WHAT IS CLAIMED IS:

1. A vertebrate cell or cell line comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates an orthogonal tRNA (O-tRNA) with para-acetyl-phenylalanine (PAF) in the vertebrate cell.

2. The cell or cell line of claim 1, wherein the O-RS aminoacylates the O-tRNA with the PAF at least 10-fold more efficiently than the O-RS aminoacylates the O-tRNA with a natural amino acid.

3. The cell or cell line of claim 1, wherein the O-RS is derived from a non-vertebrate organism.

4. The cell or cell line of claim 3, wherein the non-vertebrate organism is *Escherichia coli,* or *Bacillus stearothermophilus.*

5. The cell or cell line of claim 1, wherein the vertebrate cell or cell line is mammalian.

6. The cell line of claim 5, wherein the vertebrate cell line is a human cell line.

7. The cell or cell line of claim 1, wherein the O-RS has one or more improved or enhanced enzymatic properties for the at least one unnatural amino acid as compared to a natural amino acid, which properties are selected from the group consisting of: higher K_m, lower K_m, higher k_{cat}, lower k_{cat}, lower k_{cat}/k_m, and higher k_{cat}/k_m.

8. The cell or cell line of claim 1, wherein the O-tRNA is derived from a non-vertebrate organism.

9. The cell or cell line of claim 8, wherein the non-vertebrate organism is *Escherichia coli,* or *Bacillus stearothermophilus.*

10. The cell or cell line of claim 1, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises a selector codon that is recognized by the O-tRNA.

11. The cell or cell line of claim 10, wherein the selector codon is selected from the group consisting of: amber codon, ochre codon, opal codon, or four or more base codons.

12. The cell or cell line of claim 10, wherein the selector codon is amber codon.

13. The cell or cell line of claim 10, wherein the selector codon is ochre codon.

14. The cell or cell line of claim 10, wherein the selector codon is opal codon.

15. The cell or cell line of claim 10, wherein the selector codon contains four or more base codons.

16. The cell or cell line of claim 10, wherein the yield of the polypeptide of interest comprising the at least one unnatural amino acid is at least 5% of that obtained for the naturally occurring polypeptide of interest from a cell in which the polynucleotide lacks the selector codon.

17. The cell or cell line of claim 1, wherein the cell produces the polypeptide of interest in the absence of the at least one unnatural amino acid with a yield that is less than 30% of the yield of the polypeptide in the presence of the at least one unnatural amino acid.

18. The cell or cell line of claim 10, wherein the polypeptide of interest is a therapeutic protein, a diagnostic protein, an industrial enzyme, or portion thereof.

19. A vertebrate cell or cell line comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates an orthogonal tRNA (O-tRNA) with para-amino-phenylalanine in the vertebrate cell.

20. The cell or cell line of claim 19, wherein the O-RS aminoacylates the O-tRNA with the para-amino-phenylalanine at least 10-fold more efficiently than the O-RS aminoacylates the O-tRNA with a natural amino acid.

21. The cell or cell line of claim 19, wherein the O-RS is derived from a non-vertebrate organism.

22. The cell or cell line of claim 21, wherein the non-vertebrate organism is *Escherichia coli,* or *Bacillus stearothermophilus.*

23. The cell or cell line of claim 19, wherein the vertebrate cell or cell line is mammalian.

24. The cell line of claim 19, wherein the vertebrate cell line is a human cell line.

25. The cell or cell line of claim 19, wherein the O-RS has one or more improved or enhanced enzymatic properties for the at least one unnatural amino acid as compared to a natural amino acid, which properties are selected from the group consisting of: higher K_m, lower K_m, higher k_{cat}, lower k_{cat}, lower k_{cat}/k_m, and higher k_{cat}/k_m.

26. The cell or cell line of claim 19, wherein the O-tRNA is derived from a non-vertebrate organism.

27. The cell or cell line of claim 26, wherein the non-vertebrate organism is *Escherichia coli,* or *Bacillus stearothermophilus.*

28. The cell or cell line of claim 19, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises a selector codon that is recognized by the O-tRNA.

29. The cell or cell line of claim 28, wherein the selector codon is selected from the group consisting of: amber codon, ochre codon, opal codon, or four or more base codons.

30. The cell or cell line of claim 28, wherein the selector codon is amber codon.

31. The cell or cell line of claim 28, wherein the selector codon is ochre codon.

32. The cell or cell line of claim 28, wherein the selector codon is opal codon.

33. The cell or cell line of claim 28, wherein the selector codon contains four or more base codons.

34. The cell or cell line of claim 28, wherein the yield of the polypeptide of interest comprising the at least one unnatural amino acid is at least 5% of that obtained for the naturally occurring polypeptide of interest from a cell in which the polynucleotide lacks the selector codon.

35. The cell or cell line of claim 19, wherein the cell produces the polypeptide of interest in the absence of the at least one unnatural amino acid with a yield that is less than 30% of the yield of the polypeptide in the presence of the at least one unnatural amino acid.

36. The cell or cell line of claim 28, wherein the polypeptide of interest is a therapeutic protein, a diagnostic protein, an industrial enzyme, or portion thereof.

37. The cell or cell line of claim 28, wherein the polypeptide of interest comprises a protein or a portion of a protein selected from the group consisting of: a cytokine, a growth factor, a growth factor receptor, an interferon, an interleukin, an inflammatory molecule, an

oncogene product, a peptide hormone, a signal transduction molecule, a steroid hormone receptor, insulin, human growth hormone, an Alpha-1 antitrypsin, an Angiostatin, an Antihemolytic factor, an antibody, an Apolipoprotein, an Apoprotein, an Atrial natriuretic factor, an Atrial natriuretic polypeptide, an Atrial peptide, a C-X-C chemokine, T39765, NAP-2, ENA-78, a Gro-a, a Gro-b, a Gro-c, an IP-10, a GCP-2, an NAP-4, an SDF-1, a PF4, a MIG, a Calcitonin, a c-kit ligand, a CC chemokine, a Monocyte chemoattractant protein-1, a Monocyte chemoattractant protein-2, a Monocyte chemoattractant protein-3, a Monocyte inflammatory protein-1 alpha, a Monocyte inflammatory protein-1 beta, RANTES, I309, R83915, R91733, HCC1, T58847, D31065, T64262, a CD40, a CD40 ligand, a C-kit Ligand, a Collagen, a Colony stimulating factor (CSF), a Complement factor 5a, a Complement inhibitor, a Complement receptor 1, DHFR, an epithelial Neutrophil Activating Peptide-78, a GROα/MGSA, a GROβ, a GROγ a MIP-1α, a MIP-1δ, a MCP-1, an Epidermal Growth Factor (EGF), an epithelial Neutrophil Activating Peptide, an Erythropoietin (EPO), an Exfoliating toxin, a Factor IX, a Factor VII, a Factor VIII, a Factor X, a Fibroblast Growth Factor (FGF), a Fibrinogen, a Fibronectin, a G-CSF, a GM-CSF, a Glucocerebrosidase, a Gonadotropin, a Hedgehog protein, a Hemoglobin, a Hepatocyte Growth Factor (HGF), a Hirudin, a Human serum albumin, an ICAM-1, an ICAM-1 receptor, an LFA-1, an LFA-1 receptor, an Insulin, an Insulin-like Growth Factor (IGF), an IGF-I, an IGF-II, an interferon, an IFN-α, an IFN-β, an IFN-γ, an interleukin, an IL-1, an IL-2, an IL-3, an IL-4, an IL-5, an IL-6, an IL-7, an IL-8, an IL-9, an IL-10, an IL-11, an IL-12, a Keratinocyte Growth Factor (KGF), a Lactoferrin, a leukemia inhibitory factor, a Luciferase, a Neurturin, a Neutrophil inhibitory factor (NIF), an oncostatin M, an Osteogenic protein, a Parathyroid hormone, a PD-ECSF, a PDGF, a Pleiotropin, a Protein A, a Protein G, a Pyrogenic exotoxins A, B, or C, a Relaxin, a Renin, an SCF, a Soluble complement receptor I, a Soluble I-CAM 1, a Soluble interleukin receptor, a Soluble TNF receptor, a Somatomedin, a Somatostatin, a Somatotropin, a Streptokinase, a Superantigen, a Staphylococcal enterotoxins, an SEA, an SEB, an SEC1, an SEC2, an SEC3, an SED, an SEE, a steroid hormone receptor, a Superoxide dismutase (SOD), a Toxic shock syndrome toxin, a Thymosin alpha 1, a Tissue plasminogen activator, a tumor growth factor (TGF), a TGF-α, a TGF-β, a Tumor Necrosis Factor, a Tumor Necrosis Factor alpha, a Tumor necrosis factor beta, a Tumor necrosis factor receptor (TNFR), a VLA-4 protein, a VCAM-1 protein, a Vascular Endothelial Growth Factor (VEGEF), a Urokinase, a Mos, a Ras, a Raf, a Met; a p53, a Tat, a Fos, a Myc, a Jun, a Myb, a

Rel, an estrogen receptor, a progesterone receptor, a testosterone receptor, an aldosterone receptor, an LDL receptor, a SCF/c-Kit, a CD40L/CD40, a VLA-4/VCAM-1, an ICAM-1/LFA-1, a hyaluurin/CD44, and a corticosterone.

38. The vertebrate cell line from claim 1 wherein the cell line has been transiently transfected.

39. The vertebrate cell line from claim 1 wherein the cell line has been stably transfected.

40. A vertebrate cell or cell line comprising an orthogonal tRNA (O-tRNA), wherein the O-tRNA mediates incorporation of para-acetyl-phenylalanine into a protein that is encoded by a polynucleotide that comprises a selector codon that is recognized by the O-tRNA in vivo.

41. The cell or cell line of claim 40, wherein the protein comprises at least two unnatural amino acids.

42. The cell or cell line of claim 40, wherein the protein comprises at least two different unnatural amino acids.

43. The cell or cell line of claim 40, wherein the protein further comprises a pharmaceutically acceptable excipient.

44. A cell or cell line comprising an orthogonal tRNA (O-tRNA), wherein the O-tRNA mediates incorporation of para-amino-phenylalanine into a protein that is encoded by a polynucleotide that comprises a selector codon that is recognized by the O-tRNA in vivo.

45. The cell or cell line of claim 44, wherein the protein comprises at least two unnatural amino acids.

46. The cell or cell line of claim 44, wherein the protein comprises at least two different unnatural amino acids.

47. The cell or cell line of claim 44, wherein the protein further comprises a pharmaceutically acceptable excipient.

48. A kit for producing a protein that comprises at least one unnatural amino acid in a cell, the kit comprising: a container containing a polynucleotide sequence encoding an O-tRNA, and a polynucleotide sequence encoding an O-RS or an O-RS.

49. The kit of claim 48, wherein the kit further comprises at least one unnatural amino acid.

50. The kit of claim 48, wherein the kit further comprises instructional materials for producing the protein.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A vertebrate cell or cell line comprising (i) an orthogonal aminoacyl-tRNA synthetase (O-RS) comprising an amino acid sequence set forth in SEQ ID NO: 45 and (ii) an orthogonal tRNA (O-tRNA) comprising a transcript of the nucleotide sequence set forth in SEQ ID NO: 88, wherein said O-RS is capable of preferentially aminoacylating the O-tRNA with para-acetyl-phenylalanine (*p*AF) or *p*-amino-phenylalanine.

2. The vertebrate cell or cell line according to claim 1, wherein the O-RS is capable of preferentially aminoacylating the O-tRNA with *p*AF.

3. The vertebrate cell or cell line according to claim 1 or claim 2, when used to preferentially aminoacylate and O-tRNA with *p*AF, or when used in the production of a polypeptide comprising *p*AF.

4. The vertebrate cell or cell line according to any one of claims 1 to 3, wherein the O-RS aminoacylates the O-tRNA with the *p*AF at least 10-fold more efficiently than the O-RS aminoacylates the O-tRNA with a natural amino acid.

5. The vertebrate cell or cell line according to claim 1, wherein the O-RS is capable of preferentially aminoacylating the O-tRNA with *p*-amino-phenylalanine.

6. The vertebrate cell or cell line according to claim 1 or 5, when used to preferentially aminoacylate an O-tRNA with *p*-amino-phenylalanine, or when used in the production of a polypeptide comprising *p*-amino-phenylalanine.

7. The vertebrate cell or cell line according to claim 5 or 6, wherein the O-RS aminoacylates the O-tRNA with the *p*-amino-phenylalanine at least 10-fold more efficiently than the O-RS aminoacylates the O-tRNA with a natural amino acid.

8. The vertebrate cell or cell line according to any one of claims 1 to 7, wherein the O-RS has one or more improved or enhanced enzymatic properties for *p*AF or *p*-amino-phenylalanine compared to a natural amino acid, which properties are selected from the group consisting of: higher K_m, lower K_m, higher k_{cat}, lower k_{cat}, lower k_{cat}/k_m, and higher k_{cat}/k_m.

9. The vertebrate cell or cell line according to any one of claims 1 to 8, further comprising a nucleic acid that comprises a nucleotide sequence that encodes a polypeptide, wherein the nucleotide sequence comprises a selector codon that is recognisable by an aminoacylated O-tRNA.

10. The vertebrate cell or cell line according to claim 9, wherein the selector codon is selected from the group consisting of: amber codon, ochre codon, opal codon, or four or more base codons.

11. The vertebrate cell or cell line according to claim 9, wherein the selector codon is amber codon.

12. The vertebrate cell or cell line according to claim 9, wherein the selector codon is ochre codon.

13. The vertebrate cell or cell line according to claim 9, wherein the selector codon is opal codon.

14. The vertebrate cell or cell line according to claim 9, wherein the selector codon is a a four-base codon or a codon comprising more than four bases.

15. The vertebrate cell or cell line according to any one of claims 1 to 11, wherein the O-tRNA is capable of mediating incorporation of *p*-acetyl-phenylalanine (*p*AF) or *p*-amino-phenylalanine into a polypeptide encoded by a polynucleotide that comprises a selector codon recognisable by an aminoacylated form of the O-tRNA.

16. The vertebrate cell or cell line according to claim 15, wherein the O-tRNA is capable of mediating incorporation of *p*AF into a polypeptide.

17. The vertebrate cell or cell line according to claim 15 or claim 16, when used to mediate incorporation of *p*AF into a polypeptide.

18. The vertebrate cell or cell line according to claim 15, wherein the O-tRNA is capable of mediating incorporation of *p*-amino-phenylalanine into a polypeptide.

19. The vertebrate cell or cell line according to claim 15 or claim 18, when used to mediate incorporation of *p*-amino-phenylalanine into a polypeptide.

20. The vertebrate cell or cell line according to any one of claims 1 to 19, wherein the O-RS and O-tRNA are derived from a non-vertebrate organism.

21. The vertebrate cell or cell line according to claim 20, wherein the non-vertebrate organism is *Escherichia coli* or *Bacillus stearothermophilus*.

22. The vertebrate cell or cell line according to claim 17 or 19, wherein said cell or cell line yields a polypeptide comprising *p*AF or *p*-amino-phenylalanine at a level which is at least 5% of the yield obtainable for a corresponding polypeptide comprising natural amino acids instead of *p*AF or *p*-amino-phenylalanine.

23. The vertebrate cell or cell line according to claim 17 or 19, wherein said cell or cell line yields a polypeptide in the absence of *p*AF or *p*-amino-phenylalanine at a level that is less than 30% of the yield of the polypeptide obtained in the presence of *p*AF or *p*-amino-phenylalanine.

24. The vertebrate cell or cell line according to claim 17 or 19, when used to produce a polypeptide comprising *p*AF or *p*-amino-phenylalanine, and wherein said polypeptide is a therapeutic protein, a diagnostic protein, an industrial enzyme, or a functional portion thereof.

25. The vertebrate cell or cell line according to any one of claims 17, 19 or 24, wherein the polypeptide is selected from the group consisting of: a cytokine, a growth factor, a growth factor receptor, an interferon, an interleukin, an inflammatory molecule, an oncogene product, a peptide hormone, a signal transduction molecule, a steroid hormone receptor, erythropoietin (EPO), insulin, human growth hormone, an Alpha-1 antitrypsin, an Angiostatin, an Antihemolytic factor, an antibody, an Apolipoprotein, an Apoprotein, an Atrial natriuretic factor, an Atrial natriuretic polypeptide, an Atrial peptide, a C-X-C chemokine, T39765, NAP-2, ENA-78, a Gro-a, a Gro-b, a Gro-c, an IP-10, a GCP-2, an NAP-4, an SDF-1, a PF4, a MIG, a Calcitonin, a c-kit ligand, a cytokine, a CC chemokine, a Monocyte chemoattractant protein-1, a Monocyte chemoattractant protein-2, a Monocyte chemoattractant protein-3, a Monocyte inflammatory protein-1 alpha, a Monocyte inflammatory protein-1 beta, RANTES, I309, R83915, R91733, HCC1, T58847, D31065, T64262, a CD40, a CD40 ligand, a C-kit Ligand, a Collagen, a Colony stimulating factor (CSF), a Complement factor 5a, a Complement inhibitor, a Complement receptor 1, a cytokine, DHFR, an epithelial Neutrophil Activating Peptide-78, a GROα/MGSA, a GROβ, a GROγ a MIP-1α, a MIP-1δ, a MCP-1, an Epidermal Growth Factor (EGF), an epithelial Neutrophil Activating Peptide, an Erythropoietin (EPO), an Exfoliating toxin, a Factor IX, a Factor VII, a Factor VIII, a Factor X, a Fibroblast Growth Factor (FGF), a Fibrinogen, a Fibronectin, a G-CSF, a GM-CSF, a Glucocerebrosidase, a Gonadotropin, a growth factor, a growth factor receptor, a Hedgehog protein, a Hemoglobin, a Hepatocyte Growth Factor (HGF), a Hirudin, a Human serum albumin, an ICAM-1, an ICAM-1 receptor, an LFA-1, an LFA-1 receptor, an Insulin, an Insulin-like Growth Factor (IGF), an IGF-I, an IGF-II, an interferon, an IFN-α, an IFN-β, an IFN-γ, an interleukin, an IL-1, an IL-2, an IL-3, an IL-4, an IL-5, an IL-6, an IL-7, an IL-8, an IL-9, an IL-10, an IL-11, an IL-12, a Keratinocyte Growth Factor (KGF), a Lactoferrin, a leukemia inhibitory factor, a Luciferase, a Neurturin, a Neutrophil inhibitory factor (NIF), an oncostatin M, an Osteogenic protein, an oncogene product, a Parathyroid hormone, a PD-ECSF, a PDGF, a peptide hormone, a Human Growth Hormone, a Pleiotropin, a Protein A, a Protein G, a Pyrogenic exotoxins A, B, or C, a Relaxin, a Renin, an SCF, a Soluble complement receptor I, a Soluble I-CAM 1, a Soluble interleukin receptor, a Soluble TNF receptor, a Somatomedin, a Somatostatin, a Somatotropin, a Streptokinase, a Superantigen, a Staphylococcal enterotoxins, an SEA, an SEB, an SEC1, an SEC2, an SEC3, an SED, an SEE, a steroid hormone

receptor, a Superoxide dismutase (SOD), a Toxic shock syndrome toxin, a Thymosin alpha 1, a Tissue plasminogen activator, a tumor growth factor (TGF), a TGF-α, a TGF-β, a Tumor Necrosis Factor, a Tumor Necrosis Factor alpha, a Tumor necrosis factor beta, a Tumor necrosis factor receptor (TNFR), a VLA-4 protein, a VCAM-1 protein, a Vascular Endothelial Growth Factor (VEGEF), a Urokinase, a Mos, a Ras, a Raf, a Met; a p53, a Tat, a Fos, a Myc, a Jun, a Myb, a Rel, an estrogen receptor, a progesterone receptor, a testosterone receptor, an aldosterone receptor, an LDL receptor, a SCF/c-Kit, a CD40L/CD40, a VLA-4/VCAM-1, an ICAM-1/LFA-1, a hyalurin/CD44, and a corticosterone.

26. The vertebrate cell or cell line according to any one of claims 17, 19, 24 or 25, wherein the polypeptide comprises *p*AF or *p*-amino-phenylalanine.

27. The vertebrate cell or cell line according to any one of claims 17, 19 or 24 to 26, wherein the polypeptide further comprises at least one unnatural amino acid other than *p*AF or *p*-amino-phenylalanine.

28. The vertebrate cell or cell line according to any one of claims 1 to 27, wherein the cell or cell line has been transiently transfected.

29. The vertebrate cell or cell line according to any one of claims 1 to 28, wherein the cell or cell line has been stably transfected.

30. The vertebrate cell or cell line according to any one of claims 1 to 29, wherein the cell or cell line is mammalian.

31. The vertebrate cell line according to any one of claims 1 to 30, wherein the cell or cell line is human.

32. A kit for producing the vertebrate cell or cell line according to any one of claims 1 to 31, wherein said kit comprises:(i) an isolated nucleic acid comprising the sequence set forth in SEQ ID NO: 88 or an orthogonal tRNA (O-tRNA) comprising a transcript of the sequence set forth in SEQ ID NO: 88; and (ii) an isolated nucleic acid comprising a nucleotide sequence encoding an orthogonal aminoacyl-tRNA synthetase (O-RS) or an O-RS, wherein said O-RS comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the O-RS is capable of preferentially aminoacylating the O-tRNA with at least one unnatural amino acid selected from *p*-acetyl-phenylalanine (pAF) or *p*-amino-phenylalanine.

33. The kit according to claim 32, further comprising pAF and/or *p*-amino-phenylalanine.

34. The kit according to claim 33, comprising *p*AF.

35. The kit according to claim 33, comprising *p*-amino-phenylalanine.

36. The kit according to any one of claim 32 to 35 when used to produce the vertebrate cell or cell line according to any one of claims 1 to 32.