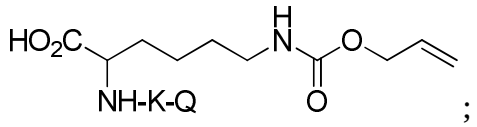
Claims

1. A process for stabilizing a eukaryotic cell line which expresses PylRS and tRNAPyl and which is suitable for incorporation of a gene encoding a target protein containing one or more non-natural amino acids encoded by a nonsense codon which comprises culturing said cell line in the presence of a decoy amino acid which is a substrate for PylRS but which is incapable of incorporation into an extending protein chain, the decoy amino acid having a formula of:

(a) formula VIIA:

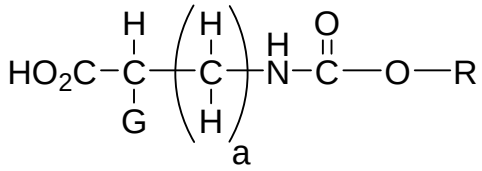


;

wherein K is CO or SO_2;

Q=H, C_{1-6}alkyl, aryl, heteroaryl, OC_{1-6}alkyl, OCH_2aryl, OCH_2heteroaryl, C_2-$_6$alkenyl or OC_2-6alkenyl; or

(b) formula VIIB:



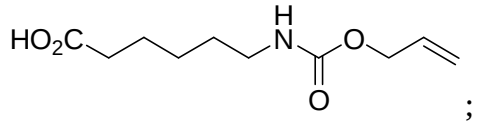
wherein G=H;

a=4 or 5; and

R= C_{1-6}alkyl, C_{2-6} alkenyl,-CH_2aryl, C_{2-6}alkynyl, C_{1-6}haloalkyl or C_{1-6}azidoalkyl.

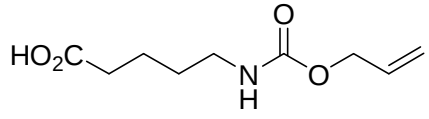
2. A process according to claim 1 wherein the decoy amino acid is selected from:

Formula VIIB.1,



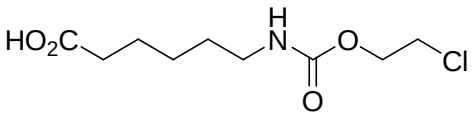
;

Formula VIIB.2,



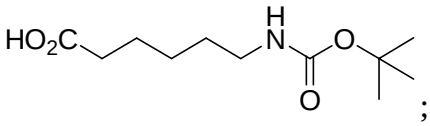
;

Formula VIIB.3,



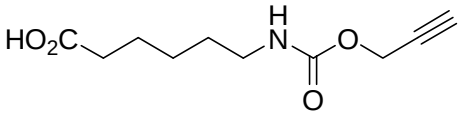
;

Formula VIIB.4,



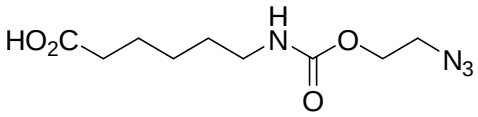
;

Formula VIIB.5



; and

Formula VIIB.6

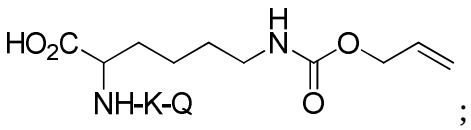


3. A process for production of a stable eukaryotic cell line which is capable of expressing PylRS and tRNAPyl and which is suitable for incorporation of a gene encoding a target protein containing one or more non-natural amino acids encoded by an nonsense codon which comprises:

(a) introducing into the eukaryotic cell line genes encoding PylRS and tRNAPyl such that PylRS and tRNAPyl are stably expressed in said cell line; and

(b) culturing or selecting the resultant cell line in the presence of a decoy amino acid which is a substrate for PylRS but which is incapable of incorporation into an extending protein chain thereby to reduce the adverse effect of tRNAPyl on cell viability and/or cell growth, wherein the decoy amino acid has a formula selected from:

(i) formula VIIA:

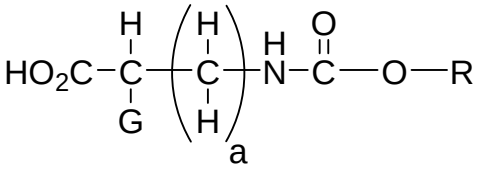


;

wherein K is CO or SO_2;

Q=H, C_{1-6}alkyl, aryl, heteroaryl, OC_{1-6}alkyl, OCH_2aryl, OCH_2heteroaryl, C_2-$_6$alkenyl or OC_2-6alkenyl; or

(ii) formula VIIB:



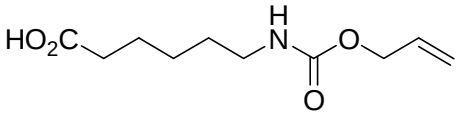
wherein G=H;

a=4 or 5; and

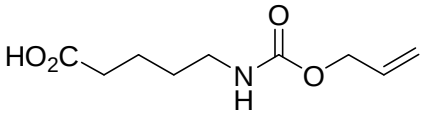
R= C_{1-6}alkyl, C_{2-6} alkenyl,-CH_2aryl, C_{2-6}alkynyl, C_{1-6}haloalkyl or C_{1-6}azidoalkyl.

4. A process according to claim 7 wherein the decoy amino acid is selected from:

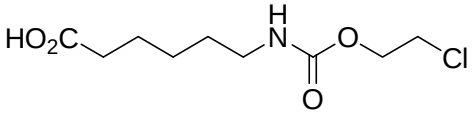
Formula VIIB.1,


;

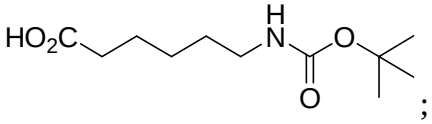
Formula VIIB.2,


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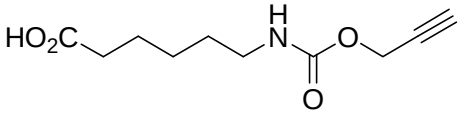
Formula VIIB.3,


;

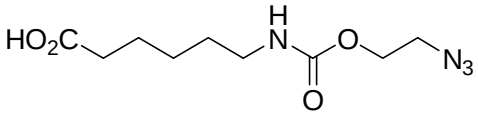
Formula VIIB.4,


;

Formula VIIB.5


; and

Formula VIIB.6



5. A process according to claim 3 or 4, further comprising introducing into said eukaryotic cell line a gene encoding a target protein containing one or more non-natural amino acids; and expressing the target protein in the absence of said decoy amino acid.

6. A process for preparing a target protein containing one or more non-natural amino acids encoded by an nonsense codon which comprises introducing into a stable eukaryotic cell line a gene encoding a target protein containing one or more non-natural amino acids encoded by an nonsense codon such that the target protein is stably expressed in said cell line and expressing said target protein in the presence of a source of the one or more non-natural amino acids, wherein the stable eukaryotic cell line is stabilized by the process according to any one of claims 1 to 5.

7. A process according to any one of claims 1 to 6 wherein the eukaryotic cell line is a mammalian cell line e.g. selected from CHO, HEK293, PERC6, COS-1, COS-7, HeLa, VERO, mouse hybridoma and mouse myeloma cell lines.

8. A process according to any one of claims 1 to 7 wherein the nonsense codon is an amber codon.

9. A process according to claims 6 or 7, wherein the target protein is an antibody of the IgG type which comprises one or more non-natural amino acids which are a substrate for pylRS, the positions of such non-natural amino acids being selected from positions 157 and 242 of SEQ ID No 82 in the heavy chain and positions 70 and 81 in the framework region of the light chain of SEQ ID Nos 52 and 79, or the corresponding position in different IgGs.