PRODUCTION METHOD OF POLYPEPTIDE, TAG, EXPRESSION VECTOR, EVALUATION METHOD OF POLYPEPTIDE, PRODUCTION METHOD OF NUCLEIC ACID DISPLAY LIBRARY, AND SCREENING METHOD

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present disclosure relates to a production method of a polypeptide, a tag, an expression vector, an evaluation method of a polypeptide, a production method of a nucleic acid display library, and a screening method.

2. Description of the Related Art

[0002] In a case of expressing a target protein by genetic engineering, there is a technique of attaching a relatively low molecular weight polypeptide, which is called a tag, to the terminal of the target protein. The tag is used to detect, isolate, immobilize, or the like a target protein.

[0003] It is being examined to use the tag to increase the expression level of a target protein.

WO2016/204198A discloses a tag (SEQ ID NO: 107) consisting of an amino acid sequence of SKIK as a tag that increases an expression level of a target protein and a method of adding the tag to the N-terminal of the target protein to express the target protein.

Protein Expression and Purification 2010; 74: 248-256 describes that an expression level of a protein is increased by incorporating Ala or Ser immediately after a start codon.

[0004] The optimization of the base sequence of the gene has been also examined in order to increase the expression level of the protein.

Science 2013; 342: 475-479 describes that the use of rare codons at the N-terminal of a protein increases the expression level thereof in Escherichia coli.

Protein Expression and Purification 2010; 70: 224-230 describes that regarding human peptide deformylase (hPDF), a reduction of the GC content of the hPDF gene and removal of rare codons increases an expression level in Escherichia coli.

SUMMARY

[0005] The example according to the present disclosure relates to a production method of a polypeptide having high productivity, a tag and an expression vector capable of increasing an expression level of a polypeptide, an efficient evaluation method of a polypeptide, a production method of a nucleic acid display library in which an expression level is high and a variation of the expression level is small, and a screening method with a high reliability degree.

[0006] The specific methods for achieving the object include the following examples.

selecting a nucleic acid-polypeptide conjugate having a target activity from the nucleic acid display library and identifying a base sequence of a nucleic acid of the selected nucleic acid-polypeptide conjugate.

[0008] According to the example according to the present disclosure, a production method of a polypeptide having high productivity, a tag and an expression vector capable of increasing an expression level of a polypeptide, an efficient evaluation method of a polypeptide, a production method of a nucleic acid display library in which an expression level is high and a variation of the expression level is small, and a screening method with a high reliability degree are provided.

[0008a] In one aspect, the present disclosure relates to a production method of a polypeptide in which expressing a polypeptide as a tagged polypeptide is included, the production method comprising: expressing the tagged polypeptide from a nucleic acid containing a base sequence encoding a tag consisting of an amino acid sequence set forth in SEQ ID NO: 1, which is arranged immediately after a start codon, and a base sequence encoding the polypeptide that is arranged in-frame downstream of the base sequence encoding the tag, SEQ ID NO: 1: Val-Lys-Lys-(Xaa)n (Xaa)n is a chain of n pieces of any amino acids, where n is an integer of 1 to 8, and the amino acids constituting (Xaa)n may be one type of amino acid or two or more types of amino acids.

[0008b] In another aspect, the present disclosure relates to a tag consisting of an amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 1: Val-Lys-Lys-(Xaa)n (Xaa)n is a chain of n pieces of any amino acids, where n is an integer of 1 to 8, and the amino acids constituting (Xaa)n may be one type of amino acid or two or more types of amino acids.

[0008c] In another aspect, the present disclosure relates to a tag consisting of an amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 2: Val-Lys-Lys-Xaa-(Xaa)m Xaa at a fourth position from an N-terminal is one type of amino acid selected from the group consisting of Ile, Lys, Arg, His, Ser, and Thr, (Xaa)m is a chain of m pieces of any amino acids, where m is an integer of 0 to 7, and the amino acids constituting (Xaa)m may be one type of amino acid or two or more types of amino acids.

[0008d] In another aspect, the present disclosure relates to a tag consisting of an amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 3: Val-Lys-Lys-Xaa-(Xaa)k Xaa at a fourth position from an N-terminal is one type of amino acid selected from the group consisting of Ile, Lys, and Thr, (Xaa)k is a chain of k pieces of amino acids selected from the group consisting of Lys, Thr, and Asp, where k is an integer of 0 to 6, and the amino acids constituting (Xaa)k may be one type of amino acid or two or more types of amino acids.

[0008e] In another aspect, the present disclosure relates to an expression vector comprising: a base sequence encoding a tag consisting of an amino acid sequence set forth in SEQ ID NO: 1, which is arranged immediately after a start codon; and a base sequence encoding a polypeptide that is arranged in-frame downstream of the base sequence encoding the tag, SEQ ID NO: 1: Val-Lys-Lys-(Xaa)n (Xaa)n is a chain of n pieces of any amino acids, where n is an integer of 1 to 8, and the amino acids constituting (Xaa)n may be one type of amino acid or two or more types of amino acids.

[0008f] In another aspect, the present disclosure relates to an evaluation method of a polypeptide, comprising: producing a polypeptide by the production method of a polypeptide as disclosed herein; and evaluating binding property of the polypeptide to a target substance.

[0008g] In another aspect, the present disclosure relates to a production method of a nucleic acid display library comprising: producing a nucleic acid-polypeptide conjugate by expressing a tagged polypeptide from a nucleic acid containing a base sequence encoding a tag consisting of an amino acid sequence set forth in SEQ ID NO: 1, which is arranged immediately after a start codon, and a base sequence encoding a polypeptide that is arranged in-frame downstream of the base sequence encoding the tag, SEQ ID NO: 1: Val-Lys-Lys-(Xaa)n (Xaa)n is a chain of n pieces of any amino acids, where n is an integer of 1 to 8, and the amino acids constituting (Xaa)n may be one type of amino acid or two or more types of amino acids.

[0008h] In another aspect, the present disclosure relates to a screening method comprising: producing a nucleic acid display library by the production method of a nucleic acid display library as disclosed herein; and selecting a nucleic acid-polypeptide conjugate having a target activity from the nucleic acid display library and identifying a base sequence of a nucleic acid of the selected nucleic acid-polypeptide conjugate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Fig. 1 is a graph showing a biosynthesis amount of a fusion protein for tags having SEQ ID NOs: 7 to 26, in which the tags are arranged in the order of the biosynthesis amounts of the fusion proteins.

Fig. 2 is a graph showing a biosynthesis amount of a fusion protein for the tags having SEQ ID NOs: 27, 28, 31, 37, 48, and 52.

Fig. 3 is a graph showing a binding performance of an integrin binding peptide to which a tag of SEQ ID NO: 27 is added.

Fig. 4 is a diagram showing structures of compounds introduced into SEQ ID NOs: 133 to 136.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0010] Hereinafter, embodiments according to the present disclosure will be described. These descriptions and Examples are only illustrative of one embodiment and do not limit the ranges of the embodiments of the present disclosure. The action mechanism mentioned in the present disclosure includes estimation, and the accuracy thereof does not limit the ranges of the embodiments of the present disclosure.

[0011] In the present disclosure, a numerical range expressed using "to" indicates a range including numerical values before and after "to" as a minimum value and a maximum value.

In the numerical ranges described stepwise in the present disclosure, the upper limit value or the lower limit value described in one numerical range may be replaced with the upper limit value or the lower limit value of the numerical range described stepwise in other stages. In addition, in a numerical range described in the present disclosure, an upper limit value or a lower limit value described in the numerical range may be replaced with a value described in an example.