the Sequence Listing; A7-15 corresponds to the polynucleotide sequence of nucleotides 2401 to 7400 of SEQ ID NO: 2 in the Sequence Listing; A7-16 corresponds to the polynucleotide sequence of nucleotides 3401 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-17 corresponds to the polynucleotide sequence of

nucleotides 1 to 6400 of SEQ ID NO: 2 in the Sequence Listing; and A7-18 corresponds to the polynucleotide sequence of nucleotides 1501 to 7400 of SEQ ID NO: 2 in the Sequence Listing.

As for the polynucleotide sequences of the respective fragments of A18, A18-1 corresponds to the polynucleotide sequence of nucleotides 1 to 5040 of SEQ ID NO: 3 in the Sequence Listing; A18-2 corresponds to the polynucleotide sequence of nucleotides 1001 to 6002 of SEQ ID NO: 3 in the Sequence Listing; A18-3 corresponds to the polynucleotide sequence of nucleotides 2001 to 7000 of SEQ ID NO: 3 in the Sequence Listing; and A18-4 corresponds to the polynucleotide sequence of nucleotides 3000 to 7000 of SEQ ID NO: 3 in the Sequence Listing.

The start and end points of the respective fragments of A2, A7 and A18 are also set forth in Figure 18.

As for the polynucleotide sequences of the respective fragments of B5, B5-1 corresponds to the polynucleotide sequence of nucleotides 1 to 4001 of SEQ ID NO: 4 in the Sequence Listing; B5-2 corresponds to the polynucleotide sequence of nucleotides 1 to 3200 of SEQ ID NO: 4 in the Sequence Listing; B5-3 corresponds to the polynucleotide sequence of nucleotides 2491 to 5601 of SEQ ID NO: 4 in the Sequence Listing; B5-4 corresponds to the polynucleotide sequence of nucleotides 5373 to 8401 of SEQ ID NO: 4 in the Sequence Listing; B5-5 corresponds to the polynucleotide sequence of nucleotides 901 to 4001 of SEQ ID NO: 4 in the Sequence Listing; and B5-6 corresponds to

the polynucleotide sequence of nucleotides 4001 to 7000 of SEQ ID NO: 4 in the Sequence Listing.

As for the polynucleotide sequences of the respective fragments of C14, C14-1 corresponds to the polynucleotide sequence of nucleotides 960 to 4015 of SEQ ID NO: 5 in the Sequence Listing; C14-2 corresponds to the polynucleotide sequence of nucleotides 1987 to 5014 of SEQ ID NO: 5 in the Sequence Listing; C14-3 corresponds to the polynucleotide sequence of nucleotides 4020 to 7119 of SEQ ID NO: 5 in the Sequence Listing; C14-4 corresponds to the polynucleotide sequence of nucleotides 960 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-5 corresponds to the polynucleotide sequence of nucleotides 960 to 6011 of SEQ ID NO: 5 in the Sequence Listing; C14-6 corresponds to the polynucleotide sequence of nucleotides 4939 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-7 corresponds to the polynucleotide sequence of nucleotides 960 to 5014 of SEQ ID NO: 5 in the Sequence Listing; C14-8 corresponds to the polynucleotide sequence of nucleotides 2994 to 7119 of SEQ ID NO: 5 in the Sequence Listing; C14-9 corresponds to the polynucleotide sequence of nucleotides 4020 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-10 corresponds to the polynucleotide sequence of nucleotides 1 to 5014 of SEQ ID NO: 5 in the Sequence Listing; C14-11 corresponds to the polynucleotide sequence of nucleotides 1987 to 7119 of SEQ ID NO: 5 in the Sequence Listing;

C14-12 corresponds to the polynucleotide sequence of nucleotides 2994 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-13 corresponds to the polynucleotide sequence of nucleotides 960 to 7119 of SEQ ID NO: 5 in the Sequence Listing; and C14-14 corresponds to the polynucleotide sequence of nucleotides 1987 to 8141 of SEQ ID NO: 5 in the Sequence Listing.

The start and end points of the respective fragments of B5 and C14 are also set forth in Figure 19.

2. Acquisition of polynucleotide

In the invention, a polynucleotide containing a foreign gene encoding a foreign protein the production of which is to be increased, which will be described later, can be obtained by common procedures as described below. For example, such a polynucleotide can be isolated by screening a cDNA library derived from cells or tissues expressing the foreign gene using a DNA probe synthesized by being based on a fragment of the foreign gene. mRNA can be prepared by methods commonly used in this technical field. For example, the cells or tissues are treated with a guanidine reagent, a phenol reagent, etc., thereby obtaining total RNA, and thereafter, poly(A)+ RNA (mRNA) is obtained by an affinity column method using an oligo(dT) cellulose column or a poly U-Sepharose column containing Sepharose 2B as a carrier, or the like, or by a batch

method. Also, the poly(A)+ RNA may further be fractionated by sucrose density-gradient centrifugation or the like. Then, a single-stranded cDNA is synthesized using the thus obtained mRNA as a template, and also using oligo dT primers and a reverse transcriptase. From the thus obtained single-stranded cDNA, a double-stranded cDNA is synthesized using DNA polymerase I, DNA ligase, RNase H, and the like. The thus synthesized double-stranded cDNA is blunted using T4 DNA polymerase, followed by ligation to an adapter (such as EcoRI adapter), phosphorylation, and the like, and the resulting DNA is incorporated into a lambda phage such as λgt11 to achieve in vivo packaging, whereby a cDNA library can be prepared. It is also possible to prepare a cDNA library using a plasmid vector other than lambda phages. Thereafter, a clone containing a target DNA (a positive clone) may be selected from the cDNA library.

In cases where the above-mentioned DNA element to be used for increasing the production of a protein or a polynucleotide containing a foreign gene is isolated from genomic DNA, or a polynucleotide containing promoter and terminator regions is isolated from genomic DNA, according to a common procedure (Molecular Cloning (1989), Methods in Enzymology 194 (1991)), genomic DNA is extracted from a cell line of an organism to be used as a collection source, and a polynucleotide is selected and isolated. The extraction of genomic DNA can be performed

according to, for example, the method of Cryer et al. (Methods in Cell Biology, 12, 39-44 (1975)) or the method of P. Philippsen et al. (Methods Enzymol., 194, 169-182 (1991)).

The target DNA element or the polynucleotide containing a foreign gene can also be obtained by, for example, the PCR method (PCR Technology. Henry A. Erlich, Atockton press (1989)). In the amplification of a polynucleotide using the PCR method, 20- to 30-mer synthetic single-stranded DNAs are used as primers and genomic DNA is used as a template. The amplified gene is used after the polynucleotide sequence of the gene is confirmed. As the template for PCR, a genomic DNA library such as a bacterial artificial chromosome (BAC) can be used.

On the other hand, the polynucleotide containing a foreign gene whose sequence is not known can be obtained by (a) preparing a gene library according to a common procedure, and (b) selecting a desired polynucleotide from the prepared gene library and amplifying the polynucleotide. The gene library can be prepared by partially digesting chromosomal DNA obtained by a common procedure from a cell line of an organism to be used as a collection source using an appropriate restriction enzyme to fragment the chromosomal DNA, ligating the obtained fragments to an appropriate vector, and introducing the vector into an appropriate host. The gene library can also be prepared by extracting mRNA from the cells, synthesizing cDNA from the mRNA, ligating the cDNA to an

appropriate vector, and introducing the vector into an appropriate host. As the vector to be used in such preparation, a plasmid generally known as a vector for gene library preparation, a phage vector, a cosmid, or the like can also be used. As the host to be transformed or transfected, a host suitable for the type of the above-mentioned vector may be used. The polynucleotide containing the foreign gene is selected from the above-mentioned gene library by a colony hybridization method, a plaque hybridization method, or the like using a labeled probe containing a sequence specific for the foreign gene.

Further, the polynucleotide containing the foreign gene can also be produced by total chemical synthesis. For example, the gene can be synthesized by a method in which two pairs of complementary oligonucleotides are prepared and annealed, a method in which several annealed DNA strands are ligated by a DNA ligase, a method in which several partially complementary polynucleotides are prepared and gaps are filled by PCR, or the like.

The determination of a polynucleotide sequence can be performed by a conventional technique, for example, a dideoxy method (Sanger et al., Proc. Natl. Acad. Sci., USA, 74, 5463-5467, (1977)), or the like. Further, the above determination of a polynucleotide sequence can also be easily performed using a commercially available sequencing kit or the

like.

3. Foreign gene expression vector, element vector

As a foreign gene expression vector of the invention, a vector containing one type of the above-mentioned DNA elements, two or more copies of one type of the above-mentioned DNA elements, or two or more different types of the above-mentioned DNA elements in combination, and further containing a foreign gene expression unit is provided. When a foreign gene is expressed in a host cell using the above-mentioned foreign gene expression vector, the DNA element may be located immediately upstream or downstream of the gene expression unit, or may be located at a position away from the gene expression unit. Further, one foreign gene expression vector containing a plurality of such DNA elements may be used. Incidentally, the DNA element may be inserted in either forward or reverse orientation with respect to the gene expression unit.

Further, as the vector to be used in the invention, a vector containing one type of the above-mentioned DNA elements, two or more copies of one type of the above-mentioned DNA elements, or two or more different types of the above-mentioned DNA elements in combination, and containing no gene expression unit (hereinafter also referred to as an "element vector") is also included. Such an element vector can be used in

combination with the above-mentioned foreign gene expression vector containing the DNA element or a foreign gene expression vector containing no DNA element and containing only the foreign gene expression unit. By allowing the element vector to coexist with the foreign gene expression vector, the expression of the foreign gene is enhanced as compared with cases where the foreign gene expression vector is used alone and, therefore, the combination of the above-mentioned vectors is also included in the foreign gene expression vector of the invention.

The gene encoding the foreign protein is not particularly limited, however, examples thereof include reporter genes such as secretory alkaline phosphatase (SEAP), a green fluorescent protein (GFP), and luciferase; various enzyme genes such as an α-amylase gene and an α-galactosidase gene; genes of various interferons which are pharmaceutically useful and physiologically active proteins such as interferon α and interferon γ; genes of various interleukins such as IL-1 and IL-2; various cytokine genes such as an erythropoietin (EPO) gene and a granulocyte colony-stimulating factor (G-CSF) gene; growth factor genes; and antibody genes. These genes may be obtained by any method.

The invention is particularly effective in relation to a protein which is highly hydrophobic and a protein which is difficult to get secreted and produced due to composite

formation. Thus, the above-mentioned foreign protein includes a multimeric protein such as a heteromultimer which is an antibody or a functional fragment thereof. The "functional fragment of an antibody" refers to a partial fragment of an antibody having an antigen-binding activity and includes Fab, F(ab')2, Fv, scFv, diabodies, linear antibodies, polyspecific antibodies formed from antibody fragments, and the like. The functional fragment of an antibody also includes Fab' which is a monovalent fragment in a variable region of an antibody obtained by treating F(ab')2 under reducing conditions. However, the functional fragment is not limited to these molecules as long as the fragment has a binding affinity for an antigen. Further, these functional fragments include not only a fragment obtained by treating a full-length molecule of an antibody protein with an appropriate enzyme, but also a protein produced in an appropriate host cell using a genetically modified antibody gene.

The gene expression unit has, in the direction of the reading frame of transcription, at least a promoter region, a foreign gene, and a transcription terminator region (poly(A) addition signal). The promoter which can be used here may be a constitutive expression promoter or an inducible expression promoter. Examples of a constitutive expression promoter include various natural promoters such as an SV40 early promoter, an adenovirus E1A promoter, a CMV (cytomegalovirus)

promoter, an EF-1α (human elongation factor-1α) promoter, an HSP70 promoter, an MT promoter, an RSV promoter, a UBC promoter, and an actin promoter; and artificial (fusion) promoters such as an SRα promoter and a CAG promoter. Further, the poly(A) addition sequence may be a sequence having the activity of causing transcription termination for the transcription from the promoter, and may be a sequence from a gene the same as or different from the promoter.

It is necessary to use a strong promoter in order to increase the production of a foreign protein. However, when it is attempted to produce a protein which is difficult to have fold or a protein which is difficult to get secreted using a highly active promoter, the protein may instead fail to be secreted. This is because when the protein is produced in an amount exceeding the capacity of the ribosome in which translation is performed and the endoplasmic reticulum where folding and secretion are performed, the excessively produced protein is denatured, accumulated, and ubiquitinated in cells, and then degraded by proteosomes. Accordingly, it is preferred that a promoter, which can attain an expression level to such an extent that the resulting protein is not denatured or aggregated or the amount of the resulting protein does not exceed the secretion capacity, is appropriately selected. Alternatively, the promoter is used by adjusting (for example, decreasing) the activity of the promoter. Among the

multimeric proteins, a molecule forming a heteromultimer is susceptible to the above-described effect, and, in particular a molecule, such as an antibody, which is a heterotetramer. An antibody has two heavy chain molecules and two light chain molecules which are associated with one another, and therefore, in order to appropriately associate the molecules, the expression level thereof is an important factor.

Further, the foreign gene expression vector and the element vector of the invention can each contain a selection marker for selecting a transformant. By using, for example, a drug-resistant marker which imparts resistance to a drug such as cerulenin, aureobasidin, Zeocin, canavanine, cycloheximide, hygromycin, puromycin, blasticidin, tetracycline, kanamycin, ampicillin, or neomycin, a transformant can be selected. Further, where a gene which imparts resistance to a solvent such as ethanol, resistance to the osmotic pressure of glycerol, a salt, or the like, resistance to a metal ion such as a copper ion, or the like is used as a marker, a transformant can also be selected.

The foreign gene expression vector and the element vector of the invention may each be a vector which is not incorporated into the chromosomal DNA. In general, the foreign gene expression vector is transfected into a host cell, and thereafter randomly incorporated into the chromosome. However, by using a constituent component derived from a

mammalian virus such as simian virus 40 (SV40), a papillomavirus (BPV, HPV), or EBV, the vector can be used as an episomal vector which is self-replicable in the transfected host cell. For example, a vector containing an SV40-derived replication origin (oriP) and a sequence encoding an SV40 large T antigen which is a trans-acting factor, a vector containing an EBV-derived oriP and a sequence encoding EBNA-1, or the like can be used. The effect of the DNA element can be expressed by the activity of enhancing foreign gene expression regardless of the type of vector or the presence or absence of incorporation thereof into the chromosome.

4. Transformed cell

The transformed cell of the invention is a transformed cell into which the foreign gene expression vector described in the above item "3" containing the DNA element described in the above item "1" has been introduced. As the foreign gene expression vector, only a foreign gene expression vector containing a DNA element may be introduced (A), or a foreign gene expression vector containing a DNA element and also an element vector described in the above item "3" may be introduced in combination (B). Alternatively, a foreign gene expression vector containing no DNA element and an element vector may be introduced in combination (C).

The expression of a foreign gene in a host cell using

the above combination of (B) or (C) can be performed according to, for example, the method of Girod et al. (Biotechnology and Bioengineering, 91, 2-11 (2005)) and the method of Otte et al. (Biotechnol. Prog., 2007, 23, 801-807 (2007)).

Examples of the host cell to be transformed include eucaryotic cells, preferred examples thereof include mammalian cells, more preferred examples include cells derived from humans, mice, rats, hamsters, monkeys, or cattle. Examples of such mammalian cells include COS-1 cells, 293 cells, and CHO cells (CHO-K1, DG44, CHO dhfr-, CHO-S), however, the host cell is not limited thereto.

In the invention, any method may be used for introducing the expression vector into the host cell as long as the method allows the introduced gene to be stably present in the host cell and to be adequately expressed therein. Examples of the method which is generally used include a calcium phosphate method (Ito et al., (1984) Agric. Biol. Chem., 48, 341), an electroporation method (Becker, D. M. et al., 1990; Methods. Enzymol., 194, 182-187), a spheroplast method (Creggh et al., Mol. Cell. Biol., 5, 3376 (1985)), a lithium acetate method (Itoh, H. (1983) J. Bacteriol. 153, 163-168), and a lipofection method.

5. Method for producing foreign protein

In the invention, a foreign protein can be produced by

culturing the transformed cell described in the above item "4", into which a gene encoding the foreign protein has been introduced using the vector described in the above item "3" by a known method, collecting the protein from the resulting culture product, followed by purification of the protein. The term "culture product" as used herein refers to cultured cells or a cell homogenate in addition to a culture supernatant. Incidentally, as the foreign protein which can be produced using the transformed cell described in the above item "4", not only a monomeric protein, but also a multimeric protein can be selected. In cases where a hetero-multimeric protein formed of a plurality of different subunits is produced, it is necessary to introduce a plurality of genes encoding these subunits into the host cell described in the above item "4", respectively.

The method for culturing the transformed cell can be performed according to conventional methods for culturing host cells.

In cases where the transformed cell is a mammalian cell, the cell is cultured under conditions of, for example, 37°C and 5% or 8% CO_2 for a culture time of from about 24 to 1000 hours. The culturing can be performed through batch culture, fed-batch culture, continuous culture, or the like under static, shaking, stirring, or aeration conditions.

The confirmation of the expression product of the gene

encoding the foreign protein from the above-mentioned culture product (culture solution) can be performed by SDS-PAGE, a Western analysis, ELISA, or the like. In order to isolate and purify the produced protein, a conventional protein isolation and purification method may be used. After completion of the culturing, in cases where the target protein is produced in the cells, the cells are homogenized using an ultrasonic homogenizer, a French press, a Manton-Gaulin homogenizer, Dinomil, or the like, thereby obtaining the target protein. Further, cases where the target protein is produced outside the cells, the culture solution is used as such, or the cells are removed by centrifugation or the like. Thereafter, the target protein is collected by extraction or the like using an organic solvent, and then the collected target protein may be isolated and purified by using techniques such as various chromatography techniques (hydrophobic chromatography, reverse-phase chromatography, affinity chromatography, ion exchange chromatography, etc.), gel filtration using a molecular sieve, and electrophoresis using a polyacrylamide gel or the like alone or in combination according to need.

The above-mentioned culturing methods and purification methods are only examples, and the methods are not limited thereto. The amino acid sequence of the purified gene product can be confirmed by a known amino acid analysis technique, such as automated amino acid sequence determination using the Edman

degradation method.

6. Method for producing antibody protein

As the hetero-multimeric protein to be produced using the production method described in the above item "5", an antibody protein can be exemplified. The antibody protein is a tetrameric protein comprising two molecules of heavy chain polypeptides and two molecules of light chain polypeptides. Accordingly, in order to obtain such an antibody protein in a state of maintaining an antigen-binding affinity, it is necessary to introduce both heavy and light chain genes into the transformed cell described in the above item "4". In this case, the heavy and light chain gene expression units may be present on the same expression vector or different expression vectors.

As the antibody to be produced in the invention, an antibody prepared by immunizing an experimental animal such as a rabbit, a mouse, or a rat with a desired antigen can be exemplified. Further, a chimeric antibody and a humanized antibody obtained by using the above-mentioned antibody as a starting material can be also exemplified as the antibody to be produced in the invention. Further, a human antibody obtained using a genetically modified animal or a phage display method is also included in the antibody to be produced in the invention.

The antibody gene to be used for the production of the antibody is not limited to an antibody gene having a specific polynucleotide sequence as long as a combination of a heavy chain polypeptide and a light chain polypeptide to be transcribed and translated from the antibody gene has an activity of binding to a given antigen protein.

Further, it is not necessary that the antibody gene encodes the full-length molecule of the antibody, and a gene encoding a functional fragment of the antibody can be used. Such a gene encoding a functional fragment thereof can be obtained by genetically modifying a gene encoding the full-length molecule of an antibody protein.

7. Production method for other foreign proteins

Examples of the foreign protein to be produced using the production method of the invention include, in addition to the above-mentioned antibodies, various proteins derived from humans or non-humans, functional fragments thereof, and modified products thereof. Examples of such proteins and the like include peptide hormones such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), vasopressin, somatostatin, growth hormone (GH), insulin, oxytocin, ghrelin, leptin, adiponectin, renin, calcitonin, osteoprotegerin, and insulin-like growth factor (IGF); cytokines such as interleukin, chemokine,

interferon, tumor necrosis factors (such as TNF-α, TNF-β, and TNF super family), nerve growth factors (such as NGF), cell growth factors (such as EGF, FGF, PDGF, HGF, and TGF), hematopoietic growth factors (such as CSF, G-CSF, and erythropoietin), and adipokine; receptors such as TNF receptors; enzymes such as lysozyme, protease, proteinase, and peptidase; functional fragments thereof (fragments having part or all of the biological activity of the original protein), and fusion proteins comprising any of these proteins. However, the proteins are not limited thereto.

Examples

Hereinafter, the invention will be specifically described with reference to the Examples. However, these Examples do not limit the technical scope of the invention. The plasmids, restriction enzymes, DNA modification enzymes, and the like to be used in the Examples of the invention are commercially available products and can be used according to common procedures. Further, procedures used for DNA cloning, polynucleotide sequence determination, transformation of a host cell, culturing of a transformed host cell, collection of a protein from the resulting culture product, purification of a protein, and the like are also well known to those skilled in the art or can be found in the literature.

Example 1

Extraction of DNA element

(1-1) Chromatin immunoprecipitation using anti-acetylated histone H3 antibody

ChIP using an anti-acetylated histone antibody was performed using EZ ChIP (Upstate) according to the following procedure. Incidentally, unless otherwise stated, as the antibodies, buffers, and the like used in the following procedure, Upstate's products were used.

First, 293F cells (Invitrogen) were cultured using GIBCO (registered trademark) FreeStyle™ 293 Medium (Invitrogen) under conditions of 37°C and 8% CO_2, followed by centrifugation (1000 rpm, 5 min, room temperature), whereby cells in the growth phase were collected. After 2×10^7 cells were fixed in a medium containing 1% formaldehyde for 10 minutes, 10x glycine was added thereto, followed by incubation at room temperature for 5 minutes. After centrifugation (3000 rpm, 5 min, 4°C), the supernatant was removed, and PBS was added to the cell pellet to suspend the cells. Then, the cell suspension was centrifuged again to remove PBS, and thereafter an SDS lysis buffer was added to the cell pellet to suspend and lyse the cells. Each sample obtained by cell lysis was subjected to DNA fragmentation using an ultrasonic homogenizer (BRANSON) while cooling the sample with ice water, and a dilution buffer containing a protease inhibitor cocktail and Protein

G-immobilized agarose were added thereto. The resulting mixture was rotated at 4°C for 1 hour, followed by centrifugation, and then the supernatant was collected. Subsequently, 10 μg of normal rabbit IgG or an α-acetyl histone H3 antibody was added thereto, followed by rotating overnight at 4°C. To the resulting solution, Protein G-immobilized agarose was added, and the resulting mixture was rotated at 4°C for 1 hour, followed by centrifugation, and then the pellet was collected. The thus obtained pellet was washed twice with Low Salt Immune Complex Wash Buffer, twice with High Salt Immune Complex Wash Buffer, twice with LiCl Immune Complex Wash Buffer, and finally four times with TE Buffer. Then an elution buffer (containing 20 μl of 1 M sodium hydrogen carbonate, 10 μl of SDS, and 170 μl of sterile water) was added thereto. After 30 minutes, the mixture was centrifuged, and the supernatant was collected.

Subsequently, 5 M sodium chloride was added to the supernatant, and the resulting mixture was heated overnight at 65°C. Then RNase A was added thereto, and the resulting mixture was incubated at 37°C for 30 minutes. Then 0.5 M EDTA, 1 M Tris-HCl, and Proteinase K were added thereto, and the resulting mixture was incubated at 45°C for 2 hours.

Finally, Reagents A, B, and C were added thereto in an amount 5 times greater than that of the solution obtained by the treatment with Proteinase K, followed by centrifugation

(10000 rpm, 30 sec, room temperature) using Spin filter, whereby chromatin-immunoprecipitated DNA was purified.

(1-2) Microarray analysis

By using GenomePlex Complete Whole Genome Amplification (WGA) Kit (Sigma), each ChIP sample obtained in (1-1) was amplified. The procedure was in accordance with Sigma's protocol accompanying the Kit.

In order to confirm ChIP, by using 320 ng of each DNA amplified by WGA as a template, and also using the following primers and SYBR (registered trademark) Premix Ex Taq™ (Perfect Real Time) (TAKARA), a glycelaldehyde-3-phosphate dehydrogenase (GAPDH) internal gene was amplified by the PCR method (95°C for 5 sec and 60°C for 20 sec x 45 cycles). Incidentally, GAPDH is a house keeping gene to be used as a positive control for confirming whether a DNA element is enriched by ChIP, and the PCR method was performed using primers attached to EZ ChIP (Upstate).

5'-TACTAGCGGTTTTACGGGCG-3'

5'-TCGAACAGGAGGAGCAGAGAGCGA-3'

As shown in Fig. 1, it was confirmed that GAPDH was amplified specifically in the sample subjected to immunoprecipitation with an anti-acetylated histone H3 antibody. Each of the DNA samples amplified by WGA was subjected to microarray analysis (NimbleGen) to perform

Chromatin Immunoprecipitation-on-chip (ChIP-on-chip). "ChIP-on-chip" is a technique for identifying each DNA element by subjecting each DNA enriched in (1-1) to microarray analysis.

(1-3) Extraction of DNA element

Based on the results of the ChIP-on-chip analysis obtained in (1-2), 5 sequences having an AT content of 62% or more were extracted.

A2: chromosome 15 (80966429 to 80974878)

A7: chromosome 11 (88992123 to 89000542)

A18: chromosome 4 (111275976 to 111284450)

B5: chromosome 1 (143034684 to 143043084)

C14: chromosome 11 (46089056 to 46097482)

Example 2

Effect of DNA element using expression of secretory alkaline phosphatase (SEAP) as index

(2-1) Construction of SEAP expression vector

By using pSEAP2-control (Clontech) as a template, the SEAP gene was amplified by the PCR method (94°C for 30 sec and 68°C for 2 min x 40 cycles) using the following primers and KOD-plus- (TOYOBO).

5'-AAAGCTAGCATGCTGCTGCTGCTGCTGCTGGGCC-3'

5'-AAAAGATCTTCATGTCTGCTCGAAGCGGCCGGCCGC-3'

Subsequently, the amplified SEAP fragment was separated by agarose gel electrophoresis and cut out from the gel, followed by purification using a QIAquick Gel Extraction Kit (Qiagen). The thus obtained DNA fragment was used as an insert. The insert was digested with the restriction enzymes NheI and BglII, and a vector pIRES hyg3 (Clontech) was digested with the restriction enzymes NheI and BamHI. The resulting DNA fragments were subjected to agarose gel electrophoresis to separate the target fragments, respectively, and the target fragments were cut out from the gel, followed by purification. Then, a ligation reaction and transformation were performed. The ligation reaction was performed using LigaFast Rapid DNA Ligation System (Promega). The transformation was performed as follows. First, frozen competent cells JM109 (TAKARA) were thawed, and 10 μl of a solution obtained after the ligation reaction was added to a solution of the thawed cells, and the resulting mixture was left to stand on ice for 30 minutes. Thereafter, a heat shock (42°C, 45 sec) was applied to the mixture, and the mixture was cooled on ice for 5 minutes. To this cell suspension, 1 ml of LB medium was added, and the resulting mixture was shaken at 37°C for 1 hour. Then, the mixture was plated on an LB plate containing 0.1 mg/ml ampicillin, and the plate was incubated at 37°C for 14 to 16 hours. Thereafter, by alkaline lysis, a target plasmid was collected from colonies cultured on the LB plate. Finally,

the polynucleotide sequence of SEAP in the plasmid obtained by alkaline lysis was determined, whereby pCMV/SEAP ires Hygro was constructed.

(2-2) Cloning of DNA element

Subsequently, each of the DNA elements extracted in Example 1 was cloned into the SEAP expression vector obtained in (2-1) using BAC SUBCLONING Kit (Gene Bridges) from a bacterial artificial chromosome (BAC) containing a polynucleotide sequence corresponding to each of the DNA elements.

First, pCMV/SEAP ires Hygro obtained in (2-1) was digested with the restriction enzyme SpeI for several hours, followed by ethanol precipitation, and the precipitate was dissolved in sterile water. By using the vector digested with SpeI as a template, the PCR method (94°C for 15 sec, 55°C for 30 sec, and 68°C for 10 min x 30 cycles) was performed using the following primers and KOD-plus- (TOYOBO).

A2D:

5′-GGAAATTGAGAAGTATCATTCACAACAGTACCACAAACATGAAATAAATGTGGAT CCTATTAATAGTAATCAATTACG-3′

A2R:

5′-CTCATTCTGTGGGTTGTCATTTCACTTCCTTGATGCTATCCTTTCAAGCAAAATC CTAGTCAATAATCAATGTCAACG-3′

A7D:

5′-CTTATTTTCTAAGTAGTATAGACTTAATTGTGAGAACAAAATAAAAACTTGGATC

CTATTAATAGTAATCAATTACG-3′

A7R:

5′-CTCTTCCCATTCTCATTTGAATCTACTTCAAAAGGTTTACCATACTAAGACCTAG

TCAATAATCAATGTCAACG-3′

A18D:

5′-CGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATCACCTGAGGTCGA

TCCTATTAATAGTAATCAATTACG-3′

A18R:

5′-CATACAGAAGCCAGTTTGAACTGAGACCTCACTCCATTTCTTACAAGTTATGCCC

TAGTCAATAATCAATGTCAACG-3′

B5D:

5′-ACCGTTTTATATTGTTTAAGCATTTCCTAGACATATTTGGCTACAAATCTAGATC

CTATTAATAGTAATCAATTACG-3′

B5R:

5′-GATCTTAGGGGGGCTGATTATATAAAACAATAGAAATGTAGTCTTAGATGAAACC

TAGTCAATAATCAATGTCAACG-3′

C14D:

5′-CACAAAGTTCACTGTCAAGGCCAGGTGATGAGGCCCACACATGCCCGGACCTTGA

TCCTATTAATAGTAATCAATTACG-3′

C14R:

5′-CAAAACCTCATCTCTACTGAAAATAGAAAATTAGCTGGGCGTGGTGGCAGGTGCC

CTAGTCAATAATCAATGTCAACG-3'

After the amplification was confirmed by agarose gel electrophoresis using a portion of the reaction solution, the rest of the reaction solution was subjected to ethanol precipitation. The precipitate was dissolved in sterile water, and the resulting solution was used as DNA for transformation.

Subsequently, preparation of *Escherichia coli* for transformation was performed.

BAC clones corresponding to the 5 sequences extracted in Example 1 are as follows.

Extracted sequence	Corresponding BAC clone
A2	RP11-152F13
A7	RP11-643G5
A18	RP11-115A14
B5	RP11-640M9
C14	RP11-702F3

10 μl of the above-mentioned BAC (Advanced GenoTechs Co.) which was thawed was inoculated into 1 ml of a medium (containing chloramphenicol at a final concentration of 15 μg/ml) and incubated overnight at 37°C. 30 μl of the culture solution was transferred to 1.4 ml of a medium (containing chloramphenicol at a final concentration of 15 μg/ml) and incubated at 37°C for 2 hours. Centrifugation and washing with sterile water were repeated twice, and the cells were suspended in 20 μl of sterile water. To a cooled cuvette (0.1 cm), 1 μl of pRED/ET (Gene Bridges) and *Escherichia coli* were added,

followed by electroporation (1350 V, 10 μF). Then, 1 ml of SOC medium was added thereto, and the resulting mixture was incubated at 30°C for 70 minutes. 100 μl of the culture solution was plated on an LB plate (containing tetracycline and chloramphenicol at final concentrations of 3 μg/ml and 15 μg/ml, respectively), and incubated overnight at 30°C. On the subsequent day, each colony thus obtained was inoculated into 1 ml of a medium (containing tetracycline and chloramphenicol at final concentrations of 3 μg/ml and 15 μg/ml, respectively), and incubated overnight at 30°C. 30 μl of the culture solution was transferred to 1.4 ml of a medium (containing tetracycline and chloramphenicol at final concentrations of 3 μg/ml and 15 μg/ml, respectively), and incubated at 30°C for 2 hours. Then, 50 μl of 10% L-arabinose was added thereto, and incubation was further performed at 37°C for 1 hour. Thereafter, washing with sterile water was repeated twice, and *Escherichia coli* which was suspended in 30 μl of sterile water and 1 μl of the DNA for transformation were added to a cooled cuvette (0.1 cm), followed by electroporation (1350 V, 10 μF). Then, 1 ml of SOC medium was added thereto, and the resulting mixture was incubated at 37°C for 90 minutes. The total amount of the culture solution was plated on an LB plate (containing 100 μg/ml ampicillin), and the plate was incubated. Thereafter, a target plasmid was obtained by alkaline lysis. Finally, the sequence of the obtained plasmid and the restriction enzyme

sites thereof were confirmed, whereby a target plasmid was constructed. The vector construct is shown in Fig. 2.

(2-3) Evaluation using SEAP expression as index

Each plasmid constructed in (2-2) was evaluated using the host cell CHO-K1 (ATCC) and transfection reagent Lipofectamine 2000 (Invitrogen).

Antibiotic selection with hygromycin at 800 μg/ml was performed for about 2 weeks starting 2 days after transfection, whereby a stably expressing polyclonal cell line was established. The thus established cell line was subjected to medium replacement on the day before measurement, and a given number of the cells were seeded into a 24-well plate (IWAKI). At 24 hours after plating the cells, the culture supernatant was collected, and the activity of SEAP was measured. The activity of SEAP in the culture supernatant was measured using SensoLyte™ pNPP Secreted Alkaline Phosphatase Reporter Assay (ANASPEC).

The measured results are shown in Fig. 3. When the activity of SEAP of the control with no element was normalized to 1, the activity of SEAP in the culture supernatant of the stably expressing CHO cell line having the DNA element A2, A7, A18, B5, or C14 showed a numerical value five times or more higher than that of the control. Based on the results, it was confirmed that all the 5 types of DNA elements dramatically

enhance SEAP expression. Incidentally, the polynucleotide sequences of the above 5 types of DNA elements are represented by SEQ ID NOS: 1 to 5 in the Sequence Listing, respectively.

Example 3

Generality of promoter to be used in combination

The promoter for the vector used in the evaluation of the DNA elements in Example 2 was a CMV promoter, and thus the use of DNA elements in combination with other general promoters was studied in Example 3.

(3-1) Construction of SEAP expression vector using EF-1α and SV40 promoters

By using pSEAP2-control (Clontech) as a template, the SEAP gene was amplified by the PCR method (94°C for 30 sec and 68°C for 2 min x 40 cycles) using the primers described in (2-1) and KOD-plus-. The amplified SEAP was prepared as an insert in the same manner as in (2-1). The insert was digested with the restriction enzymes NheI and BglII, and a vector pIRES puro3 (Clontech) was digested with the restriction enzymes NheI and BamHI, and pCMV/SEAP ires Puro was constructed in the same manner as in (2-1).

Subsequently, by using pEF1/V5-His A (Invitrogen) as a template, an EF-1α promoter was amplified by the PCR method (94°C for 15 sec, 60°C for 30 sec, and 68°C for 2 min x 30 cycles) using the following primers and KOD-plus-.

5'-AAAACTAGTCAGAGAGGAATCTTTGCAGCTAATGGACC-3'

5'-AAAGATATCCCTAGCCAGCTTGGGTGGTACCAAGC-3'

By using the above-constructed pCMV/SEAP ires Puro as a vector, digestion with the restriction enzymes SpeI and EcoRV was performed for the vector and the promoter, and pEF/SEAP ires Puro was constructed according to the method described in (2-1).

Similarly, by using pcDNA3.1+ (Invitrogen) as a template, an SV40 promoter was amplified by the PCR method (94°C for 15 sec, 60°C for 30 sec, and 68°C for 1 min x 30 cycles) using the following primers and KOD-plus-.

5'-AAAACTAGTCTGTGGAATGTGTGTCAGTTAGGGTG-3'

5'-AAAGATATCAGCTTTTTGCAAAAGCCTAGGCCTC-3'

By using the above-constructed pCMV/SEAP ires Puro as a vector, digestion with the restriction enzymes SpeI and EcoRV was performed for the vector and the promoter, and pSV40/SEAP ires Puro was constructed according to the method described in (2-1).

(3-2) Cloning of DNA element A2 or A7

Subsequently, cloning of the DNA element A2 or A7 was performed using pEF/SEAP ires Puro and pSV40/SEAP ires Puro constructed in (3-1) as basic structures.

First, pEF/SEAP ires Puro and pSV40/SEAP ires Puro were digested with the restriction enzyme SpeI for several hours,

followed by ethanol precipitation, and the precipitate was dissolved in sterile water. By using the respective vectors digested with SpeI as templates, DNA for transformation was prepared by the PCR method (94°C for 15 sec, 55°C for 30 sec, and 68°C for 10 min x 30 cycles) using the following primers and KOD-plus-.

A2 (EF/D):

5′-GGAAATTGAGAAGTATCATTCACAACAGTACCACAAACATGAAATAAATGTGCTA
GTCAGAGAGGAATCTTTGCAGC-3′

A2 (SV40/D):

5′-GGAAATTGAGAAGTATCATTCACAACAGTACCACAAACATGAAATAAATGTGCTA
GTCTGTGGAATGTGTGTCAGTTAG-3′

A2 (EF and SV40/R):

5′-CTCATTCTGTGGGTTGTCATTTCACTTCCTTGATGCTATCCTTTCAAGCAAAATT
TTAAAACTTTATCCATCTTTGCA-3′

A7 (EF/D):

5′-CTTATTTTCTAAGTAGTATAGACTTAATTGTGAGAACAAAATAAAAACTTGCTAG
TCAGAGAGGAATCTTTGCAGC-3′

A7 (SV40/D):

5′-CTTATTTTCTAAGTAGTATAGACTTAATTGTGAGAACAAAATAAAAACTTGCTAG
TCTGTGGAATGTGTGTCAGTTAG-3′

A7 (EF and SV40/R):

5′-CTCTTCCCATTCTCATTTGAATCTACTTCAAAAGGTTTACCATACTAAGAACTAG
TTTTAAAACTTTATCCATCTTTGCA-3′

By using the thus prepared DNA for transformation and BAC transfected with pRed/ET, the DNA element A2 or A7 was cloned into the vector described in (3-1). The vector construct is shown in Fig. 2. Incidentally, the procedure was performed according to the method described in (2-2).

(3-3) Evaluation using SEAP expression as index

Each plasmid constructed in (3-2) was evaluated using the host cell CHO-K1 (ATCC) and transfection reagent Lipofectamine 2000 (Invitrogen).

Antibiotic selection with puromycin at 8 μg/ml was performed for about 2 weeks starting 2 days after transfection, whereby a stably expressing polyclonal cell line was established. The thus established cell line was subjected to medium replacement on the day before measurement, and a given number of the cells were seeded into a 24-well plate. At 24 hours after plating the cells, the culture supernatant was collected, and the activity of SEAP was measured. The activity of SEAP in the culture supernatant was measured using SensoLyte™ pNPP Secreted Alkaline Phosphatase Reporter Assay (ANASPEC).

The measurement results are shown in Fig. 4. When the activity of SEAP of the control with no element was normalized to 1, the DNA element A2 or A7 exhibited an effect on enhancement of expression such that the activity of SEAP was twice or more

as high in the case of use with the EF-1α promoter, and four times or more higher in the case of use with the SV40 promoter than that of the control. Based on the results, it was confirmed that these DNA elements exhibit the effect of enhancing foreign gene expression when used in combination with a general promoter.

Example 4

Evaluation using antibody expression as index

(4-1) Construction of human light chain expression vector pEF6KCL

By using a plasmid pEF6/V5-HisB (Invitrogen) as a template, a DNA fragment between position 2174 (immediately downstream of BGHpA) and position 2958 (SmaI) (a DNA fragment containing an f1 origin of replication and SV40 promoter and origin, hereinafter referred to as "fragment A", the polynucleotide sequence of fragment A being represented by SEQ ID NO: 6 in the Sequence Listing) was obtained by the PCR method using the following primers and KOD-plus-.

5'-CCACGCGCCCTGTAGCGGCGCATTAAGC-3'

5'-AAACCCGGGAGCTTTTTGCAAAAGCCTAGG-3'

The obtained fragment A and a DNA fragment containing a DNA sequence encoding a human κ chain secretory signal, a human κ chain constant region, and a human poly(A) addition signal (hereinafter referred to as "fragment B") were ligated

by overlapping PCR. The thus obtained DNA fragment in which fragment A and fragment B were ligated was digested with the restriction enzymes KpnI and SmaI, and the resulting fragment was ligated to plasmid pEF6/V5-HisB (Invitrogen) which was digested with the restriction enzymes KpnI and SmaI, whereby a human light chain expression vector pEF6KCL having a signal sequence, a cloning site, a human κ chain constant region, and a human poly(A) addition signal sequence downstream of the EF-1α promoter was constructed.

A DNA fragment obtained by cleaving the pEF6KCL obtained by the above-mentioned method with the restriction enzymes KpnI and SmaI was ligated to pEF1/myc-HisB (Invitrogen) which was digested with KpnI and SmaI, followed by transformation alkaline lysis, and its sequence confirmation, whereby a plasmid pEF1KCL was constructed.

(4-2) Construction of human heavy chain expression vector pEF1FCCU

A DNA fragment (the polynucleotide sequence of this DNA fragment is represented by SEQ ID NO: 7 in the Sequence Listing) containing a DNA sequence encoding a human IgG1 signal sequence and a constant region amino acid sequence was digested with the restriction enzymes NheI and PmeI, and the resulting fragment was ligated to a plasmid pEF1KCL which was digested with NheI and PmeI, whereby a human heavy chain expression

vector pEF1FCCU having a signal sequence, a cloning site, a human heavy chain constant region, and a human poly(A) addition signal sequence downstream of the EF-1α promoter was constructed.

(4-3) Construction of single humanized antibody gene X expression vector (humanized antibody gene X/pEF_LHN#)

By ligating the L-chain or H-chain expression vector constructed in (4-1) or (4-2), a single humanized antibody expression vector (pEF_LHN (lacking a variable region)) was constructed.

A restriction enzyme SalI site was added by the PCR method to both ends of the gene expression unit from upstream of the promoter to downstream of poly(A) of pEF1KCL. Agarose gel electrophoresis, cutting out of a desired DNA fragment from the gel, and purification of the DNA fragment were then performed, whereby an insert was prepared. By digesting the pEF1FCCU constructed in (4-2) with the restriction enzyme SalI, the vector was linearized at the SalI site located upstream of the gene expression unit. Then, the linearized vector was ligated to the above insert, followed by transformation, alkaline lysis, and sequence confirmation, whereby a single humanized antibody expression vector (pEF_LHN (lacking a variable region)) was constructed.

Subsequently, the following oligonucleotides were

introduced into an AatII site of the vector pEF_LHN (lacking a variable region).

5′-CGCGGCCGCACTAGTGACGT-3′

5′-CACTAGTGCGGCCGCGACGT-3′

The respective oligonucleotides were diluted to 5 pmol, and by using T4 Polynucleotide Kinase (TAKARA), a reaction was allowed to proceed at 37°C for 1 hour. Then, 10x buffer (TAKARA) was added thereto, and annealing was performed at 96°C for 1 minute at room temperature. These oligonucleotides and the vector pEF_LHN which was digested with the restriction enzyme AatII were ligated, followed by transformation, alkaline lysis, and sequence confirmation, whereby pEF_LHN# (lacking a variable region) was constructed.

By integrating a variable region of the humanized antibody gene X into the above-constructed universal vector (pEF_LHN# (lacking a variable region)), the construction of a humanized antibody gene X expression single vector (humanized antibody gene X/pEF_LHN#) was completed.

First, by using the following primers and KOD-plus-, an L-chain variable region of the humanized antibody gene X was amplified by the PCR method (94°C for 15 sec, 55°C for 30 sec, and 68°C for 1 min x 30 cycles).

L-chain variable region:

5′-AAACATATGGCGACATCCAGATGAC-3′

5′-AAACGTACGCTTGATCTCCACCTTGG-3′

The amplified L-chain variable region fragment and the universal vector (pEF_LHN# (lacking a variable region)) were digested with the restriction enzymes NdeI and BsiWI, followed by agarose gel electrophoresis, cutting out of a desired fragment from the gel, purification, ligation reaction, transformation, alkaline lysis, and sequence confirmation, whereby the L-chain variable region was integrated into the vector. In the same manner, by using the following primers and KOD-plus-, an H-chain variable region of the humanized antibody gene X was amplified by the PCR method (94°C for 15 sec, 55°C for 30 sec, and 68°C for 1 min x 30 cycles).

H-chain variable region:

5'-AAAGCTGAGCCAGGTGCAGCTGCAGG-3'

5'-AAAGCTGAGCTCACGGTCACCAGGGTTC-3'

The amplified H-chain variable region fragment and the vector having the L-chain variable region inserted therein were digested with the restriction enzyme BlpI, followed by agarose gel electrophoresis, cutting out of a desired fragment from the gel, purification, ligation reaction, transformation, alkaline lysis, and sequence confirmation, whereby the H-chain variable region was integrated into the vector and a single humanized antibody gene X expression vector (humanized antibody gene X/pEF_LHN#) was constructed.

(4-4) Construction of single humanized antibody gene X

expression vector (humanized antibody gene X/pCMV_LHN#)

By using the single humanized antibody gene X expression vector (humanized antibody gene X/pEF_LHN#) constructed in (4-3) as a basic vector structure, another single humanized antibody gene X expression vector (humanized antibody gene X/pCMV_LHN#) was constructed by replacing the promoter according to the following procedure.

By using pIRES puro3 as a template, a CMV promoter fragment was amplified by the PCR method (94°C for 30 sec and 68°C for 3 min x 40 cycles) using the following primers and KOD-plus-.

Upstream of H-chain:

5′-CTTTTGCAAAAAGCTTCGCGTTACATAACTTACGGTAAATGGCC-3′

5′-TTCATGGTGGCGCTAGCCCGCAGATATCGATCCGAGCTCGGTA-3′

Upstream of L-chain:

5′-TGACGTCGACAAGCTTCGCGTTACATAACTTACGGTAAATGGCC-3′

5′-CTGGATGTCGCCATATGCGCCGGAGATCCACAGCAGCAGGGAGATGAACACCTGG

GTCTGCAGCACCATGGTGGCGCTAGCCCGCAGATATCGATCCGAGCTCGGTA-3′

To the PCR reaction solution, the restriction enzyme DpnI was added, and a reaction was allowed to proceed at 37°C for 1 hour, followed by purification using miniElute reaction Cleanup kit (Qiagen), whereby a sample for use in In-Fusion was prepared. Meanwhile, the humanized antibody gene X/pEF_LHN# was digested with the restriction enzymes HindIII, NheI, NdeI, and FseI, followed by agarose gel electrophoresis,

whereby two large fragments among the resulting fragments were separated. Each of the fragments was cut out from the gel, and the DNA was extracted from the gel, whereby a sample for use in In-Fusion was prepared. All the samples for use in In-Fusion were put together, and cloning was performed using In-Fusion™ Advantage PCR Cloning Kit (TAKARA), followed by transformation, alkaline lysis, and sequence confirmation, whereby a single humanized antibody gene X expression vector (humanized antibody gene X/pCMV_LHN#) was constructed.

(4-5) Cloning of DNA element A7

A7 was selected from the 5 types of the DNA elements which were confirmed to have an effect of enhancing SEAP expression, and cloned into an antibody expression vector.

In the same manner as in (2-2), by using each of the humanized antibody gene X expression single vectors (humanized antibody gene X/pEF_LHN# and humanized antibody gene X/pCMV_LHN#) digested with the restriction enzyme NotI as a template, DNA for transformation was prepared by the PCR method (94°C for 15 sec, 55°C for 30 sec, and 68°C for 11 min x 30 cycles) which was performed using the following primers and KOD-plus-.

Humanized antibody gene X/pEF_LHN# D:

5'-CTCTTCCCATTCTCATTTGAATCTACTTCAAAAGGTTTACCATACTAAGACTCGA GGCACTAGTGACGTCAGGTGGCACT-3'

Humanized antibody gene X/pEF_LHN# R:

5′-CTCTTCCCATTCTCATTTGAATCTACTTCAAAAGGTTTACCATACTAAGAGCACT
AGTGACGTCAGGTGGCACTTTTCGG-3′

Humanized antibody gene X/pCMV_LHN# D:
Humanized antibody gene X/pEF_LHN# D was used.
Humanized antibody gene X/pCMV_LHN# R:
Humanized antibody gene X/pEF_LHN# R was used.

By using the above-prepared DNA for transformation and BAC transfected with pRed/ET, the DNA element A7 was cloned into the single humanized antibody gene X expression vectors described in (4-3) and (4-4). The vector construct is shown in Fig. 5. Incidentally, the procedure was performed according to the method described in (2-2).

(4-6) Evaluation using antibody expression as index

Each plasmid constructed in (4-5) was evaluated using the host cell CHO-K1 (ATCC) and transfection reagent Lipofectamine 2000 (Invitrogen).

Antibiotic selection with Geneticin (Roche) at 800 μg/ml was performed for about 2 weeks starting 2 days after transfection, whereby a stably expressing polyclonal cell line was established. The thus established cell line was subjected to medium replacement on the day before measurement, and a given number of the cells were seeded into a 24-well plate. At 24

hours after plating the cells, the culture supernatant was collected, and the expression level of the antibody in the culture supernatant was measured by the ELISA method. Incidentally, the ELISA was performed as follows. To a 96-well plate coated with anti-kappa light chain at 50 ng/well, 100 μl of the cell-free culture supernatant was added to each well, and the plate was incubated at 37°C for 1 hour. Subsequently, the sample (culture supernatant) was removed, and each well was washed with 200 μl of PBS-Tween (0.05%). Then, 100 μl of HRP-labeled anti-human IgG (Fc) was added to each well and the plate was incubated at 37°C for an additional 1 hour. Thereafter, the HRP-labeled anti-human IgG (Fc) was removed, and each well was washed with PBS-Tween (0.05%). Then, a color was developed using a POD Substrate ABTS Kit (Nacalai), and an absorbance at a measurement wavelength of 405 nm was measured. For the dilution of the anti-kappa light chain, the anti-human IgG (Fc), and the sample, PBS-Tween (0.05%) was used. By using human IgG serially diluted to 12 ng, 6 ng, 3 ng, 1.5 ng, 0.75 ng, 0.375 ng, and 0.1875 ng as a standard, the concentration of the sample was calculated.

The results are shown in Fig. 6. It was confirmed that the sample having the DNA element A7 has a higher effect of enhancing antibody production as compared with a control with no element when the EF-1α promoter or the CMV promoter was used in the antibody expression vector.

Example 5

Length of sequence exhibiting activity of enhancing foreign gene expression

(5-1) Cloning of DNA elements having different sequence lengths

Based on the length of the sequence used in Example 2, vectors containing each of the DNA elements but having different sequence lengths were constructed.

The details of the DNA elements having different sequence lengths which were designed based on the full length of each of the DNA elements A2, A7, A18, B5, and C14 are shown in Figs. 7, 9, 11, 13, 15, 18, and 19 respectively. The pCMV/SEAP ires Hygro described in (2-1) was digested with the restriction enzyme SpeI for several hours, followed by ethanol precipitation, and the precipitate was dissolved in sterile water. By using the vector digested with SpeI as a template, DNA for transformation was prepared by the PCR method (94°C for 15 sec, 55°C for 30 sec, and 68°C for 10 min x 30 cycles) using the following primers and KOD-plus-. By using the thus prepared DNA for transformation and the corresponding BAC transfected with pRed/ET, each DNA element having a different sequence length was cloned into the pCMV/SEAP ires Hygro described in (2-1). The vector construct is shown in Fig. 2. Incidentally, the procedure was performed according to the method described in (2-2).

A2-1D:

5′-CATGCACAGATTAGCCATTTAGTACTTACTAAATCAAACTCAATTTCTGAAGTCT

AGTTATTAATAGTAATCAATTACG-3′

A2-1R:

5′-CTCATTCTGTGGGTTGTCATTTCACTTCCTTGATGCTATCCTTTCAAGCAAAATT

CAATAATCAATGTCAACGCGTATAT-3′

A2-2D:

5′-ACACTGGTCAAAGGGACAGGTCATTGTTATGCTGGCAATGCAGGCTGCTGAAAAC

TAGTTATTAATAGTAATCAATTACG-3′

A2-2R:

5′-ACTGTAGCTTCTTATTTTTTACCTGCAGTGCATTCCTGTAAAAGTAGTGTGGAGT

CAATAATCAATGTCAACGCGTATAT-3′

A2-3D:

5′-CTGGAAATTGAGAAGTATCATTCACAACAGTACCACAAACATGAAATAAATGTGC

TAGTTATTAATAGTAATCAATTACG-3′

A2-3R:

5′-CCAAGCTTGTCCAACCGCGGCCTGCAGGCTGCATGCAGCCTGTGAAGGCTTTGAT

CAATAATCAATGTCAACGCGTATAT-3′

A2-4D:

5′-TCAATCATTTATCAATTTTATCTTCAAAGTCCCTCACTTCAGGGAGATGATATAC

TAGTTATTAATAGTAATCAATTACG-3′

A2-4R:

5′-ATATATAAAAGTTCATGTATATATAAAATCATGCAATACACGGCCTTTTGTGACT

CAATAATCAATGTCAACGCGTATAT-3′

A2-5D:

5′-CGCATAAAAGGAAAAGCATCCTTAAAATAAACACCATCAATGGCTCCTCGGTGGC

TAGTTATTAATAGTAATCAATTACG-3′

A2-5R:

A2-4R was used.

A2-6D:

5′-GGGAGGCTACAGCTTGCCTCTCTAACCACTAAAAGGCATGACCCTCCTCAAAGCT

AGTTATTAATAGTAATCAATTACG-3′

A2-6R:

A2-4R was used.

A2-7D:

5′-TCTGGCTTCCCTGGGCCACGCTGGAAGAAGAATTGTCTTGCGCCACACATAAAAC

TAGTTATTAATAGTAATCAATTACG-3′

A2-7R:

5′-AGCTGATTTTTACGTTAAATGTAACATGTAAAGAAATATATGTGTGTTTTTAGAT

CAATAATCAATGTCAACGCGTATAT-3′

A2-8D:

5′-GTGAAGAGGAGGAGATGTCAAAATTCAAAGTCTTAAATGATGTAGTTTTAAGTAC

TAGTTATTAATAGTAATCAATTACG-3′

A2-8R:

5'-ATGACACTTGATATTGTTGTTTATATTGCTGGTTAGTATGTGCCTTCATTTACCT

CAATAATCAATGTCAACGCGTATAT-3'

A2-9D:

A2-6D was used.

A2-9R:

A2R was used.

A2-10D:

A2-2D was used.

A2-10R:

A2-7R was used.

A2-11D:

A2-8D was used.

A2-11R:

A2-2R was used.

A2-12D:

A2-2D was used.

A2-12R:

A2-4R was used.

A2-13D:

A2-8D was used.

A2-13R:

A2-7R was used.

A2-14D:

A2D was used.

A2-14R:

A2-2R was used.

A2-15D:

A2-2D was used.

A2-15R:

A2R was used.

A2-16D:

A2-8D was used.

A2-16R:

A2-4R was used.

A2-17D:

A2D was used.

A2-17R:

A2-7R was used.

A7-1D:

5′-AAAAACAAAACTGGAGTAAACAAGATGAATTGTTTTAATAGAGGCACTGTATTAC

TAGTTATTAATAGTAATCAATTACG-3′

A7-1R:

5′-ATACAATGTTCCATGTATTCTGTGCCTGAACCTATGCAGCTGATGTAGCTGAAGT

CAATAATCAATGTCAACGCGTATAT-3′

A7-2D:

5′-GATCTTATTTTCTAAGTAGTATAGACTTAATTGTGAGAACAAAATAAAAACTTGC

TAGTTATTAATAGTAATCAATTACG-3′

A7-2R:

5′-TGTTGTTTTCAGCCACTAAGTTTGAGGTGATTTGTTCTGGCAGTCCTAGGAAACT

CAATAATCAATGTCAACGCGTATAT-3′

A7-3D:

A7-2D was used.

A7-3R:

5′-AGCCTACACTACCCTTTGCAGCCTTTGGTAACTATCCTTCTGCTGTCTACCTCCT

CAATAATCAATGTCAACGCGTATAT-3′

A7-4D:

5′-AGGAGCTCCTGAATGAAGGACATCACTCAGCTGTGTTAAGTATCTGGAACAATAC

TAGTTATTAATAGTAATCAATTACG-3′

A7-4R:

5′-GACATAAAATGTAAGATATGATATGCTATGTAAGATATGATACCTGCCTTAAAAT

CAATAATCAATGTCAACGCGTATAT-3′

A7-5D:

5′-CACTGCTTGATACTTACTGTGGACTTTGAAAATTATGAATGTGTGTGTGTGTGTC

TAGTTATTAATAGTAATCAATTACG-3′

A7-5R:

5′-CAATTACATTCCAGTGATCTGCTACTTAGAATGCATGACTGAACTCCTGGGTGGT

CAATAATCAATGTCAACGCGTATAT-3′

A7-6D:

5′-TTATTTTGAAGAGAAACTCCTGGTTCCCACTTAAAATCCTTTCTTGTTTCCAAGC

TAGTTATTAATAGTAATCAATTACG-3′

A7-6R:

5′-AAGCAGTGTGTGTTTACCTGCATGTGTATGTGAATTAACTCTGTTCCTGAGGCAT

CAATAATCAATGTCAACGCGTATAT-3′

A7-7D:

5′-ATTGCATGTTCTCATTTATTTGTGGGATGTAAAAATCAAAACAATAGAACGTATC

TAGTTATTAATAGTAATCAATTACG-3′

A7-7R:

5′-TTGGGAGGCCGCAGCTGGTAGATCACTTGAGGCCACGAATTTGACACCAGCAGGT

CAATAATCAATGTCAACGCGTATAT-3′

A7-8D:

A7-1D was used.

A7-8R:

A7R was used.

A7-9D:

A7-7D was used.

A7-9R:

A7-5R was used.

A7-10D:

A7-4D was used.

A7-10R:

A7-7R was used.

A7-11D:

A7-6D was used.

A7-11R:

A7-4R was used.

A7-12D:

A7-2D was used.

A7-12R:

A7-6R was used.

A7-13D:

A7-7D was used.

A7-13R:

A7R was used.

A7-14D:

A7-4D was used.

A7-14R:

A7-5R was used.

A7-15D:

A7-6D was used.

A7-15R:

A7-7R was used.

A7-16D:

A7-2D was used.

A7-16R:

A7-4R was used.

A7-17D:

A7-4D was used.

A7-17R:

A7R was used.

A7-18D:

A7-6D was used.

A7-18R

A7-5R was used.

A18-1:

5′-ATCCCCTGCTCTGCTAAAAAAGAATGGATGTTGACTCTCAGGCCCTAGTTCTTGA

TCCTATTAATAGTAATCAATTACG-3′

A18-1R:

A18R was used.

A18-2D:

5′-CTAAAGTGCTGGGATTACAGGCATAAGCCACCGTGCCCGGCTGGAGCATTGGGAT

CCTATTAATAGTAATCAATTACG-3′

A18-2R:

5′-ACTACTTACACATTTCGAGTTTTAAATAAGGCGTTCAATATAGAGTGAACACCTA

GTCAATAATCAATGTCAACG-3′

A18-3D:

5′-CAGGCATAAGCCACCGCACCCGGCCACCCCTTACTAATTTTTAGTAACGTCGATC

CTATTAATAGTAATCAATTACG-3′

A18-3R:

5′-CTGATTGACTTTGACCTCTGCTTTCCAACTTTGCCCCAAAGAAAGTTAGTCACCT

AGTCAATAATCAATGTCAACG-3′

A18-4D:

A18-3D was used.

A18-4R:

5′-TTCAATGAAACAAGCTCTGTGAGGCTCATTTGTACCCATTTTGTTCAGTACTGCC

TAGTCAATAATCAATGTCAACG-3′

B5-1D:

5′-ACATACCCAGAGACACTGAGAGAGACAGACAGACAGTAAACAGAGGAGCACGATC

CTATTAATAGTAATCAATTACG-3′

B5-1R:

B5R was used.

B5-2D:

5′-GCTCAATTGTATCTTATGAAAACAATTTTTCAAAATAAAACAAGAGATATGATCC

TATTAATAGTAATCAATTACG-3′

B5-2R:

B5R was used.

B5-3D:

5′-CCTGTGCTGAATACCGTCTGCATATGTATAGGAAAGGGTTAACTCAGCAGGGATC

CTATTAATAGTAATCAATTACG-3′

B5-3R:

5′-TATGTGAATGGAAATAAAATAATCAAGCTTGTTAGAATTGTGTTCATAATGACCC

TAGTCAATAATCAATGTCAACG-3′

B5-4D:

B5D was used.

B5-4R:

5′-GAAAGTCTACAATTTTTTCAGTTTAAAATGGTATTTATTTGTAACATGTACCCTA
GTCAATAATCAATGTCAACG-3′

B5-5D:

B5-1D was used.

B5-5R:

5′-CAAAGATGAAGGATGAGAGTGACTTCTGCCTTCATTATGTTATGTGTTCATATCC
TAGTCAATAATCAATGTCAACG-3′

B5-6D:

5′-CAGTGAATTATTCACTTTGTCTTAGTTAAGTAAAAATAAAATCTGACTGTGATCC
TATTAATAGTAATCAATTACG-3′

B5-6R:

5′-GAACAGACAGGTGAATGAGCACAGAGGTCATTTGTAAACCGTTTGTGGTTAGCCT
AGTCAATAATCAATGTCAACG-3′

C14-1D:

5′-CTTTTTGGCTTCTGTGTTTAAGTTATTTTTCCCCTAGGCCCACAAACAGAGTCGA
TCCTATTAATAGTAATCAATTACG-3′

C14-1R:

5′-AACCTTGGAAAAATTCTGTTGTGTTTAGAAGCATGTACCAATCTATCACTCCTAG
TCAATAATCAATGTCAACG-3′

C14-2D:

5′-CTATTCACTGTCTGTAGGATGAAAAAGTTAATAACACCCTGAGAGGTTTCGATCC

TATTAATAGTAATCAATTACG-3′

C14-2R:

5′-CCTTAGATTAGTTTATTGTATTTTTTATCAGCTACTATAAGGTTTACACACCCTA

GTCAATAATCAATGTCAACG-3′

C14-3D:

5′-CAAGACCCTCAAAATTCAAAAATTTCCTTTATCTTGCTGTAGCACCTCCTGCGAT

CCTATTAATAGTAATCAATTACG-3′

C14-3R:

5′-GGAGGGGATAGGAAGGGGATGAGGCCTAACAGGTTGATGATCTAGGCTTTACCTA

GTCAATAATCAATGTCAACG-3′

C14-4D:

5′-CTCAAAAAGGAGATAATTCCAGCCCCTCGCCTTAAAGAATCCCTATCAAGTGATC

CTATTAATAGTAATCAATTACG-3′

C14-4R:

C14-1R was used.

C14-5D:

5′-CGCTTGAACCTGGGAGGCAGAGGTTGCAGTGAGCCGAGATCACGCCGTTGGATCC

TATTAATAGTAATCAATTACG-3′

C14-5R:

C14-1R was used.

C14-6D:

C14-4D was used.

C14-6R:

5′-TTAACTTTTTCATCCTACAGACAGTGAATAGTAAAGCTTTCTGTGAAGACATACC

CTAGTCAATAATCAATGTCAACG-3′

C14-7D:

C14-2D was used.

C14-7R:

C14-1R was used.

C14-8D:

C14-3D was used.

C14-8R:

5′-AAATTATTTCCTGGTGGGCAATATTAGAATATGGGGAATGTTTGCTTCTGAGCCT

AGTCAATAATCAATGTCAACG-3′

C14-9D:

C14-4D was used.

C14-9R:

C14-3R was used.

C14-10D:

C14-2D was used.

C14-10R:

C14R was used.

C14-11D:

C14-3D was used.

C14-11R:

C14-2R was used.

C14-12D:

C14-4D was used.

C14-12R:

C14-8R was used.

C14-13D:

C14-3D was used.

C14-13R:

C14-1R was used.

C14-14D:

C14-4D was used.

C14-14R:

C14-2R was used.

As for the polynucleotide sequences of the respective fragments of A2, A2-1 corresponds to the polynucleotide sequence of nucleotides 1 to 3000 of SEQ ID NO: 1 in the Sequence Listing; A2-2 corresponds to the polynucleotide sequence of

nucleotides 2801 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-3 corresponds to the polynucleotide sequence of nucleotides 5401 to 8450 of SEQ ID NO: 1 in the Sequence Listing; A2-4 corresponds to the polynucleotide sequence of nucleotides 701 to 2700 of SEQ ID NO: 1 in the Sequence Listing; A2-5 corresponds to the polynucleotide sequence of nucleotides 701 to 2200 of SEQ ID NO: 1 in the Sequence Listing; A2-6 corresponds to the polynucleotide sequence of nucleotides 701 to 3700 of SEQ ID NO: 1 in the Sequence Listing; A2-7 corresponds to the polynucleotide sequence of nucleotides 2001 to 5000 of SEQ ID NO: 1 in the Sequence Listing; A2-8 corresponds to the polynucleotide sequence of nucleotides 4001 to 7000 of SEQ ID NO: 1 in the Sequence Listing; A2-9 corresponds to the polynucleotide sequence of nucleotides 1 to 3700 of SEQ ID NO: 1 in the Sequence Listing; A2-10 corresponds to the polynucleotide sequence of nucleotides 2001 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-11 corresponds to the polynucleotide sequence of nucleotides 2801 to 7000 of SEQ ID NO: 1 in the Sequence Listing; A2-12 corresponds to the polynucleotide sequence of nucleotides 701 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-13 corresponds to the polynucleotide sequence of nucleotides 2001 to 7000 of SEQ ID NO: 1 in the Sequence Listing; A2-14 corresponds to the polynucleotide sequence of nucleotides 2801 to 8450 of SEQ ID NO: 1 in the Sequence Listing; A2-15 corresponds to the

polynucleotide sequence of nucleotides 1 to 5800 of SEQ ID NO: 1 in the Sequence Listing; A2-16 corresponds to the polynucleotide sequence of nucleotides 701 to 7000 of SEQ ID NO: 1 in the Sequence Listing; and A2-17 corresponds to the polynucleotide sequence of nucleotides 2001 to 8450 of SEQ ID NO: 1 in the Sequence Listing.

As for the polynucleotide sequences of the respective fragments of A7, A7-1 corresponds to the polynucleotide sequence of nucleotides 601 to 3600 of SEQ ID NO: 2 in the Sequence Listing; A7-2 corresponds to the polynucleotide sequence of nucleotides 3601 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-3 corresponds to the polynucleotide sequence of nucleotides 5401 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-4 corresponds to the polynucleotide sequence of nucleotides 3401 to 6400 of SEQ ID NO: 2 in the Sequence Listing; A7-5 corresponds to the polynucleotide sequence of nucleotides 1501 to 4500 of SEQ ID NO: 2 in the Sequence Listing; A7-6 corresponds to the polynucleotide sequence of nucleotides 4401 to 7400 of SEQ ID NO: 2 in the Sequence Listing; A7-7 corresponds to the polynucleotide sequence of nucleotides 2401 to 5400 of SEQ ID NO: 2 in the Sequence Listing; A7-8 corresponds to the polynucleotide sequence of nucleotides 1 to 3600 of SEQ ID NO: 2 in the Sequence Listing; A7-9 corresponds to the polynucleotide sequence of nucleotides 1501 to 5400 of SEQ ID NO: 2 in the Sequence Listing;

A7-10 corresponds to the polynucleotide sequence of nucleotides 2401 to 6400 of SEQ ID NO: 2 in the Sequence Listing; A7-11 corresponds to the polynucleotide sequence of nucleotides 3401 to 7400 of SEQ ID NO: 2 in the Sequence Listing; A7-12 corresponds to the polynucleotide sequence of nucleotides 4401 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-13 corresponds to the polynucleotide sequence of nucleotides 1 to 5400 of SEQ ID NO: 2 in the Sequence Listing; A7-14 corresponds to the polynucleotide sequence of nucleotides 1501 to 6400 of SEQ ID NO: 2 in the Sequence Listing; A7-15 corresponds to the polynucleotide sequence of nucleotides 2401 to 7400 of SEQ ID NO: 2 in the Sequence Listing; A7-16 corresponds to the polynucleotide sequence of nucleotides 3401 to 8420 of SEQ ID NO: 2 in the Sequence Listing; A7-17 corresponds to the polynucleotide sequence of nucleotides 1 to 6400 of SEQ ID NO: 2 in the Sequence Listing; and A7-18 corresponds to the polynucleotide sequence of nucleotides 1501 to 7400 of SEQ ID NO: 2 in the Sequence Listing.

As for the polynucleotide sequences of the respective fragments of A18, A18-1 corresponds to the polynucleotide sequence of nucleotides 1 to 5040 of SEQ ID NO: 3 in the Sequence Listing; A18-2 corresponds to the polynucleotide sequence of nucleotides 1001 to 6002 of SEQ ID NO: 3 in the Sequence Listing; A18-3 corresponds to the polynucleotide sequence of nucleotides 2001 to 7000 of SEQ ID NO: 3 in the Sequence Listing;

and A18-4 corresponds to the polynucleotide sequence of nucleotides 3000 to 7000 of SEQ ID NO: 3 in the Sequence Listing.

As for the polynucleotide sequences of the respective fragments of B5, B5-1 corresponds to the polynucleotide sequence of nucleotides 1 to 4001 of SEQ ID NO: 4 in the Sequence Listing; B5-2 corresponds to the polynucleotide sequence of nucleotides 1 to 3200 of SEQ ID NO: 4 in the Sequence Listing; B5-3 corresponds to the polynucleotide sequence of nucleotides 2491 to 5601 of SEQ ID NO: 4 in the Sequence Listing; B5-4 corresponds to the polynucleotide sequence of nucleotides 5373 to 8401 of SEQ ID NO: 4 in the Sequence Listing; B5-5 corresponds to the polynucleotide sequence of nucleotides 901 to 4001 of SEQ ID NO: 4 in the Sequence Listing; and B5-6 corresponds to the polynucleotide sequence of nucleotides 4001 to 7000 of SEQ ID NO: 4 in the Sequence Listing.

As for the polynucleotide sequences of the respective fragments of C14, C14-1 corresponds to the polynucleotide sequence of nucleotides 960 to 4015 of SEQ ID NO: 5 in the Sequence Listing; C14-2 corresponds to the polynucleotide sequence of nucleotides 1987 to 5014 of SEQ ID NO: 5 in the Sequence Listing; C14-3 corresponds to the polynucleotide sequence of nucleotides 4020 to 7119 of SEQ ID NO: 5 in the Sequence Listing; C14-4 corresponds to the polynucleotide sequence of nucleotides 960 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-5 corresponds to the polynucleotide

sequence of nucleotides 960 to 6011 of SEQ ID NO: 5 in the Sequence Listing; C14-6 corresponds to the polynucleotide sequence of nucleotides 4939 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-7 corresponds to the polynucleotide sequence of nucleotides 960 to 5014 of SEQ ID NO: 5 in the Sequence Listing; C14-8 corresponds to the polynucleotide sequence of nucleotides 2994 to 7119 of SEQ ID NO: 5 in the Sequence Listing; C14-9 corresponds to the polynucleotide sequence of nucleotides 4020 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-10 corresponds to the polynucleotide sequence of nucleotides 1 to 5014 of SEQ ID NO: 5 in the Sequence Listing; C14-11 corresponds to the polynucleotide sequence of nucleotides 1987 to 7119 of SEQ ID NO: 5 in the Sequence Listing; C14-12 corresponds to the polynucleotide sequence of nucleotides 2994 to 8141 of SEQ ID NO: 5 in the Sequence Listing; C14-13 corresponds to the polynucleotide sequence of nucleotides 960 to 7119 of SEQ ID NO: 5 in the Sequence Listing; and C14-14 corresponds to the polynucleotide sequence of nucleotides 1987 to 8141 of SEQ ID NO: 5 in the Sequence Listing.

The start and end points of the respective fragments on the full-length sequence are also shown in Figs. 18 and 19.

(5-2) Evaluation of DNA elements having different sequence lengths

Each plasmid constructed in (5-1) was evaluated using

the host cell CHO-K1 (ATCC) and transfection reagent Lipofectamine 2000 (Invitrogen).

In the same manner as in (2-3), antibiotic selection with hygromycin was performed after transfection, whereby a stably expressing polyclonal cell line was established. The thus established cell line was subjected to medium replacement on the day before measurement, and a given number of the cells were seeded into a 24-well plate. At 24 hours after plating the cells, the culture supernatant was collected, and the activity of SEAP was measured.

The measurement results are shown in Figs. 8, 10, 12, 14, and 16. It was confirmed that not only the full-length DNA element, but also clones having a sequence length shorter than the full length have an effect of enhancement of expression. Based on the results, it was confirmed that the DNA elements A2, A7, A18, B5, and C14 have an activity of enhancing foreign gene expression even cases where they have a sequence length shorter than the full length. However, they exhibit the highest effect when the sequence length is the full length.

Example 6

Effect using host cells other than CHO cell line

A CHO cell line was used as the cell line in the evaluation in Examples 2 to 5. However, in Example 6 an HEK293 cell line was selected as a cell line other than the CHO cell line. The

HEK293 cell line was subjected to static culture at 37°C in the presence of 5% CO_2 using DMEM medium (Invitrogen) containing 10% FCS, and a given number of the cells were seeded into a 6-well plate on the day before transfection. In order to evaluate the SEAP expression vector containing each DNA element constructed in (3-2), transfection was performed using each plasmid and transfection reagent Lipofectamine 2000 (Invitrogen). Antibiotic selection with hygromycin was performed for about 2 weeks starting 2 days after transfection, whereby a stably expressing polyclonal cell line was established. The thus established cell line was subjected to medium replacement on the day before measurement, and a given number of the cells were seeded into a 24-well plate. At 24 hours after plating the cells, the culture supernatant was collected, and the activity of SEAP was measured. The activity of SEAP in the culture supernatant was measured using SensoLyte™ pNPP Secreted Alkaline Phosphatase Reporter Assay (ANASPEC).

The measurement results are shown in Fig. 17. In the same manner as in Example 3, it was confirmed that each element is also highly effective in enhancing the expression of a foreign gene (SEAP) in the HEK293 cell line.

Industrial Applicability

By introducing a foreign gene expression vector using

the DNA element according to the invention into mammalian host cells, it becomes possible to improve the productivity of a foreign gene of a therapeutic protein, an antibody, or the like.

CLAIMS

1. A polynucleotide consisting of a polynucleotide sequence represented by SEQ ID NO: 2 in the Sequence Listing.

2. A polynucleotide comprising at least 3000 consecutive nucleotides of a polynucleotide sequence represented by SEQ ID NO: 2 in the Sequence Listing.

3. A polynucleotide comprising at least 2000 consecutive nucleotides of a polynucleotide sequence represented by SEQ ID NO: 2 in the Sequence Listing.

4. A polynucleotide comprising at least 1500 consecutive nucleotides of a polynucleotide sequence represented by SEQ ID NO: 2 in the Sequence Listing.

5. A polynucleotide consisting of a polynucleotide sequence having a homology of 95% or more to the polynucleotide sequence of the polynucleotide according to any one of claims 1 to 4.

6. A polynucleotide consisting of a polynucleotide sequence having a homology of 99% or more to the polynucleotide sequence of the polynucleotide according to any one of claims 1 to 4.

7. A polynucleotide consisting of:
(i) a polynucleotide sequence containing two or more sequences of the polynucleotide sequence of the polynucleotide according to any one of claims 1 to 6; and/or
(ii) two or more types of polynucleotides selected from the polynucleotides according to claims 1 to 6.

8. A polynucleotide consisting of a polynucleotide sequence containing:

- at least one sequence of the polynucleotide sequence of the polynucleotide according to any one of claims 1 to 6; and
- at least one sequence of

(a) polynucleotide sequence represented by SEQ ID NO: 1 in the Sequence Listing or a partial fragment thereof;

(b) polynucleotide sequence represented by SEQ ID NO: 3 in the Sequence Listing or a partial fragment thereof;

(c) polynucleotide sequence represented by SEQ ID NO: 4 in the Sequence Listing or a partial fragment thereof; and/or

(d) polynucleotide sequence represented by SEQ ID NO: 5 in the Sequence Listing or a partial fragment thereof.

9. A foreign gene expression vector comprising the polynucleotide sequence of a polynucleotide according to any one of claims 1 to 8.

10. The foreign gene expression vector according to claim 9, wherein the protein encoded by the foreign gene is a multimeric protein.

11. The foreign gene expression vector according to claim 9, wherein the protein encoded by the foreign gene is a hetero-multimeric protein.

12. The foreign gene expression vector according to claim 11, wherein the protein encoded by the foreign gene is an antibody or a functional fragment thereof.

13. A transformed cell into which the foreign gene expression vector according to any one of claims 9 to 12 has been introduced.

14. The transformed cell according to claim 13, wherein the cell is a cultured cell derived from a mammal.

15. The transformed cell according to claim 14, wherein the cultured cell derived from a mammal is a cell selected from the group consisting of COS-1 cells, 293 cells, and CHO cells.

16. The transformed cell according to any one of claims 13 to 15, wherein the protein encoded by the foreign gene is a multimeric protein.

17. The transformed cell according to claim 16, wherein the protein encoded by the foreign gene is a hetero-multimeric protein.

18. The transformed cell according to claim 17, wherein the protein encoded by the foreign gene is an antibody or a functional fragment thereof.

19. A method for producing a protein characterized by comprising culturing the transformed cell according to any one of claims 13 to 18 and obtaining the protein encoded by the foreign gene from the resulting culture product.

20. A method for enhancing foreign gene expression in a transformed cell into which a foreign gene or a foreign gene expression vector has been introduced, characterized by using a polynucleotide according to any one of claims 1 to 8 or a foreign gene expression vector according to any one of claims 9 to 12.

21. Use of the polynucleotide according to any one of claims 1 to 8 for enhancing foreign gene expression in a transformed cell.