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*).

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~~an *E. coli*, 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 an *E. coli*~~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 an *E. coli* ~~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.

antibody or the anti-VEGF antibody fragment is free of norleucine misincorporation, the method comprising expressing in the bacteria host cell a nucleic acid encoding the anti-VEGF antibody or the anti-VEGF 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 anti-VEGF antibody or an anti-VEGF antibody fragment free of norleucine misincorporation. In some embodiments, the method comprises expressing in the bacteria host cell a nucleic acid encoding an anti-VEGF antibody heavy chain polypeptide or an anti-VEGF antibody fragment heavy chain polypeptide or fragment thereof and a nucleic acid encoding an anti-VEGF antibody light chain polypeptide or an anti-VEGF antibody fragment light chain polypeptide or fragment thereof. In some embodiments , the anti-VEGF antibody heavy chain and the anti-VEGF antibody light chain are full-length heavy chain and light chain anti-VEGF antibody polypeptides. In other embodiments , the anti-VEGF antibody heavy chain is an antibody Fab fragment heavy chain polypeptide, and the anti-VEGF antibody light chain is an antibody Fab fragment light chain polypeptide. In some embodiments, the anti-VEGF antibody heavy chain comprises the amino acid sequence of SEQ ID NO:47 and the anti-VEGF antibody light chain comprises the amino acid sequence of SEQ ID NO:46. In some embodiments, the nucleic acid encoding the amino acid sequence of SEQ ID NO:47 is the nucleic acid sequence of SEQ ID NO:34. In some embodiments, the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:46 is the nucleic acid sequence of SEQ ID NO:33. 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. In some embodiments, 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~~Disclosed is an anti-VEGF antibody or anti-VEGF antibody fragment produced according to the methods as described herein.

The disclosure further provides an anti-VEGF antibody or anti-VEGF antibody fragment produced by any of the methods described herein, wherein the anti-VEGF antibody or anti-VEGF antibody fragment is free of norleucine misincorporation.

In some embodiments, the present disclosure provides a method for producing an anti-Factor D antibody or an anti-Factor D antibody fragment in a bacterial host cell, wherein the anti-Factor D antibody or the anti-Factor D antibody fragment is free of norleucine

misincorporation, the method comprising expressing in the bacteria host cell a nucleic acid encoding the anti-Factor D antibody or the anti-Factor D 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 anti-Factor D antibody or an anti-Factor D antibody fragment free of norleucine misincorporation. In some embodiments, the method comprises expressing in the bacteria host cell a nucleic acid encoding an anti-Factor D antibody heavy chain polypeptide or an anti-Factor antibody fragment heavy chain polypeptide or fragment thereof and a nucleic acid encoding an anti-Factor D antibody light chain polypeptide or an anti-Factor D antibody fragment light chain polypeptide or fragment thereof. In some embodiments , the anti-Factor D antibody heavy chain and the anti-Factor D antibody light chain are full-length heavy chain and light chain anti-Factor D antibody polypeptides. In other embodiments, the anti-Factor D antibody heavy chain is an antibody Fab fragment heavy chain polypeptide, and the anti-Factor D antibody light chain is an antibody Fab fragment light chain polypeptide. In some embodiments, the anti-Factor D antibody heavy chain comprises the amino acid sequence of SEQ ID NO:49 and the anti-Factor D antibody light chain comprises the amino acid sequence of SEQ ID NO:48. 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. In some embodiments, 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~~Disclosed is an anti-Factor D antibody or anti-Factor D antibody fragment produced according to the methods as described herein.

The disclosure further provides an anti-Factor D antibody or anti-Factor D antibody fragment produced by any of the methods described herein, wherein the anti-Factor D antibody or anti-Factor D antibody fragment is free of norleucine misincorporation.

In some embodiments, the present disclosure provides a method for producing an anti-MET antibody or an anti-MET antibody fragment in a bacterial host cell, wherein the anti-MET antibody or the anti-MET antibody fragment is free of norleucine misincorporation, the method comprising expressing in the bacteria host cell a nucleic acid encoding the anti-MET antibody or the anti-MET 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 anti-MET antibody or an anti-MET antibody fragment free of

norleucine misincorporation. In some embodiments, the method comprises expressing in the bacteria host cell a nucleic acid encoding an anti-MET antibody heavy chain polypeptide or an anti-MET antibody fragment heavy chain polypeptide or fragment thereof and a nucleic acid encoding an anti-MET antibody light chain polypeptide or an anti-MET antibody fragment light chain polypeptide or fragment thereof. In some embodiments , the anti-MET antibody heavy chain and the anti-MET antibody light chain are full-length heavy chain and light chain anti-MET antibody polypeptides. In other embodiments , the anti-MET antibody heavy chain is an antibody Fab fragment heavy chain polypeptide, and the anti-MET antibody light chain is an antibody Fab fragment light chain polypeptide. In some embodiments, the anti-MET antibody heavy chain comprises the amino acid sequence of SEQ ID NO:51, the anti-MET antibody heavy chain fragment comprises the amino acid sequence of SEQ ID NO:52, and the anti-MET antibody light chain comprises the amino acid sequence of SEQ ID NO:50. 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. In some embodiments, 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~~Disclosed is an anti-MET antibody or anti-MET antibody fragment produced according to the methods as described herein.

The disclosure further provides an anti-MET antibody or anti-MET antibody fragment produced by any of the methods described herein, wherein the anti-MET antibody or anti-MET antibody fragment is free of norleucine misincorporation.

In various embodiments , a mutant microorganism comprising any one or more of the nucleic acid sequences provided by the present disclosure is bacteria; in other embodiments , the microorganism is *E. coli*. The present disclosure specifically provides for the use of a mutant microorganism described herein for the production of heterologous (*e.g.*, recombinant) polypeptides and heterologous (*e.g.*, recombinant) proteins, wherein the misincorporation of norleucine into the heterologous polypeptides and heterologous proteins is reduced, substantially reduced, substantially eliminated, or prevented.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleic acid sequence of *metA*(R27C) corresponding to SED ID NO:23.

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 an *E. coli*, 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 an *E. coli*, 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 an *E. coli*, 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. 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.

5. 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.

6. The method of claim 4 or claim 5, wherein the protein or the polypeptide is an antibody or an antibody fragment.

7. The method of claim 4 or claim 5, wherein the antibody or the antibody fragment is an anti-VEGF antibody or an anti-VEGF antibody fragment.

8. The method of claim 4 or claim 5, 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.

9. The method of claim 4 or claim 5, 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.

10. The method of claim 4 or claim 5, wherein the antibody or the antibody fragment is an anti-Factor D antibody or an anti-Factor D antibody fragment.

11. The method of claim 4 or claim 5, 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.

12. The method of claim 4 or claim 5, wherein the antibody or the antibody fragment is an anti-MET antibody or an anti-MET antibody fragment.

13. The method of claim 4 or claim 5, 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.

14. The method of claim 4 or claim 5, 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.

15. 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.

16. The *E. coli* host cell of claim 15, 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.

17. The *E. coli* host cell of claim 15, 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.

18. 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.

19. The *E. coli* host cell of claim 18, 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.

20. The *E. coli* host cell of claim 18, 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.

21. 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.

22. The *E. coli* host cell of claim 21, 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.

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-Factor D antibody or an anti-Factor D antibody fragment.

24. The *E. coli* host cell of claim 23, 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.

25. 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.

26. The *E. coli* host cell of claim 25, 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.

27. 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.

28. The *E. coli* host cell of claim 27, 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 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.