Claims

1. A DNA construct which comprises a *tRNApyl* coding sequence and a RNA polymerase III promoter sequence which is capable of acting to express functional tRNApyl sufficiently to support nonsense suppression in a eukaryotic expression system.

2. A DNA construct according to claim 1 wherein the nonsense suppression is amber suppression.

3. A DNA construct according to claim 1 or claim 2 comprising a terminator sequence which is downstream of the *tRNApyl* coding sequence.

4. A DNA construct according to any one of claims 1 to 3 wherein the construct comprises a *tRNApyl* gene comprising the *tRNApyl* coding sequence and a functional intragenic RNA polymerase III promoter.

5. A DNA construct according to claim 4 wherein the tRNApyl gene contains mutations in 1 or 2 nucleotide positions in the putative A box and/or the putative B box of the intragenic RNA polymerase III promoter.

6. A DNA construct according to claim 5 wherein the tRNApyl gene contains mutations in 1 or 2 nucleotide positions (e.g. 1 position) in the putative B box.

7. A DNA construct according to any one of claims 4 to 6 wherein the *tRNApyl* gene has or comprises a sequence of SEQ ID Nos 7, 8, 9, 10, or 11 derived from *Methanosacrina mazei* or has or comprises the sequence of an analogous *tRNApyl* gene derived from another bacterial species in which the mutations indicated by bold in SEQ ID Nos 7, 8, 9, 10, or 11 are made in equivalent positions.

8. A DNA construct according to any one of claims 1 to 6 wherein the *tRNApyl* coding sequence is operably linked to a RNA polymerase III promoter wherein said promoter is placed 5' to the t*RNApyl* coding sequence.

9. A DNA construct according to claim 7 wherein the 5' promoter and the *tRNApyl* coding sequence are separated by a spacer sequence containing a transcriptional start site.

10. A DNA construct according to any one of claims 1 to 9 which comprises an intragenic promoter element and a 5' promoter element being a RNA polymerase III promoter element placed 5' to the t*RNApyl* coding sequence such that the combination of said intragenic promoter element and said 5' promoter element constitutes a hybrid promoter.

11. A DNA construct according to claim 10 wherein said 5' promoter element is the 5' element of a Type 4 RNA polymerase III promoter.

12. A DNA construct according to claim 11 wherein the 5' promoter element of a Type 4 RNA Polymerase III promoters is selected from 5' promoter elements of the EBER RNA gene promoter, the 7SL RNA gene promoter and the Vault RNA gene promoter.

13. A DNA construct according to claim 11 wherein the 5' promoter element is selected from SEQ ID Nos 12, 20, 26, 57, 58, 59, 60, 61, 62, 63, 64 and 65.

14. A DNA construct according to claim 11 wherein the 5' promoter element of a Type 4 RNA Polymerase III promoters is a7SL RNA gene promoter.

15. A DNA construct according to any one of claims 10 to 14 wherein the 5' promoter element and the *tRNApyl* coding sequence are separated by a spacer sequence containing a transcriptional start site.

16. A DNA construct according to claim 14 wherein the spacer sequence is selected from SEQ ID NOs 13, 21 and 27.

17. A multimeric DNA construct comprising multiple copies (e.g. 2 to 60 copies) of the DNA construct according to any one of claims 1 to 16.

18. A DNA construct which comprises a eukaryotic tRNAglu gene or a eukaryotic tRNAasp gene 5' to a tRNApyl gene, said tRNAglu gene or tRNAasp gene being deprived of a termination sequence thus leading to a bicistronic message upon transcription.

19. A DNA construct according to claim 13 wherein the tRNApyl gene is a wildtype gene.

20. A DNA construct according to claim 13 wherein the tRNApyl gene contains mutations in 1 or 2 nucleotide positions in the putative A box and/or the putative B box.

21. A DNA construct according to any one of claims 1 to 15 which further comprises a PylRS coding sequence or gene.

22. A eukaryotic host cell which is transformed with a vector comprising a DNA construct according to any one of claims 1 to 16.

23. A eukaryotic host cell according to claim 17 which is transformed with a vector comprising a gene encoding a target protein containing one or more non-natural amino acids encoded by a nonsense codon.

24. A eukaryotic cell line which expresses or is capable of expressing functional PylRS and functional tRNApyl in which functional tRNApyl expression occurs under the control of a an intragenic promoter element and a 5' promoter element being a RNA polymerase III promoter

element placed 5' to the tRNApyl coding sequence such that the combination of said intragenic promoter element and said 5' promoter element constitute a hybrid promoter sufficiently to support nonsense suppression.

25. A eukaryotic cell line according to claim 19 wherein the nonsense suppression is amber suppression.

26. A eukaryotic host cell according to any one of claims 17 to 20 wherein the host cell is a mammalian cell.

27. A process for preparing a target protein containing one or more non-natural amino acids encoded by a nonsense codon which comprises expressing said target protein in a eukaryotic cell line which is transformed with a gene encoding a target protein containing one or more non-natural amino acids encoded by a nonsense codon and which is also transformed with genes encoding PylRS and tRNApyl such that PylRS and tRNApyl are expressed and function sufficiently to support nonsense suppression.

28. A process according to claim 27 wherein the cell line is transformed with a DNA construct according to any one of claims 1 to 21.

29. A process for preparing a chemically modified target protein which comprises preparing a target protein according to the process of claim 27 or 28 and chemically modifying the resultant target protein.

30. A cell, cell line or process according to any one of claims 22 to 29 wherein the nonsense codon is an amber codon.

31. A cell, cell line or process according to any one of claims 22 to 30 wherein at least one of the non-natural amino acids comprises an alkyne or azide moiety.

32. An protein conjugate such as an antibody conjugate prepared using a process according to any one of claims 27 to 31 in which the protein such as an antibody is conjugated to one or more other moieties selected from protein, drug and PEG moieties via linkers comprising a triazole moiety.

33. A process for increasing the viability of a eukaryotic cell line stably transformed to express a target protein containing one or more non-natural amino acids and to express PylRS and tRNApyl which comprises transforming the eukaryotic cell line with genes encoding PylRS and tRNApyl such that PylRS and tRNApyl are expressed and function sufficiently to support nonsense suppression.

34. A process according to claim 33 wherein the nonsense suppression is amber suppression.

35. A DNA construct, cell, cell line, process or protein conjugate according to any one of claims 1 to 34 wherein the tRNApyl is not a tRNApyl derived from *Methanosarcina barkeri*.

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Claims

1. A DNA construct which comprises a *tRNApyl* gene comprising a *tRNApyl* coding sequence and a functional intragenic RNA polymerase III promoter sequence which is capable of acting to express functional tRNApyl sufficiently to support amber suppression in a eukaryotic expression system; wherein the tRNApyl gene contains mutations in 1 or 2 nucleotide positions in the putative A box and/or the putative B box of the intragenic RNA polymerase III promoter.

2. A DNA construct according to claim 1 comprising a terminator sequence which is downstream of the *tRNApyl* coding sequence.

3. A DNA construct according to claim 1 or claim 2 wherein the tRNApyl gene contains mutations in 1 or 2 nucleotide positions (e.g. 1 position) in the putative B box.

4. A DNA construct according to any one of claims 1 to 3 wherein the *tRNApyl* gene has or comprises a sequence of SEQ ID Nos 7, 8, 9, 10, or 11 derived from *Methanosacrina mazei* or has or comprises the sequence of an analogous *tRNApyl* gene derived from another bacterial species in which the mutations indicated by bold in SEQ ID Nos 7, 8, 9, 10, or 11 are made in equivalent positions.

5. A DNA construct according to claim 4 wherein the *tRNApyl* gene has or comprises a sequence of a *tRNApyl* gene derived from *Desulfitobacterium hafniense* (SEQ ID NO: 5), *Methanosarcina acetivorans* (SEQ ID NO: 2), *Methanosarcina burtonii* (SEQ ID NO: 4) or *Methanosarcina thermophila* in which the mutations indicated by bold in SEQ ID Nos 7, 8, 9, 10, or 11 are made in equivalent positions.

6. A DNA construct according to any one of claims 1 to 5 wherein the *tRNApyl* coding sequence is operably linked to a RNA polymerase III promoter wherein said promoter is placed 5' to the t*RNApyl* coding sequence; and optionally the 5' promoter and the *tRNApyl* coding sequence are separated by a spacer sequence containing a

transcriptional start site; or

wherein the DNA construct comprises an intragenic promoter element and a 5' promoter element being a RNA polymerase III promoter element placed 5' to the t*RNApyl* coding sequence such that the combination of said intragenic promoter element and said 5' promoter element constitutes a hybrid promoter; and wherein optionally the 5' promoter element and the *tRNApyl* coding sequence are separated by a spacer sequence containing a transcriptional start site.

7. A DNA construct according to claim 6 wherein said 5' promoter element is the 5' element of a Type 4 RNA polymerase III promoter.

8. A DNA construct according to claim 6 wherein the 5' promoter element of a Type 4 RNA Polymerase III promoters is selected from:
5' promoter elements of the EBER RNA gene promoter, the 7SL RNA gene promoter and the Vault RNA gene promoter;
SEQ ID Nos 12, 20, 26, 57, 58, 59, 60, 61, 62, 63, 64 and 65.

9. A DNA construct according to claim any one of claims 5 to 8 wherein the spacer sequence is selected from SEQ ID NOs 13, 21 and 27.

10. A multimeric DNA construct comprising multiple copies (e.g. 2 to 60 copies) of the DNA construct according to any one of claims 1 to 9.

11. A DNA construct according to any one of claims 1 to 10 which further comprises a PylRS coding sequence or gene.

12. A eukaryotic host cell which is transformed with a vector comprising a DNA construct according to any one of claims 1 to 11 and which is optionally transformed with a vector comprising a gene encoding a target protein containing one or more non-natural amino acids encoded by an amber codon.

13. A eukaryotic cell line comprising a DNA construct according to any one of claims 5 to 11 which expresses or is capable of expressing functional PylRS and functional tRNApyl in which functional tRNApyl expression occurs under the control of a an intragenic promoter element and a 5' promoter element being a RNA polymerase III promoter element placed 5' to the tRNApyl coding sequence such that the combination of said intragenic promoter element and said 5' promoter element constitute a hybrid promoter sufficiently to support amber suppression

14. A eukaryotic host cell according to claim 12 or cell line according to claim 13 wherein the host cell is a mammalian cell or the cell line is a mammalian cell line.

15. A process for preparing a target protein containing one or more non-natural amino acids encoded by an amber codon which comprises expressing said target protein in a eukaryotic cell line according to claim 13, which is transformed with a gene encoding the target protein.