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.