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 ~~OF THE INVENTION~~

The present disclosure ~~invention~~ provides, in part, methods and compositions for preventing or reducing norleucine misincorporation into proteins and polypeptides. The methods and compositions of the present disclosure ~~invention~~ 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 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 some embodiments, the present disclosure ~~invention~~ 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 some embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or polypeptide in a microorganism, 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, and a proline to leucine substitution at amino acid position 298. In some embodiments, the mutant *metA* allele comprises a nucleic acid sequence encoding amino acid substitutions in MetA comprising an isoleucine to serine substitution at amino acid position 296 and a proline to leucine substitution at amino acid position 298. MetA amino acid positions described herein in the instant specification are in reference to wild-type MetA amino acid sequence as shown in Figure 7A and SEQ ID NO:29.

In other embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or 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.

As stated above, the present disclosure ~~invention~~ 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 other embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide expressed by a microorganism, the method comprising expressing the protein or polypeptide in the microorganism, wherein the microorganism is a microorganism de-repressed for methionine production. In some embodiments, the microorganism is de-repressed for methionine production due to partial loss-of-function of S-adenosylmethionine synthase. In other embodiments, the present

disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide expressed by a microorganism, the method comprising expressing the protein or polypeptide in the microorganism, wherein the microorganism comprises a mutant *metK* allele. In some embodiments, the mutant *metK* allele results in a partial loss-of-function of MetK.

In some embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or polypeptide in a microorganism, wherein the microorganism comprises a mutant *metK* allele, 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. In other embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or polypeptide in a microorganism, wherein the microorganism comprises a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence comprising a deletion of the cytosine base at nucleic acid residue position 1132 of the *metK* allele. MetK amino acid positions described herein in the instant specification are in reference to wild-type MetK amino acid sequence as shown in Figure 8A and SEQ ID NO:30. *metK* nucleic acid positions described herein in the instant specification are in reference to wild-type *metK* nucleic acid sequence as shown in Figure 8B and SEQ ID NO:32.

In some embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or 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 other embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele and a *metK* allele. In some embodiments, the mutant *metA* allele

comprises a nucleic acid sequence encoding a tyrosine to cysteine substitution at amino acid position 294 of MetA and the mutant *metK* allele comprises a nucleic acid sequence encoding a valine to glutamic acid substitution at amino acid position 185 of MetK. In other embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele and a *metK* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence encoding a tyrosine to cysteine substitution at amino acid position 294 of MetA, and wherein the mutant *metK* allele comprises a nucleic acid sequence comprising a deletion of the cytosine base at nucleic acid residue position 1132 of the *metK* allele.

In other embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele and a mutant *metK* allele, and wherein the mutant *metA* allele comprises the nucleic acid sequence of SEQ ID NO:24, and the mutant *metK* allele comprises the nucleic acid sequence of SEQ ID NO:27. In yet other embodiments, the present disclosure ~~invention~~ provides methods for reducing or preventing norleucine misincorporation in a protein or polypeptide, the method comprising expressing the protein or polypeptide in a microorganism, wherein the microorganism comprises a mutant *metA* allele and a mutant *metK* allele, wherein the mutant *metA* allele comprises the nucleic acid sequence of SEQ ID NO:24, and the mutant *metK* allele comprises the nucleic acid sequence of SEQ ID NO:28.

The present disclosure ~~invention~~ further provides microorganism host cells useful for preventing or reducing norleucine misincorporation into proteins and polypeptides expressed by a microorganism host cell. The present disclosure ~~invention~~ also provides microorganism host cells for use in the expression of proteins or polypeptides by the microorganism host cell, wherein the expressed proteins or polypeptides are free of norleucine misincorporation. In some embodiments, the microorganism host cell is a bacterium. In other embodiments, the microorganism host cell is *E. coli*.

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

The present disclosure ~~invention~~ 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 ~~invention~~ provides a microorganism, wherein the microorganism is a feedback-insensitive homoserine succinyltransferase microorganism. In other embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a mutant *metA* allele. In some embodiments, the present disclosure ~~invention~~ 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 ~~invention~~ 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 ~~invention~~ 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 ~~invention~~ 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 ~~invention~~ provides a microorganism, wherein the microorganism is a microorganism de-repressed for methionine production. In some embodiments ~~aspects~~, the microorganism de-repressed for methionine production results from a partial loss-of-function of S-adenosylmethionine synthase. In other embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a mutant *metK* allele. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a mutant *metK* allele, 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. In other embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a mutant *metK* allele, wherein the mutant *metK* allele comprises a nucleic acid sequence comprising a deletion of the cytosine base at nucleic acid residue position 1132 in the *metK* allele. 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 ~~invention~~ provides 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 some embodiments, the microorganism host cell is a bacterium. In other embodiments, the microorganism host cell is *E. coli*.

The present disclosure ~~invention~~ also provides microorganism host cells comprising various combinations of mutant *metA* alleles and a mutant *metK* alleles. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a mutant *metA* allele and a mutant *metK* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence encoding an amino acid substitution in MetA comprising a tyrosine to cysteine substitution at amino acid position 294, and 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. In other embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a mutant *metA* allele and a mutant *metK* allele, wherein the mutant *metA* allele comprises a nucleic acid sequence encoding an amino acid substitution in MetA comprising a tyrosine to cysteine substitution at amino acid position 294, and wherein the mutant *metK* allele comprises a deletion of the nucleic acid cytosine at nucleic acid residue 1132 of the

metK allele. In some embodiments, the present disclosure ~~invention~~ provides a microorganism, wherein the microorganism comprises a mutant *metA* allele and a mutant *metK* allele, wherein the mutant *metA* allele comprises the nucleic acid sequence of SEQ ID NO:24, and wherein the mutant *metK* allele comprises the nucleic acid sequence of SEQ ID NO:27. In other embodiments, the present disclosure ~~invention~~ provides a microorganism, wherein the microorganism comprises a mutant *metA* allele and a mutant *metK* allele, wherein the mutant *metA* allele comprises the nucleic acid sequence of SEQ ID NO:24, and wherein the mutant *metK* allele comprises the nucleic acid sequence of SEQ ID NO:28. In some embodiments, the microorganism host cell is a bacterium. In other embodiments, the microorganism host cell is *E. coli*.

The present disclosure ~~invention~~ also provides isolated nucleic acid molecules for use in the present methods. In some embodiments ~~aspects~~, the present disclosure ~~invention~~ provides isolated *metA* nucleic acid molecules (*i.e.*, isolated nucleic acid molecules encoding MetA). In some embodiments, the present disclosure ~~invention~~ provides an isolated *metA* nucleic acid molecule, wherein the *metA* nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid substitution in MetA selected from the group consisting of an arginine to cysteine substitution in 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 other embodiments, an isolated *metA* nucleic acid molecule provided by the present disclosure ~~invention~~ 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. The use of these isolated *metA* nucleic acid molecules and sequences thereof for the production of microorganisms for use in preventing or reducing norleucine misincorporation in proteins or polypeptides is specifically provided herein by the present disclosure ~~invention~~.

The present disclosure ~~invention~~ also provides isolated *metK* nucleic acid molecules (*i.e.*, isolated nucleic acid molecules encoding MetK). In some embodiments, the present disclosure ~~invention~~ provides a *metK* nucleic acid molecule, wherein the *metK* nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid substitution in MetK comprising a valine to glutamic acid substitution at amino acid position 185. In other embodiments, the present

disclosure ~~invention~~ provides a *metK* nucleic acid molecule, wherein the *metK* nucleic acid molecule comprises a deletion of the nucleic acid cytosine at nucleic acid residue 1132 of the *metK* allele. In other embodiments, a *metK* nucleic acid molecule provided by the present disclosure ~~invention~~ comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:28. The use of these isolated *metK* nucleic acid molecules and sequences thereof for the production of microorganisms for use in preventing or reducing norleucine misincorporation in proteins or polypeptides is specifically provided herein by the present disclosure ~~invention~~.

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele, wherein the microorganism further comprises nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 46 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 47. In some embodiments, the nucleic acid encoding the amino acid sequence of SEQ ID NO:46 is the nucleic acid sequence of SEQ ID NO:33. In some embodiments, the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:47 is the nucleic acid sequence of SEQ ID NO:34. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele, wherein the microorganism further comprises a nucleic acid having the nucleic acid sequence corresponding to SEQ ID NO:33 and a nucleic acid having the nucleic acid sequence corresponding to SEQ ID NO:34. 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 ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metK* allele, wherein the microorganism further comprises nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid

sequence of SEQ ID NO: 46 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 47. In some embodiments, the nucleic acid encoding the amino acid sequence of SEQ ID NO:46 is the nucleic acid sequence of SEQ ID NO:33. In some embodiments, the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:47 is the nucleic acid sequence of SEQ ID NO:34. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid having the nucleic acid sequence corresponding to SEQ ID NO:33 and a nucleic acid having the nucleic acid sequence corresponding to SEQ ID NO:34. 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 some embodiments ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele and a mutant *metK* allele, wherein the microorganism further comprises nucleic acid encoding an anti-VEGF antibody or an anti-VEGF antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele and a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 46 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 47. In some embodiments, the nucleic acid encoding the amino acid sequence of SEQ ID NO:46 is the nucleic acid sequence of SEQ ID NO:33. In some embodiments, the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:47 is the nucleic acid sequence of SEQ ID NO:34. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele and a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid having the nucleic acid sequence corresponding to SEQ ID NO:33 and a nucleic acid having the nucleic acid sequence corresponding to SEQ ID NO:34. 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 some embodiments ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele, wherein the microorganism further comprises nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 48 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 49. 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 ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metK* allele, wherein the microorganism further comprises nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 48 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 49. 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 some embodiments ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele and a mutant *metK* allele, wherein the microorganism further comprises nucleic acid encoding an anti-Factor D antibody or an anti-Factor D antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele and a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 48 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 49. 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 some embodiments ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele, wherein the microorganism further comprises nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 50, a nucleic acid encoding the amino acid sequence of SEQ ID NO: 51, and a nucleic acid encoding the amino acid sequence of SEQ ID NO:52. 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 ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metK* allele, wherein the microorganism further comprises nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 50, a nucleic acid encoding the amino acid sequence of SEQ ID NO: 51, and a nucleic acid encoding the amino acid sequence of SEQ ID NO:52. 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 some embodiments ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

The present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele and a mutant *metK* allele, wherein the microorganism further comprises nucleic acid encoding an anti-MET antibody or an anti-MET antibody fragment. In some embodiments, the present disclosure ~~invention~~ provides a microorganism comprising a nucleic acid comprising a mutant *metA* allele and a mutant *metK* allele, wherein the microorganism further comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 50, a nucleic acid encoding the amino acid sequence of SEQ ID NO: 51, and a nucleic acid encoding the amino acid sequence of SEQ ID NO:52. 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 some embodiments ~~aspects~~, the microorganism is a bacteria, *e.g., E. coli.*

In one aspect, there is provided 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 ~~invention~~ 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 ~~invention~~ 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 ~~aspects~~, the method for producing an antibody or an antibody fragment in a bacteria host cell free of norleucine misincorporation according to the present disclosure ~~invention~~ 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 ~~aspects~~, 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 some embodiments, the present disclosure ~~invention~~ provides a method for producing an anti-VEGF antibody or an anti-VEGF antibody fragment in a bacterial host cell, wherein the anti-VEGF

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 ~~aspects~~, 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 ~~aspects~~, 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 an anti-VEGF antibody or anti-VEGF antibody fragment produced according to the methods as described herein.

The disclosure ~~invention~~ 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 ~~invention~~ 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 ~~aspects~~, 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 ~~aspects~~, 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 an anti-Factor D antibody or anti-Factor D antibody fragment produced according to the methods as described herein.

The disclosure ~~invention~~ 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 ~~invention~~ 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 ~~aspects~~, 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 ~~aspects~~, 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 an anti-MET antibody or anti-MET antibody fragment produced according to the methods as described herein.

The disclosure ~~invention~~ 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 ~~aspects~~, a mutant microorganism comprising any one or more of the nucleic acid sequences provided by the present disclosure ~~invention~~ is bacteria; in other embodiments ~~aspects~~, the microorganism is *E. coli*. The present disclosure ~~invention~~ 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.