NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0.

[0020f]In another aspect, there is provided a method for expressing protein in a cell-free synthesis system, comprising:

i. combining a nucleic acid template with a bacterial extract comprising an RF1 protein that has been cleaved by OmpT1 at a scissile OmpT1 peptide bond located within the switch loop region corresponding to amino acids 287-303 of wild type RF1 (SEQ ID NO:1) and where the switch loop region has been modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0 under conditions that permit protein synthesis; and

ii. expressing a protein from the nucleic acid template.

[0020g] In another aspect, there is provided a functional Releasing Factor 1 protein (RF1) that is cleavable by an Outer Membrane Protein T1 (OmpT1), where a scissile OmpT1 peptide bond is located within the switch loop region corresponding to amino acids 287-304 of wild type RF1 (SEQ ID NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

[0020h] In another aspect, there is provided a nucleic acid encoding a functional Releasing Factor 1 protein (RF1) that is cleavable by an Outer Membrane Protein T1 (OmpT1) where a scissile OmpT1 peptide bond is located within the switch loop region of RF1 corresponding to amino acids 287-304 of wild type RF1 (SEQ ID NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

[0020i] In another aspect, there is provided a method for reducing RF1 competition at an amber codon in an *in vitro* cell free synthesis system, the method comprising the steps of:

i) culturing an OmpT1 positive bacteria expressing a functional Releasing Factor 1 protein (RF1) that is cleavable by an Outer Membrane Protein T1 (OmpT1);

ii) lysing the bacteria to create a cell free synthesis extract;

iii) contacting the RF1 protein in the extract with OmpT1 in an amount to sufficient to reduce intact RF1 protein by 50%;

iv) adding a nucleic acid template to the extract where the template codes for a protein of interest and includes an amber codon; and,

v) allowing the cell free synthesis system to produce the protein of interest, where the RF1 protein has a scissile OmpT1 peptide bond is located within the switch loop region corresponding to amino acids 287-304 of wild type RF1 (SEQ ID NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

[0020j] In another aspect, there is provided a cell free synthesis system comprising in a single reaction mixture:

i) components from a bacterial lysate sufficient to translate a nucleic acid template encoding a protein;

ii) a nucleic acid template encoding a protein of interest and having at least one amber codon;

iii) tRNA complementary to the amber codon; and,

iv) a functional Releasing Factor 1 (RF1) protein cleavable by an Outer Membrane Protein T1 (OmpT1), where a scissile OmpT1 peptide bond is located within the switch loop region of RF1 corresponding to amino acids 287-304 of wild type RF1 (SEQ ID NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

[0020k] In another aspect, there is provided a method for expressing protein in a cell-free synthesis system, comprising:

i. combining a nucleic acid template with a bacterial extract comprising an RF1 protein that has been cleaved by OmpT1 at a scissile OmpT1 peptide bond located within the switch loop region corresponding to amino acids 287-303 of wild type RF1 (SEQ ID NO:1) and where the switch loop region has been modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid under conditions that permit protein synthesis; and

ii. expressing a protein from the nucleic acid template.

[0020l] In another aspect, there is provided a bacterial cell comprising a nucleic acid encoding the the functional RF1 as disclosed herein incorporated into the bacterial cell's genome.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **Figure 1** shows that modified RF1 protein variants containing OmpT cleavage sites have wild-type RF1 activity when added to cell-free extracts lacking OmpT activity. The ability of exogenous RF-1 variants to reduce suppression (increase chain termination) when adding a non-native amino acid at an amber codon introduced at position S378 of the Fc protein (Fc_S378TAG) was determined. Figure 1(A) Lane 1 is a control extract with no exogenously added RF1. The extract contains wild-type RF1, which results in reduced amber suppression, producing a truncated Fc (lower band in gel). Lane 2 is a control extract containing exogenously added wild-type (WT) RF1. The addition of wild-type RF1 results in a further reduction in amber suppression, which produces relatively more truncated Fc. Lanes 3-7 show cell-free extracts containing different RF1 variants of the invention. All five

WHAT IS CLAIMED IS:

1. A functional Releasing Factor 1 protein (RF1) that is cleavable by an Outer Membrane Protein T1 (OmpT1), where a scissile OmpT1 peptide bond is located within the switch loop region corresponding to amino acids 287-304 of wild type RF1 (SEQ ID NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

2. The RF1 protein of claim 1 where the switch loop region is modified to have four or five adjacent basic amino acids.

3. The RF1 protein of claim 1 wherein the native asparagine at position 296 is substituted for one of the three adjacent basic amino acids.

4. The RF1 protein of claim 1 wherein the cleavage activity by OmpT1 is greater than 50% of the wild type RF1 (SEQ ID NO:1) after 30 minutes at 30° C when the modified and wild-type proteins are present at a similar concentration in a cell-free extract from bacteria expressing OmpT1.

5. The RF1 protein of claim 1 wherein the switch loop region comprises an amino acid sequence selected from the group of sequences consisting of:

QQAEASTRRKLLGSGDRS (SEQ ID NO:5),
QQAEASTRRRLLGSGDRS (SEQ ID NO:6),
QQAEASTRRKKLGSGDRS (SEQ ID NO:12),
QQAEASTRRKRLGSGDRS (SEQ ID NO:13),
QQAEASTRRKVLGSGDRS (SEQ ID NO:14),
QQAEASTRRRKLGSGDRS (SEQ ID NO:15),
QQAEASTRRRRLGSGDRS (SEQ ID NO:16),
QQAEASTRRRVLGSGDRS (SEQ ID NO:17),
QQAEASTRRKKKGSGDRS (SEQ ID NO:18),
QQAEASTRRKKRGSGDRS (SEQ ID NO:19),
QQAEASTRRKRKGSGDRS (SEQ ID NO:20),
QQAEASTRRKRRGSGDRS (SEQ ID NO:21),
QQAEASTRRRRRGSGDRS (SEQ ID NO:22),
QQAEASTRRRKRGSGDRS (SEQ ID NO:23),
QQAEASTRR**RRK**GSGDRS (SEQ ID NO:24), and
QQAEASTRR**RKK**GSGDRS (SEQ ID NO:25).

of wild type RF1 (SEQ ID NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

8. A method for reducing RF1 competition at an amber codon in an *in vitro* cell free synthesis system, the method comprising the steps of:

i) culturing an OmpT1 positive bacteria expressing a functional Releasing Factor 1 protein (RF1) that is cleavable by an Outer Membrane Protein T1 (OmpT1);

ii) lysing the bacteria to create a cell free synthesis extract;

iii) contacting the RF1 protein in the extract with OmpT1 in an amount to sufficient to reduce intact RF1 protein by 50%;

iv) adding a nucleic acid template to the extract where the template codes for a protein of interest and includes an amber codon; and,

v) allowing the cell free synthesis system to produce the protein of interest,

where the RF1 protein has a scissile OmpT1 peptide bond is located within the switch loop region corresponding to amino acids 287-304 of wild type RF1 (SEQ ID NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

9. The method of claim 8 wherein the OmpT1 positive bacteria is from *E. coli*.

10. The method of claim 8 wherein the oxidative phosphorylation system of the bacteria remains active after cell lysis and during the synthesis of the protein of interest.

11. The method of claim 8 where the cell free synthesis system places a non-native amino acid at the amber codon of the protein of interest.

12. A cell free synthesis system comprising in a single reaction mixture:

i) components from a bacterial lysate sufficient to translate a nucleic acid template encoding a protein;

ii) a nucleic acid template encoding a protein of interest and having at least one amber codon;

iii) tRNA complementary to the amber codon; and,

iv) a functional Releasing Factor 1 (RF1) protein cleavable by an Outer Membrane Protein T1 (OmpT1), where a scissile OmpT1 peptide bond is located within the switch loop region of RF1 corresponding to amino acids 287-304 of wild type RF1 (SEQ ID

NO:1) and where the switch loop region is modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid.

13. The cell free synthesis system of claim 12 wherein the reaction mixture further comprises a non-natural amino acid and corresponding amino acid synthetase, the synthetase able to charge the tRNA complimentary to the amber codon with the non-natural amino acid.

14. The cell free synthesis system of claim 12 wherein the system generates ATP via an active oxidative phosphorylation system.

15. A method for expressing protein in a cell-free synthesis system, comprising:

i. combining a nucleic acid template with a bacterial extract comprising an RF1 protein that has been cleaved by OmpT1 at a scissile OmpT1 peptide bond located within the switch loop region corresponding to amino acids 287-303 of wild type RF1 (SEQ ID NO:1) and where the switch loop region has been modified to include three adjacent basic amino acids selected from arginine and lysine that are positively charged at pH 7.0, wherein the modification is a substitution of a wild type amino acid with a basic amino acid under conditions that permit protein synthesis; and

ii. expressing a protein from the nucleic acid template.

16. A bacterial cell comprising a nucleic acid encoding the the functional RF1 of claim 1 or claim 6 incorporated into the bacterial cell's genome.