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 comprises a nucleic acid sequence selected from SEQ ID Nos 7, 8, 9, 10, and 11 derived from *Methanosarcina mazei* or a sequence of an analogous *tRNApyl* gene derived from another bacterial species which contains mutations in 1 or 2 nucleotide positions in a putative A box and/or putative B box of the intragenic RNA polymerase III promoter in positions equivalent to A10G, A52C, T14A, or a combination thereof as shown in SEQ ID Nos 7, 8, 9, 10, or 11.

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 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.

4. A DNA construct according to any one of claims 1 to 3 wherein the *tRNApyl* coding sequence is operably linked to a RNA polymerase III promoter wherein said promoter is placed 5' to the *tRNApyl* 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 Ill promoter element placed 5' to the *tRNApyl* 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.

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

6. A DNA construct according to claim 4 wherein the 5' promoter element of a Type 4 RNA Polymerase Ill 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.

7. A DNA construct according to claim any one of claims 3 to 6 wherein the spacer sequence is selected from SEQ ID NOs 13, 21 and 27.

8. 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 7.

9. A DNA construct according to any one of claims 1 to 8 which further comprises a PylRS coding sequence or gene.

10. A eukaryotic host cell which is transformed with a vector comprising a DNA construct according to any one of claims 1 to 9 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.

11. A eukaryotic cell line comprising a DNA construct according to any one of claims 3 to 9 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.

12. A eukaryotic host cell according to claim 10 or cell line according to claim 11 wherein the host cell is a mammalian cell or the cell line is a mammalian cell line.

13. 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 11, which is transformed with a gene encoding the target protein.