

amino acid is an unnatural amino acid, and wherein A is adenine, C is cytosine, G is guanine, and U is uracil, and wherein a tRNA body of the first tRNA is a chimeric tRNA body.

In another aspect, the present disclosure provides a method for producing the composition of the disclosure, which comprises preparing the first tRNA by attaching the first amino acid to a tRNA
5 outside a translation system, and/or preparing the second tRNA by attaching the second amino acid to a tRNA outside a translation system.

In another aspect, the present disclosure provides a method for producing a peptide, comprising translating a nucleic acid using the composition of the disclosure.

In another aspect, the present disclosure provides a method for reducing misreading of a codon
10 complementary to the anticodon in the second tRNA by the first tRNA, wherein the method comprises translating a nucleic acid using the composition of the disclosure.

In another aspect, the present disclosure provides a method for reducing misreading of a second codon by a tRNA carrying an anticodon complementary to a first codon, comprising substituting at least one base at a position selected from the group consisting of positions 32, 33, 37, and 38
15 (tRNA numbering rule) in said tRNA, wherein, the bases at the first letters of the first codon and the second codon are the same, the bases at the second letters of the first codon and the second codon are the same, and the bases at the third letters of the first codon and the second codon are different from each other.

In another aspect, the present disclosure provides a peptide produced by the method of the
20 disclosure.

In another aspect, the present disclosure provides a method for producing a peptide library, comprising translating a nucleic acid library using the composition of the disclosure.

In another aspect, the present disclosure provides a peptide library produced by the method of the disclosure.

25 In another aspect, the present disclosure provides a method of identifying a peptide that binds to a target molecule, comprising: (a) producing a peptide library using the method of the disclosure, and (b) contacting the target molecule with the peptide library.

[Technical Problem]

[0007]

30 The present invention was achieved in view of the above circumstances. An objective of the present invention is to provide compositions for translation that enable reducing the rate of mistranslation into unintended amino acids attributable to misreading of codons by tRNAs, and methods for producing peptides using the compositions for translation.

35 [Solution to Problem]

[0008]

[CLAIMS]

1. A composition for translation, comprising a first tRNA to which a first amino acid is attached and a second tRNA to which a second amino acid is attached,

5 wherein a combination of bases at positions 32, 33, 37, and 38 (tRNA numbering rule) in the first tRNA is:

(1) 32U, 33U, 37G, and 38A;

(2) 32A, 33U, 37G, and 38U;

(3) 32A, 33U, 37A, and 38U;

10 (4) 32U, 33U, 37G, and 38U;

(5) 32U, 33U, 37A, and 38U; or

(6) 32C, 33U, 37G, and 38A;

bases at the first letters of the anticodons in the first tRNA and the second tRNA are different from each other,

15 bases at the second letters of the anticodons in the first tRNA and the second tRNA are the same, bases at the third letters of the anticodons in the first tRNA and the second tRNA are the same, and

at least one selected from the first amino acid and the second amino acid is an unnatural amino acid, and

20 wherein A is adenine, C is cytosine, G is guanine, and U is uracil, and wherein a tRNA body of the first tRNA is a chimeric tRNA body.

2. The composition of claim 1, wherein

the base at the first letter of the anticodon in the first tRNA is A or G, and the base at the first letter of the anticodon in the second tRNA is C or U, or

25 the base at the first letter of the anticodon in the first tRNA is C or U, and the base at the first letter of the anticodon in the second tRNA is A or G.

3. The composition of claim 1 or 2, wherein a combination of bases at the second and third letters of the anticodons in the first and the second tRNAs is:

30 (i) G at the second letter and G at the third letter;

(ii) A at the second letter and G at the third letter;

(iii) C at the second letter and C at the third letter;

(iv) G at the second letter and C at the third letter;

(v) A at the second letter and C at the third letter;

35 (vi) G at the second letter and U at the third letter;

(vii) G at the second letter and A at the third letter; or

(viii) C at the second letter and G at the third letter.

4. The composition of any one of claims 1 to 3, which is a composition for cell-free translation.

5. The composition of any one of claims 1 to 4, wherein the chimeric tRNA body is a chimeric tRNA body in which the bases at positions 32, 33, 37, and 38 (tRNA numbering rule) and the base sequence of the other portions have different origins.

6. The composition of any one of claims 1 to 5, wherein the first tRNA is an artificial tRNA.

7. The composition of any one of claims 1 to 6 (provided that, when the first tRNA and the second tRNA have a chimeric tRNA body which has the bases specified in (4) of claim 1 at positions 32, 33, 37, and 38 (tRNA numbering rule) and which at positions 1 to 31 and positions 39 to 74 (tRNA numbering rule) has the base sequences of positions 1 to 31 and positions 39 to 74 (tRNA numbering rule) of a tRNA having the base sequence of SEQ ID NO: 255, a composition in which the combination of the anticodons in the first tRNA and the second tRNA is GCG and CCG, and a composition in which the combination of the anticodons in the first tRNA and the second tRNA is CCG and GCG, are excluded.

8. The composition of any one of claims 1 to 7, wherein the first tRNA is a tRNA carrying a chimeric anticodon loop.

9. The composition of any one of claims 1 to 8, wherein the combination of bases at positions 32, 33, 37, and 38 (tRNA numbering rule) in the first tRNA is:

- (1) 32U, 33U, 37G, and 38A;
- (2) 32A, 33U, 37G, and 38U;
- (3) 32A, 33U, 37A, and 38U; or
- (4) 32U, 33U, 37G, and 38U.

10. The composition of any one of claims 1 to 9, wherein the second amino acid is an unnatural amino acid.

11. A method for producing the composition of any one of claims 1 to 10, which comprises preparing the first tRNA by attaching the first amino acid to a tRNA outside a translation system, and/or preparing the second tRNA by attaching the second amino acid to a tRNA outside a

translation system.

12. A method for producing a peptide, comprising translating a nucleic acid using the composition of any one of claims 1 to 10.

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13. A method for reducing misreading of a codon complementary to the anticodon in the second tRNA by the first tRNA, wherein the method comprises translating a nucleic acid using the composition of any one of claims 1 to 10.

10 14. A method for reducing misreading of a second codon by a tRNA carrying an anticodon complementary to a first codon, comprising substituting at least one base at a position selected from the group consisting of positions 32, 33, 37, and 38 (tRNA numbering rule) in said tRNA, wherein,
the bases at the first letters of the first codon and the second codon are the same,
15 the bases at the second letters of the first codon and the second codon are the same, and
the bases at the third letters of the first codon and the second codon are different from each other.

15. A peptide produced by the method of claim 12.

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16. A method for producing a peptide library, comprising translating a nucleic acid library using the composition of any one of claims 1 to 10.

17. A peptide library produced by the method of claim 16.

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18. A method of identifying a peptide that binds to a target molecule, comprising:
(a) producing a peptide library using the method of claim 16, and
(b) contacting the target molecule with the peptide library.