screening process are used as free antibodies, which correspond to an actual usage form, the antibodies cannot be used due to loss of the structural stability in some cases. Moreover, a plurality of antibodies are presented to a single carrier (e.g., phage or yeast cell), and therefore, only antibodies having a low binding ability may be obtained due to the close inhibitory effect.

[0005] Monoclonal antibodies have gained attention as antibody drugs. A monoclonal antibody binds to a single antigen and has high specificity, and is thus expected to be applied to cancer cells, autoimmune diseases, infectious diseases, and the like. For example, a monoclonal antibody binds specifically to a target antigen, and thus the immune mechanism can attack and destroy cells containing the antigen (e.g., cancer cells or cells infected with an infectious factor). Accordingly, there is a demand for a method for more easily manufacturing a monoclonal antibody. Furthermore, there is also a demand for a method for more easily identifying a monoclonal antibody that binds specifically to a target antigen.

[0006] Non-Patent Documents 1 and 2 disclose that NestLink, which is a method for selecting and identifying a binding substance that can simultaneously characterize several thousand library members, was developed as a method for examining the binding ability of an antibody without immobilizing the antibody. NestLink is based on genetically encoded peptide barcodes "flycodes", and Non-Patent Documents 1 and 2 disclose that these flycodes were designed to improve the detectability of mass spectrometry and serve as unique identifiers in sequencing analysis.

[0007] However, in order to more easily identify an antibody having a binding ability, there is still a demand for a method that has improved sensitivity and specificity, and reduced identification bias in peptide detection through mass spectrometry.

Related Art Documents

Non-Patent Documents

[0008]

[Non Patent Document 1] Egloff P. et al., bioRxiv: 287813 (March 23, 2018)

[Non Patent Document 2] Egloff P. et al., NATURE METHOD VOL 16 MAY 2019 421-428

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CLAIMS

1. A method for manufacturing a monoclonal antibody, comprising:

a step of introducing a DNA fragment comprising a gene that encodes a secretory signal, a gene that encodes a nanobody, and a gene that encodes a peptide barcode, or a vector containing the DNA fragment, into a yeast cell; and

a step of collecting a polypeptide comprising the nanobody and the peptide barcode that has been expressed in the cell and secreted to the outside of the cell,

wherein the peptide barcode is represented by an amino acid sequence having 5 to 30 amino acids, and the amino acids are independently selected from the group consisting of A, F, G, K, L, P, R, V, and W.

2. The manufacturing method according to claim 1, wherein the yeast belongs to the genus *Saccharomyces*, *Pichia*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Candida*, *Torulopsis, Yarrowia*, or *Hansenula*.

3. The manufacturing method according to claim 1 or 2, wherein the secretory signal is an α-factor secretory signal, a glucoamylase secretory signal, or a PHO1 secretory signal.

4. The manufacturing method according to any one of claims 1 to 3, wherein the DNA fragment further comprises a promoter that is an AOX1 promoter, a GAP promoter, an FLD1 promoter, a PEX8 promoter, or a YPT1 promoter.

5. The manufacturing method according to any one of claims 1 to 4, wherein the DNA fragment further comprises a gene encoding at least one tag selected from the group consisting of a FLAG tag, a His tag, a calmodulin protein (CBP) tag, a Strep tag, a StrepII tag, a GST tag, a Myc tag, and a maltose binding protein (MBP) tag.

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6. The manufacturing method according to any one of claims 1 to 5, wherein the peptide barcode is represented by an amino acid sequence having 6 to 16 amino acids.

7. The manufacturing method according to any one of claims 1 to 6, further comprising:

a step of mixing the collected polypeptide and an antigen and obtaining a polypeptide including a nanobody that binds specifically to the antigen;

a step of cleaving the peptide barcode from the obtained polypeptide and identifying the cleaved peptide barcode through mass spectrometry; and

a step of identifying the nanobody included in the polypeptide from which the identified peptide barcode was cleaved, based on the base sequence of a nucleic acid encoding the identified peptide barcode.

8. The manufacturing method according to claim 7, wherein the step of identifying the peptide barcode is performed by detecting a peak using a tandem mass spectrometer (MS/MS) connected to a high performance liquid chromatograph (LC).

9. The manufacturing method according to claim 8, wherein the high performance liquid chromatograph is provided with a long monolith column.

10. The manufacturing method according to any one of claims 1 to 9, wherein the DNA fragment further comprises a specific protease cleavage site.

11. The manufacturing method according to any one of claims 1 to 10, further comprising a step of determining the base sequence of the DNA fragment.

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12. The manufacturing method according to any one of claims 1 to 11, wherein at least two DNA fragments or vectors are used in the step of introduction into a yeast cell, and genes encoding a peptide barcode included in the DNA fragments encode peptide barcodes represented by different amino acid sequences.

13. The manufacturing method according to any one of claims 1 to 12, wherein the DNA fragment includes a gene encoding two or more peptide barcodes, and a cleavage site is arranged at each position between the two or more peptide barcodes.

14. A vector for manufacturing a monoclonal antibody in yeast, wherein the vector contains a DNA fragment comprising a gene that encodes a secretory signal, a gene that encodes a nanobody, and a gene that encodes a peptide barcode, and is to be introduced into a cell of the yeast to express a polypeptide comprising the nanobody and the peptide barcode and secrete the polypeptide to the outside of the cell of the yeast,

wherein the peptide barcode is represented by an amino acid sequence having 5 to 30 amino acids, and the amino acids are independently selected from the group consisting of A, F, G, K, L, P, R, V, and W.

15. The vector according to claim 14, wherein the secretory signal is an α-factor secretory signal, a glucoamylase secretory signal, or a PHO1 secretory signal.

16. The vector according to claim 14 or 15, wherein the DNA fragment further comprises a promoter that is an AOX1 promoter, a GAP promoter, an FLD1 promoter, a PEX8 promoter, or a YPT1 promoter.

17. The vector according to any one of claims 14 to 16, wherein the DNA fragment further comprises a gene encoding at least one tag selected from the group consisting of a FLAG tag, a His tag, a calmodulin protein (CBP)

tag, a Strep tag, a StrepII tag, a GST tag, a Myc tag, and a maltose binding protein (MBP) tag.

18. The vector according to any one of claims 14 to 17, wherein the peptide barcode is represented by an amino acid sequence having 6 to 16 amino acids.

19. The vector according to any one of claims 14 to 18, wherein the DNA fragment further includes a specific protease cleavage site.

20. A screening method for a monoclonal antibody, comprising:

(i) a step of expressing an antibody library from a gene library,

the gene library comprising at least two gene members, each of the gene members comprising a DNA fragment that comprises a gene encoding a nanobody and a gene encoding at least one peptide barcode,

the DNA fragments of the gene members of the gene library encoding polypeptides of antibody members of the antibody library,

each of the polypeptides corresponding to the antibody members of the antibody library comprising a nanobody and at least one peptide barcode, the nanobody and the at least one peptide barcode being encoded by a DNA fragment comprised in a gene member of the gene library,

the peptide barcodes of the antibody members being represented by different amino acid sequences;

(ii) a step of mixing the antibody library and an antigen and selecting an antibody member of the antibody library that includes a nanobody binding to the antigen, from the antibody library;

(iii) a step of cleaving the peptide barcode included in the selected antibody member of the antibody library and identifying the cleaved peptide barcode through mass spectrometry; and

(iv) a step of determining the base sequence of the gene encoding the identified peptide barcode based on the base sequences of the gene library and identifying the nanobody of the antibody member from which the

identified peptide barcode has been cleaved,

wherein the expression step is performed by introducing the vector according to any one of claims 14 to 19 into a yeast cell.

21. The method according to claim 20, further comprising a step of determining the base sequence of the DNA fragment.