CLAIMS

1. A method of producing a selectable marker gene-free plasmid comprising the steps of:

   a) culturing a plasmid containing a selectable marker gene flanked by site specific recombinase target sites in a first host cell environment which is incapable of effecting recombination between the site specific recombinase target sites; and

   b) subsequently culturing the plasmid in a second host cell environment which is capable of effecting recombination between the site specific recombinase target sites, such that the selectable marker gene is excised.

2. The method of claim 1 further comprising the step of:

   c) maintaining the selectable marker gene-free plasmid in cell culture.

3. The method of claim 1 or claim 2 further comprising the step of:

   d) isolating the selectable marker gene-free plasmid from the second host cell environment.

4. The method of any one of claims 1-3 wherein the site specific recombinase target sites are functionally associated with accessory sequences necessary to effect site specific recombination.

5. The method of any one of the preceding claims wherein the first host cell environment and the second host cell environment are within different cells.

6. The method of claim 5, wherein the first host cell environment comprises an inactivating mutation in one or more of the genes encoding PepA, ArgR and ArcA.

7. The method according to claim 5 or claim 6 wherein the second host cell environment contains active versions of the PepA and ArgR or ArcA genes.

8. The method of claim 5, wherein the second host cell environment comprises a site specific recombinase.

9. The method of claim 7 wherein the site specific recombinase is an endogenous site specific recombinase.

10. The method of any one of claims 1-4, wherein the first host cell environment and the second host cell environment are formed within the same host cell.

11. The method of claim 9 wherein the first host cell environment and the second host cell environment are temporally separated.

12. The method of claim 9 or claim 10 wherein the second host cell environment comprises an inducible site specific recombinase.

13. The method of any one of claims 9-12, wherein the second host cell environment is formed by altering the temperature of the cell, altering the osmolarity of the cell, altering the levels of inducer in the cell or altering the levels of chemicals in the cell that alter the secondary structure or supercoiling of the plasmid.

14. The method of any one of claims 8, 9, 12 or 13, wherein the site specific recombinase is selected from Cre, Flp, R, XerC, XerD, RipX and CodV.

15. The method of any one of claims 1-14, wherein the site specific recombinase target site is a transposase target site.

16. The method of any one of claims 1-14 wherein the site specific recombinase target sites are selected from *Ecdif, cer, psi, pif, mwr, Bsdif, loxP, FRT* and *RS*.

17. The method of any one of the preceding claims wherein the selectable marker gene is an antibiotic resistance gene.

18. The method of any one of claims 1-16 wherein the selectable marker gene enables the production of a metabolite essential for but absent from the first and/or the second host cell environment.

19. The method according to any one of the preceding claims wherein the first host cell environment and/or the second host cell environment is a Gram-negative bacterial cell.

20. The method according to claim 19 wherein the first host cell environment and the second host cell environment are independently selected from the genera *Escherichia, Salmonella, Shigella, Agrobacterium, Pseudomonas* and *Vibrio*.

21. The method according to any one of claims 1-18 wherein the first host cell environment and/or the second host cell environment is a Gram-positive bacterial cell.

22. The method according to claim 21 wherein the first host cell environment and the second host cell environment are independently selected from the genera *Bacillus, Streptomyces, Listeria, Lactobacillus, Lactococcus* and *Mycobacterium.*

23. The method according the any one of claims 1-18 wherein the first host cell environment and/or the second host cell environment is an archaeon.

24. The method according to claim 23 wherein the first host cell environment and/or the second host cell environment is a yeast cell.

25. The method according to claim 24 wherein the first host cell environment and the second host cell environment are independently selected from the genera *Hansenula*, *Pichia*, *Saccharomyces* and *Schizosaccharomyces*.

26. The method according to any one of claims 1-18 wherein the first host cell environment and/or the second host cell environment is a non-fungal eukaryote capable of replicating a plasmid.

27. The method according to claim 26 wherein the first host cell environment and the second host cell environment are independently selected from the genera *Chlamydomomas*, *Dictyostelium* and *Entamoeba*.

28. The method of any one of the preceding claims wherein the plasmid encodes one or more genes of interest.

29. A plasmid produced according to the method of any one of the preceding claims.

30. An unmodified host cell containing a selectable marker gene-free plasmid.

31. A host cell containing a selectable marker gene-free plasmid having a residual site specific recombinase target site.

32. The host cell of claim 31 containing a gene encoding an inducible site specific recombinase.

33. The host cell of claim 32 wherein the inducible site specific recombinase is present on the host cell chromosome.

34. A composition comprising the unmodified host cell of claim 30 or the host cell of any one of claims 31-33 and a pharmaceutically acceptable carrier.

35. A method of vaccinating or treating a patient comprising administering to the patient an unmodified host cell according to claim 30, a host cell according to any one of claims 31-33 or a composition according to claim 34.

36. An unmodified host cell according to claim 30, a host cell according to any one of claims 31-33 or a composition according to claim 34 for use in vaccinating a patient or treating a disease in a patient.

37. An unmodified host cell according to claim 30, a host cell according to any one of claims 31-33 or a composition according to claim 34 for use in the manufacture of a medicament for vaccinating a patient or treating a disease in a patient.

38. A host cell containing a plasmid containing a selectable marker gene flanked by site specific recombinase target sites, wherein the host cell further comprises an inactivating mutation in one of more of the chromosomal genes encoding one or more of the accessory proteins PepA, ArgR or ArcA.

39. A kit comprising or consisting of:

i) a first host cell environment containing a plasmid containing a selectable marker gene flanked by site specific recombinase target sites, wherein the first host cell environment is incapable of effecting recombination between the site specific recombinase target sites and;

ii) a second host cell environment which is capable of effecting recombination between the site specific recombinase target sites, such that the selectable marker gene is excised.

CLAIMS

1. A method of producing a selectable marker gene-free plasmid comprising the steps of:

   a) culturing a plasmid containing a selectable marker gene flanked by site specific recombinase target sites selected from *Ecdif, cer, psi, pif and mwr* in a first host cell environment which is incapable of effecting recombination between the site specific recombinase target sites, wherein the first host cell environment comprises an inactivating mutation in one or more of the genes encoding PepA, ArgR and ArcA; and

   b) subsequently culturing the plasmid in a second host cell environment which is capable of effecting recombination between the site specific recombinase target sites, such that the selectable marker gene is excised, wherein the second host cell environment contains active versions of PepA and ArgR or ArcA, and comprises a site specific recombinase selected from XerC and XerD.

2. The method of claim 1 further comprising the step of:

   c) maintaining the selectable marker gene-free plasmid in cell culture.

3. The method of claim 1 or claim 2 further comprising the step of:

   d) isolating the selectable marker gene-free plasmid from the second host cell environment.

4. The method of any one of the preceding claims wherein the first host cell environment and the second host cell environment are within different cells.

5. The method of any one of claims 1-3, wherein the first host cell environment and the second host cell environment are formed within the same host cell.

6. The method of claim 5 wherein the first host cell environment and the second host cell environment are temporally separated.

7. The method of any one of the preceding claims wherein the selectable marker gene is an antibiotic resistance gene.

8. The method of any one of claims 1-6 wherein the selectable marker gene enables the production of a metabolite essential for but absent from the first and/or the second host cell environment.

9. The method according to any one of the preceding claims wherein the first host cell environment and/or the second host cell environment is a Gram-negative bacterial cell.

10. The method according to claim 9 wherein the first host cell environment and the second host cell environment are independently selected from the genera *Escherichia, Salmonella, Shigella, Agrobacterium, Pseudomonas* and *Vibrio*.

11. The method of any one of the preceding claims wherein the plasmid encodes one or more genes of interest.

Claims

1. A method of producing a selectable marker gene-free plasmid comprising the steps of:

a) culturing a plasmid containing a selectable marker gene flanked by site specific recombinase target sites selected from *Ecdif, cer psi, pif and mwr* in a first host cell environment which is incapable of effecting recombination between the site specific recombinase target sites, wherein the first host cell environment comprises an inactivating mutation in one or more of the genes encoding PepA, ArgR and ArcA; and

b) subsequently culturing the plasmid in a second host cell environment which is capable of effecting recombination between the site specific recombinase target sites, such that the selectable marker gene is excised, wherein the second host cell environment contains active versions of PepA and ArgR or ArcA, and comprises a site specific recombinase selected from XerC and XerD.

2. The method of claim 1 further comprising the step of:

c) maintaining the selectable marker gene-free plasmid in cell culture.

3. The method of claim 1 or claim 2 further comprising the step of:

d) isolating the selectable marker gene-free plasmid from the second host cell environment.

4. The method of any one of the preceding claims wherein the first host cell environment and the second host cell environment are within different cells.

5. The method of any one of claims 1-3, wherein the first host cell environment and the second host cell environment are formed within the same host cell.

6. The method of claim 5 wherein the first host cell environment and the second host cell environment are temporally separated.

7. The method of any one of the preceding claims wherein the selectable marker gene is an antibiotic resistance gene.

8. The method of any one of claims 1-6 wherein the selectable marker gene enables the production of a metabolite essential for but absent from the first and/or the second host cell environment.

9. The method according to any one of the preceding claims wherein the first host cell environment and/or the second host cell environment is a Gramnegative bacterial cell.

10. The method according to claim 9 wherein the first host cell environment and the second host cell environment are independently selected from the genera *Escherichia, Salmonella, Shigella, Agrobacterium, Pseuclomonas* and *Vibrio.*

11. The method of any one of the preceding claims wherein the plasmid encodes one or more genes of interest.