## CLAIMS

1. An expression cassette expressed in a bacterium of the genus *Bifidobacterium*, sequentially comprising the following DNAs (1) to (4).

(1) a promoter DNA functioning in the bacterium of the genus *Bifidobacterium*;

(2) a DNA encoding a secretory signal peptide consisting of an amino acid sequence represented by the following a) or b);

a) an amino acid sequence represented by any one of SEQ ID Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16;

b) an amino acid sequence obtained by deletion, replacement or addition of one or several amino acids in the amino acid sequence represented by any one of SEQ ID Nos: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16, wherein a peptide consisting of the amino acid sequence functions as a secretory signal peptide in the bacterium of the genus *Bifidobacterium*;

(3) a DNA encoding a heterogeneous polypeptide; and

(4) a terminator DNA functioning in the bacterium of the genus *Bifidobacterium*.

2. The expression cassette according to claim 1, wherein a DNA encoding a linker peptide is linked downstream of a DNA encoding a secretory signal peptide.

3. The expression cassette according to claim 2, wherein the linker peptide consists of each amino acid sequence obtained by deletion of 0 to 29 amino acid residues from a

C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35.

4. The expression cassette according to claim 2, wherein the linker peptide consists of each amino acid sequence obtained by deletion of 10 to 29 amino acid residues from a C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35.

5. The expression cassette according to any one of claims 1 to 4, wherein the heterogeneous polypeptide is a single-chain antibody.

6. The expression cassette according to claim 5, wherein the single-chain antibody is an anti-PD-1 antibody.

7. The expression cassette according to claim 5, wherein the single-chain antibody is an anti-CTLA-4 antibody.

8. A vector comprising the expression cassette according to any one of claims 1 to 7.

9. A bacterium of the genus *Bifidobacterium* transformed with the vector according to claim 8.

10. The bacterium of the genus *Bifidobacterium* according to claim 9, which is *Bifidobacterium longum*.

11. A pharmaceutical composition comprising the bacterium of the genus *Bifidobacterium* according to claim 9 or 10.

12. A secretory signal peptide consisting of the amino acid sequence represented by any one of SEQ ID Nos: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16.

13. A DNA encoding the secretory signal peptide according to claim 12.

14. A secretory signal peptide-linker conjugate consisting of each amino acid sequence obtained by deletion of 0 to 29 amino acid residues from a C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114 and 115.

15. The secretory signal peptide-linker conjugate consisting of each amino acid sequence obtained by deletion of 10 to 29 amino acid residues from a C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114 and 115.

16. A DNA encoding the secretory signal peptide-linker conjugate according to claim 14 or 15.

## CLAIMS

1. An expression cassette expressed in a bacterium of the genus *Bifidobacterium*, sequentially comprising the following DNAs (1) to (4).

(1) a promoter DNA functioning in the bacterium of the genus *Bifidobacterium*;

(2) a DNA encoding a secretory signal peptide consisting of an amino acid sequence represented by the following a) or b);

a) an amino acid sequence represented by any one of SEQ ID Nos. 8, 12, 14, 15, 16, 6, 7, 9, 10, 11, and 13;

b) an amino acid sequence obtained by deletion, replacement or addition of one or several amino acids in the amino acid sequence represented by any one of SEQ ID Nos: 8, 12, 14, 15, 16, 6, 7, 9, 10, 11, and 13, wherein a peptide consisting of the amino acid sequence functions as a secretory signal peptide in the bacterium of the genus *Bifidobacterium*;

(3) a DNA encoding a heterogeneous polypeptide; and

(4) a terminator DNA functioning in the bacterium of the genus *Bifidobacterium*.

2. The expression cassette according to claim 1, wherein a DNA encoding a linker peptide is linked downstream of a DNA encoding a secretory signal peptide.

3. The expression cassette according to claim 2, wherein the linker peptide consists of each amino acid sequence obtained by deletion of 0 to 29 amino acid residues from a

C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 27, 31, 33, 34, 35, 25, 26, 28, 29, 30, and 32.

4. The expression cassette according to claim 2, wherein the linker peptide consists of each amino acid sequence obtained by deletion of 10 to 29 amino acid residues from a C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 27, 31, 33, 34, 35, 25, 26, 28, 29, 30, and 32.

5. The expression cassette according to any one of claims 1 to 4, wherein the heterogeneous polypeptide is a single-chain antibody.

6. The expression cassette according to claim 5, wherein the single-chain antibody is an anti-PD-1 antibody.

7. The expression cassette according to claim 5, wherein the single-chain antibody is an anti-CTLA-4 antibody.

8. A vector comprising the expression cassette according to any one of claims 1 to 7.

9. A bacterium of the genus *Bifidobacterium* transformed with the vector according to claim 8.

10. The bacterium of the genus *Bifidobacterium* according to claim 9, which is *Bifidobacterium longum*.

11. A pharmaceutical composition comprising the bacterium of the genus *Bifidobacterium* according to claim 9 or 10.

12. A secretory signal peptide consisting of the amino acid sequence represented by any one of SEQ ID Nos: 8, 12, 14, 15, 16, 6, 7, 9, 10, 11, and 13.

13. A DNA encoding the secretory signal peptide according to claim 12.

14. A secretory signal peptide-linker conjugate consisting of each amino acid sequence obtained by deletion of 0 to 29 amino acid residues from a C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 107, 111, 113, 114, 115, 105, 106, 108, 109, 110, and 112.

15. The secretory signal peptide-linker conjugate consisting of each amino acid sequence obtained by deletion of 10 to 29 amino acid residues from a C terminal of an amino acid sequence represented by any one of SEQ ID Nos: 107, 111, 113, 114, 115, 105, 106, 108, 109, 110, and 112.

16. A DNA encoding the secretory signal peptide-linker conjugate according to claim 14 or 15.