therefore, a need exists for novel methods useful for preventing or reducing norleucine misincorporation in to proteins, in particular during recombinant protein production in microorganisms, such as *E. coli*.

The present invention meets this need by providing engineered microorganism host cells effective at preventing norleucine misincorporation during recombinant protein production in microorganisms, such as, for example, bacteria. The present invention provides, *inter alia*, *E. coli* host cells comprising mutated *metA* and *metK* alleles (*i.e.*, altered *metA* and *metK* nucleic acid sequences) which result in methionine production by the microorganism to a degree or extent sufficient to reduce or prevent norleucine misincorporation into proteins and polypeptides. Analysis of recombinant proteins produced utilizing such host cells showed that misincorporation of norleucine residues in place of methionine residues was eliminated. The present invention further demonstrates that fermentation process performance using such *E. coli* host cells, including growth of the host cells and recombinant protein product titers utilizing such *E. coli* host cells, was comparable to that observed in control host cells.

SUMMARY

The present disclosure provides, in part, methods and compositions for preventing or reducing norleucine misincorporation into proteins and polypeptides. The methods and compositions of the present disclosure are useful for preventing or reducing norleucine misincorporation in heterologous (*e.g.*, recombinant) proteins and polypeptides expressed by a microorganism, such as, for example, bacteria (*e.g., E. coli*).

~~In one aspect, there is provided~~Disclosed is a method for preventing or reducing norleucine misincorporation into a protein or polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism is a mutant microorganism which produces methionine to a degree or extent sufficient to prevent or reduce norleucine misincorporation into the protein or polypeptide.

In one aspect, there is provided a method for preventing or reducing norleucine misincorporation into a protein or a polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.

In one aspect, there is provided a method for preventing or reducing norleucine misincorporation into a protein or a polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism comprises a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

In one aspect, there is provided a method for preventing or reducing norleucine misincorporation into a protein or a polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele and a mutant *metK* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and further wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

In some embodiments, the present disclosure provides methods for preventing or reducing norleucine misincorporation into a protein or polypeptide expressed by a microorganism, wherein the microorganism produces methionine to a degree or extent sufficient to prevent or reduce norleucine misincorporation into the protein or polypeptide. In some embodiments, the microorganism is a feedback-resistant or feedback-insensitive homoserine succinyltransferase microorganism. In other embodiments, the microorganism is a microorganism comprising a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele.

~~In one aspect there is provided~~Disclosed is a microorganism comprising a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele.

In one aspect, there is provided an *E. coli* host cell comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment.

In one aspect, there is provided an *E. coli* host cell comprising a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment.

In one aspect, there is provided an *E. coli* host cell comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment.

In one aspect, there is provided an *E. coli* host cell comprising a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment.

In one aspect, there is provided an *E. coli* host cell comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment.

In one aspect, there is provided an *E. coli* host cell comprising a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment.

The present disclosure provides a microorganism (*e.g.*, a microorganism host cell), wherein the microorganism produces methionine to a degree or extent sufficient to prevent or reduce norleucine misincorporation into proteins or polypeptides expressed by the microorganism. In some embodiments, the present disclosure provides a microorganism, wherein the microorganism is a feedback-insensitive homoserine succinyltransferase microorganism. In other embodiments, the present disclosure provides a microorganism comprising a mutant *metA* allele. In some embodiments, the present disclosure provides a microorganism comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence encoding an amino acid substitution in MetA selected from the group consisting of an arginine to cysteine substitution at amino acid position 27, a glutamine to glutamic acid substitution at amino acid position 64, a tyrosine to cysteine substitution at amino acid position 294, an isoleucine to serine substitution at amino acid position 296, and a proline to leucine substitution at amino acid position 298. In some embodiments, the microorganism host cell is a bacterium. In other embodiments, the microorganism host cell is *E. coli*.

In some embodiments, the present disclosure provides a microorganism comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence encoding more than one amino acid substitution in MetA. In some embodiments, the mutant *metA* allele comprises a nucleic acid sequence encoding an isoleucine to serine substitution at amino acid position 296 in MetA and a proline to leucine substitution at amino acid position 298 in MetA. In some embodiments, the microorganism host cell is a bacterium. In other embodiments, the microorganism host cell is *E. coli*.

In some embodiments, the present disclosure provides a microorganism, wherein the microorganism comprises a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26. In some embodiments, the microorganism host cell is a bacterium. In other embodiments, the microorganism host cell is *E. coli*.

The present disclosure provides a microorganism (*e.g.*, a microorganism host cell), wherein the microorganism produces methionine to a degree or extent sufficient to prevent or reduce norleucine misincorporation into proteins or polypeptides expressed by the microorganism.

sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28. In some embodiments , the microorganism is a bacteria, *e.g., E. coli.*

~~In one aspect, there is provided~~Disclosed is a method for producing a protein or a polypeptide in a bacteria host cell, wherein the protein or the polypeptide is free of norleucine misincorporation, the method comprising expressing in the bacteria host cell a nucleic acid encoding the protein or the polypeptide under culture conditions suitable to allow for expression of the protein or the polypeptide, wherein the bacteria host cell comprises a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele, thereby producing a protein or a polypeptide free of norleucine misincorporation.

The present disclosure further provides a method for producing in a bacteria host cell a protein or a polypeptide free of norleucine misincorporation, the method comprising expressing in the bacteria host cell a nucleic acid encoding the protein or the polypeptide, wherein the bacteria host cell comprises a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele, thereby producing a protein or polypeptide free of norleucine misincorporation. In some embodiments, the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

The present disclosure also provides a method for producing an antibody or an antibody fragment in a bacteria host cell, wherein the antibody or the antibody fragment is free of norleucine misincorporation, the method comprising expressing in the bacteria host cell a nucleic acid encoding the antibody or the antibody fragment, wherein the bacteria host cell comprises a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele, thereby producing an antibody or an antibody fragment free of norleucine misincorporation. In some embodiments , the method for producing an antibody or an antibody fragment in a bacteria host cell free of norleucine misincorporation according to the present disclosure comprises expressing in the bacteria host cell a nucleic acid encoding an antibody heavy chain polypeptide and a nucleic acid encoding an antibody light chain polypeptide. In some embodiments , the antibody heavy chain polypeptide is an antibody Fab fragment heavy chain polypeptide, and the antibody light chain polypeptide is an antibody Fab fragment light chain polypeptide. In some embodiments, the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

In one aspect, there is provided a method for producing a protein or a polypeptide in an *E. coli* host cell, wherein the protein or the polypeptide is free of norleucine misincorporation, the method comprising expressing in the *E. coli* host cell a nucleic acid encoding the protein or the polypeptide under culture conditions suitable to allow for expression of the protein or the polypepetide, wherein the *E. coli* host cell comprises a mutant *metA* allele, wherein the mutant *metA* allele encodes for MetA having an amino acid sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.
In one aspect, there is provided a method for producing a protein or a polypeptide in an *E. coli* host cell, wherein the protein or the polypeptide is free of norleucine misincorporation, the method comprising expressing in the *E. coli* host cell a nucleic acid encoding the protein or the polypeptide under culture conditions suitable to allow for expression of the protein or the polypepetide, wherein the *E. coli* host cell comprises a mutant *metK* allele, wherein the mutant *metK* allele encodes for MetK having an amino acid sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

In some embodiments, the present disclosure provides a method for producing an anti-VEGF antibody or an anti-VEGF antibody fragment in a bacterial host cell, wherein the anti-VEGF

CLAIMS

What is claimed is:

1. A method for preventing or reducing norleucine misincorporation into a protein or a polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.

2. A method for preventing or reducing norleucine misincorporation into a protein or a polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism comprises a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

3. A method for preventing or reducing norleucine misincorporation into a protein or a polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele and a mutant *metK* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and further wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

4. The method of claim 1, claim 2, or claim 3, wherein the microorganism is a bacteria.

5. The method of claim 4, wherein the bacteria is *E. coli*.

6. A method for producing a protein or a polypeptide in an *E. coli* host cell, wherein the protein or the polypeptide is free of norleucine misincorporation, the method comprising expressing in the *E. coli* host cell a nucleic acid encoding the protein or the polypeptide under culture conditions suitable to allow for expression of the protein or the polypepetide, wherein the *E. coli* host cell comprises a mutant *metA* allele, wherein the mutant *metA* allele encodes for MetA having an amino acid sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.

7. A method for producing a protein or a polypeptide in an *E. coli* host cell, wherein the protein or the polypeptide is free of norleucine misincorporation, the method comprising expressing in the *E. coli* host cell a nucleic acid encoding the protein or the polypeptide under culture conditions suitable to allow for expression of the protein or the polypepetide, wherein the *E. coli* host cell comprises a mutant *metK* allele, wherein the mutant *metK* allele encodes for MetK having an amino acid sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

8. The method of claim 6 or claim 7, wherein the protein or the polypeptide is an antibody or an antibody fragment.

9. The method of claim 6 or claim 7, wherein the antibody or the antibody fragment is an anti-VEGF antibody or an anti-VEGF antibody fragment.

10. The method of claim 6 or claim 7, wherein the protein or the polypeptide is an antibody or an antibody fragment selected from the group consisting of an anti-VEGF antibody or an anti-VEGF antibody fragment, and wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:46 and nucleic acid encoding the amino acid sequence of SEQ ID NO:47.

11. The method of claim 6 or claim 7, wherein the protein or the polypeptide is an antibody or an antibody fragment selected from the group consisting of an anti-VEGF antibody or an anti-VEGF antibody fragment, and wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is selected from the group consisting of the nucleic acid sequence comprising SEQ ID NO: 33 and the nucleic acid sequence comprising SEQ ID NO:34.

12. The method of claim 6 or claim 7, wherein the antibody or the antibody fragment is an anti-Factor D antibody or an anti-Factor D antibody fragment.

13. The method of claim 6 or claim 7, wherein the protein or the polypeptide is an antibody or an antibody fragment selected from the group consisting of an anti-Factor D antibody and an anti-Factor D antibody fragment, and wherein nucleic acid encoding the anti-Factor D antibody or the anti-Factor D antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:48 and nucleic acid encoding the amino acid sequence of SEQ ID NO:49.

14. The method of claim 6 or claim 7, wherein the antibody or the antibody fragment is an anti-MET antibody or an anti-MET antibody fragment.

15. The method of claim 6 or claim 7, wherein the protein or the polypeptide is an antibody or an antibody fragment selected from the group consisting of an anti-MET antibody and an anti-MET antibody fragment, and wherein nucleic acid encoding the anti-MET antibody or the anti-MET antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:50, nucleic acid encoding the amino acid sequence of SEQ ID NO:51, and nucleic acid encoding the amino acid sequence of SEQ ID NO:52.

16. The method of claim 6 or claim 7, wherein expressing the protein or the polypeptide in the *E. coli* host cell is performed in the absence of exogenously added methionine to the culture medium or without a methionine feed.

17. An anti-VEGF antibody or anti-VEGF antibody fragment produced according to the method of claim 9, claim 10, or claim 11.

18. An anti-Factor D antibody or anti-Factor D antibody fragment produced according to the method of claim 12 or claim 13.

19. An anti-MET antibody or anti-MET antibody fragment produced according to the method of claim 14 or claim 15.

20. An *E. coli* host cell comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment.

21. The *E. coli* host cell of claim 20, wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is selected from the group consisting of nucleic acid sequence comprising SEQ ID NO: 33 and nucleic acid sequence comprising SEQ ID NO:34.

22. The *E. coli* host cell of claim 20, wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:46 and nucleic acid encoding the amino acid sequence of SEQ ID NO:47.

23. An *E. coli* host cell comprising a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment.

24. The *E. coli* host cell of claim 23, wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is selected from the group consisting of the nucleic acid sequence comprising SEQ ID NO: 33 and nucleic acid sequence comprising SEQ ID NO:34.

25. The *E. coli* host cell of claim 23, wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:46 and nucleic acid encoding the amino acid sequence of SEQ ID NO:47.

26. An *E. coli* host cell comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment.

27. The *E. coli* host cell of claim 26, wherein nucleic acid encoding the anti-Factor D antibody or the anti-Factor D antibody fragment is selected from the group consisting of

nucleic acid encoding the amino acid sequence of SEQ ID NO:48 and nucleic acid encoding the amino acid sequence of SEQ ID NO:49.

28. An *E. coli* host cell comprising a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment.

29. The *E. coli* host cell of claim 28, wherein nucleic acid encoding the anti-Factor D antibody or the anti-Factor D antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:48 and nucleic acid encoding the amino acid sequence of SEQ ID NO:49.

30. An *E. coli* host cell comprising a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment.

31. The *E. coli* host cell of claim 30, wherein nucleic acid encoding the anti-MET antibody or the anti-MET antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:50, nucleic acid encoding the amino

acid sequence of SEQ ID NO:51, and nucleic acid encoding the amino acid sequence of SEQ ID NO:52.

32. An *E. coli* host cell comprising a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28, wherein the *E. coli* host cell further comprises nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment.

33. The *E. coli* host cell of claim 32, wherein nucleic acid encoding the anti-MET antibody or the anti-MET antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:50, nucleic acid encoding the amino acid sequence of SEQ ID NO:51, and nucleic acid encoding the amino acid sequence of SEQ ID NO:52.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for preventing or reducing norleucine misincorporation into a protein or polypeptide, the method comprising expressing the protein or the polypeptide in a microorganism, wherein the microorganism is a mutant microorganism which produces methionine to a degree or extent sufficient to prevent or reduce norleucine misincorporation into the protein or polypeptide.

2. The method of claim 1, wherein the microorganism is a bacteria.

3. The method of claim 1, wherein the microorganism is *E. coli*.

4. The method of claim 1, wherein the microorganism is a feedback-resistant or feedback-insensitive homoserine succinyltransferase microorganism.

5. The method of claim 1, wherein the microorganism is de-repressed for methionine production.

6. The method of claim 1, wherein the microorganism comprises a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele.

7. The method of claim 1, wherein expressing the protein or the polypeptide in the microorganism is performed in the absence of exogenously added methionine to the culture medium or without a methionine feed.

8. A microorganism comprising a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele.

9. The microorganism of claim 8, wherein the microorganism comprises a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence encoding an amino acid substitution in MetA selected from the group consisting of an arginine to

cysteine substitution at amino acid position 27, a glutamine to glutamic acid substitution at amino acid position 64, a tyrosine to cysteine substitution at amino acid position 294, an isoleucine to serine substitution at amino acid position 296, a proline to leucine substitution at amino acid position 298, and an isoleucine to serine substitution at amino acid position 296 and a proline to leucine substitution at amino acid position 298.

10. The microorganism of claim 9, wherein the microorganism comprises a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.

11. The microorganism of claim 8, wherein the mutant *metK* allele comprises a nucleic acid sequence encoding an amino acid substitution in MetK comprising a valine to glutamic acid substitution at amino acid position 185 or a nucleic acid sequence comprising a deletion of the cytosine base at nucleic acid residue position 1132 of the *metK* allele.

12. The microorganism of claim 11, wherein the microorganism comprises a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

13. The microorganism of claim 8, wherein the microorganism comprises a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence encoding an amino acid substitution in MetA selected from the group consisting of an arginine to cysteine substitution at amino acid position 27, a glutamine to glutamic acid substitution at amino acid position 64, a tyrosine to cysteine substitution at amino acid position 294, an isoleucine to serine substitution at amino acid position 296, a proline to leucine substitution at amino acid position 298, and an isoleucine to serine substitution at amino acid position 296 and a proline to leucine substitution at amino acid position 298, and further wherein the mutant *metK* allele comprises a nucleic acid sequence encoding an amino acid substitution in MetK comprising a valine to

glutamic acid substitution at amino acid position 185 or a nucleic acid sequence comprising a deletion of the cytosine base at nucleic acid residue position 1132 of the *metK* allele.

14. The microorganism of claim 13, wherein the microorganism comprises a mutant *metA* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and further wherein the microorganism comprises a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28.

15. The microorganism of claim 8, further comprising nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment.

16. The microorganism of claim 15, wherein nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment is nucleic acid encoding the amino acid sequence of SEQ ID NO:46 and nucleic acid encoding the amino acid sequence of SEQ ID NO:47.

17. The microorganism of claim 15, wherein nucleic acid encoding an anti-VEGF antibody of an anti-VEGF antibody fragment is selected from the group consisting of the nucleic acid sequence comprising SEQ ID NO: 33 and the nucleic acid sequence comprising SEQ ID NO:34.

18. The microorganism of claim 8, further comprising nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment.

19. The microorganism of claim 18, wherein nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment is nucleic acid encoding the amino acid sequence of SEQ ID NO:48 and nucleic acid encoding the amino acid sequence of SEQ ID NO:49.

20. The microorganism of claim 8, further comprising nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment.

21. The microorganism of claim 20, wherein nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:50, nucleic acid encoding the amino acid sequence of SEQ ID NO:51, and nucleic acid encoding the amino acid sequence of SEQ ID NO:52.

22. A method for producing a protein or a polypeptide in a bacteria host cell, wherein the protein or the polypeptide is free of norleucine misincorporation, the method comprising expressing in the bacteria host cell a nucleic acid encoding the protein or the polypeptide under culture conditions suitable to allow for expression of the protein or the polypeptide, wherein the bacteria host cell comprises a mutant *metA* allele, a mutant *metK* allele, or a mutant *metA* allele and a mutant *metK* allele, thereby producing a protein or a polypeptide free of norleucine misincorporation.

23. The method of claim 22, wherein the bacteria host cell is selected from the group consisting of the microorganism of claim 9, the microorganism of claim 10, the microorganism of claim 11, the microorganism of claim 12, the microorganism of claim 13, and the microorganism of claim 14.

24. The method of claim 22, wherein the protein or the polypeptide is an antibody or an antibody fragment.

25. The method of claim 23, wherein the protein or the polypeptide is an antibody or an antibody fragment.

26. The method of claim 24, wherein the antibody or the antibody fragment is an anti-VEGF antibody or an anti-VEGF antibody fragment.

27. The method of claim 26, wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is nucleic acid encoding the amino acid sequence of SEQ ID NO:46 and nucleic acid encoding the amino acid sequence of SEQ ID NO:47.

28. The method of claim 26, wherein nucleic acid encoding the anti-VEGF antibody or the anti-VEGF antibody fragment is selected from the group consisting of the nucleic acid sequence comprising SEQ ID NO: 33 and the nucleic acid sequence comprising SEQ ID NO:34.

29. The method of claim 24, wherein the antibody or the antibody fragment is an anti-Factor D antibody or an anti-Factor D antibody fragment.

30. The method of claim 29, wherein nucleic acid encoding the anti-Factor D antibody or the anti-Factor D antibody fragment is nucleic acid encoding the amino acid sequence of SEQ ID NO:48 and nucleic acid encoding the amino acid sequence of SEQ ID NO:49.

31. The method of claim 24, wherein the antibody or the antibody fragment is an anti-MET antibody or an anti-MET antibody fragment.

32. The method of claim 31, wherein nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment is selected from the group consisting of nucleic acid encoding the amino acid sequence of SEQ ID NO:50, nucleic acid encoding the amino acid sequence of SEQ ID NO:51, and nucleic acid encoding the amino acid sequence of SEQ ID NO:52.

33. The method of claim 22, wherein expressing the protein or the polypeptide in the bacteria host cell is performed in the absence of exogenously added methionine to the culture medium or without a methionine feed.

34. An anti-VEGF antibody or anti-VEGF antibody fragment produced according to the method of claim 26, claim 27, or claim 28.

35. An anti-Factor D antibody or anti-Factor D antibody fragment produced according to the method of claim 29 or claim 30.

36. An anti-MET antibody or anti-MET antibody fragment produced according to the method of claim 31 or claim 32.