

CLAIMS

1. A modified phenylalanine tRNA synthetase (PheS), wherein the modified PheS has the sequence of SEQ ID NO: 21, except with glycine instead of alanine at amino acid position 311.
- 5 2. The modified PheS of claim 1, wherein the modified PheS aminoacylates tRNA with DL-4-chlorophenylalanine.
3. A nucleic acid encoding the modified PheS of claim 1.
4. A method for producing a recombinant microorganism, comprising introducing into a parental microorganism a vector comprising a nucleic acid encoding the modified
10 PheS of claim 1.
5. The method of claim 4, wherein the method further comprises selecting against the expression of the modified PheS.
6. The method of claim 5, wherein the selecting against the expression of the modified PheS involves culturing the recombinant microorganism in the presence of DL-4-
15 chlorophenylalanine.
7. The method of claim 6, wherein the modified PheS aminoacylates tRNA with DL-4-chlorophenylalanine.
8. The method of claim 4, wherein the vector further comprises homology arms that allow for homologous recombination between the vector and the genome of the
20 parental microorganism.
9. The method of claim 4, wherein the vector further comprises a nucleic acid of interest.
10. The method of claim 4, wherein the vector further comprises a positive selection marker.
11. The method of claim 10, wherein the method further comprises selecting for the
25 expression of the positive selection marker.
12. The method of claim 11, wherein the positive selection marker is selected from the group consisting of CatP, ErmB, and TetA.
13. Use of the PheS of claim 1 as a counter-selection marker.