Claims

1. A transfer vector for inserting a gene into a genetic locus of a baculovirus sequence comprising:
   a. an expression cassette comprising a eukaryotic promoter operably linked to the gene;
   b. a removable bipartite selection cassette flanked on each side by LoxP recombination sequences wherein said LoxP sequences are modified to ensure that only a single round of recombination can occur comprising:
      (i) an expressible sequence encoding a first selectable marker; and
      (ii) an expressible sequence encoding a second selectable marker; and
   c. sequences flanking the expression and bipartite selection cassettes, wherein the sequences substantially correspond to sequences of the genetic locus within the baculovirus sequence.

2. The transfer vector of claim 1, wherein the first selectable marker is a visual marker.

3. The transfer vector of claim 2, wherein the first maker is the *Lac*Zalpha fragment.

4. The transfer vector of claim 1, wherein the second selectable marker confers resistance to an antibiotic.

5. The transfer vector of claim 4, wherein the antibiotic resistance gene confers resistance to phleomycin.

6. The transfer vector of any one of the preceding claims, wherein the sequences flanking the expression and bipartite selection cassettes substantially correspond to sequences of any one of the following genetic loci: *ctx*, *egt*, *39k*, *orf51*, *gp37*, *iap2*, *odv-e56* and *p10*.

7. A method for producing a recombinant bacmid comprising:
   a. bringing a bacmid and the transfer vector according to any one of claims 1 to 6 together to allow homologous recombination; and
   b. selecting for a recombinant bacmid that comprises the expression cassette and the bipartite selection cassette.

8. The method of claim 7additionally comprising causing recombination between the LoxP sequences to remove the selection cassette from the bacmid.

9. A method for producing a recombinant baculovirus comprising producing a recombinant bacmid by the method of claim 7 or claim 8 and culturing a eukaryotic cell containing the bacmid so that a baculovirus is produced.

10. ~~A recombinant bacmid obtained by the method of claim 7.~~

11. A recombinant baculovirus obtained by the method of claim 9 when dependent upon claim 7.

12. A recombinant bacmid according to claim 10 that expresses a plurality of proteins, wherein each protein is expressed from a separate genetic locus of the bacmid.

13. A recombinant baculovirus according to claim 11 that expresses a plurality of proteins, wherein each protein is expressed from a separate genetic locus of the baculovirus.

14. The recombinant bacmid of claim 12 or the recombinant baculovirus of claim 13, wherein the plurality of proteins interact to form protein complex.

15. The recombinant bacmid or the recombinant baculovirus of claim 14, wherein the protein complex is a virus like particle.

16. The recombinant bacmid of claim 12 or the recombinant baculovirus of claim 13, wherein the separate genetic loci are selected from *ctx*, *egt*, *39k*, *orf51*, *gp37*, *iap2*, and *odv-e56*.

17. A cell containing a transfer vector according to any one of claims 1 to 6, a bacmid according to any one of claims 10, 12, 14 to 16, or a baculovirus according to any one of claims 11, 13 to 16.

18. A method for producing one or more proteins comprising culturing the recombinant bacmid according to any one of claims 10, 12, 14 to 16, or a baculovirus according to any one of claims 11, 13 to 16 under suitable conditions.

Claims

1. A transfer vector for inserting a gene into a genetic locus of a baculovirus sequence comprising:
   a. an expression cassette comprising a eukaryotic promoter operably linked to the gene;
   b. a removable bipartite selection cassette flanked on each side by modified LoxP recombination sequences capable of only a single round of recombination comprising:
      (i) an expressible sequence encoding a first selectable marker; and
      (ii) an expressible sequence encoding a second selectable marker; and
   c. sequences flanking the expression and bipartite selection cassettes, wherein the sequences substantially correspond to sequences of the genetic locus within the baculovirus sequence

2. The transfer vector of claim 1, wherein the first selectable marker is a visual marker.

3. The transfer vector of claim 2, wherein the first maker is the *LacZ*alpha fragment.

4. The transfer vector of claim 1, wherein the second selectable marker confers resistance to an antibiotic.

5. The transfer vector of claim 4, wherein the antibiotic resistance gene confers resistance to phleomycin.

6. The transfer vector of any one of the preceding claims, wherein the sequences flanking the expression and bipartite selection cassettes substantially correspond to sequences of any one of the following genetic loci: *ctx*, *egt*, *39k*, *orf51*, *gp37*, *iap2*, *odv-e56* and *p10*.

7. A method for producing a recombinant bacmid comprising:
    a. bringing a bacmid and the transfer vector according to any one of claims 1 to 6 together to allow homologous recombination; and
    b. selecting for a recombinant bacmid that comprises the expression cassette and the bipartite selection cassette.

8. The method of claim 7 additionally comprising causing recombination between the LoxP sequences to remove the selection cassette from the bacmid.

9. A method for producing a recombinant baculovirus comprising producing a recombinant bacmid by the method of claim 7 or claim 8 and culturing a eukaryotic cell containing the bacmid so that a baculovirus is produced.

10. A recombinant bacmid obtained by the method of claim 7 comprising the expression cassette and the bipartite expression cassette as characterized in any one of claims 1-6.

11. A recombinant baculovirus obtained by the method of claim 9 when dependent upon claim 7 comprising the expression cassette and the bipartite expression cassette as characterized in any one of claims 1-6..

12. A recombinant bacmid according to claim 10 that expresses a plurality of proteins, wherein each protein is expressed from a separate genetic locus of the bacmid.

13. A recombinant baculovirus according to claim 11 that expresses a plurality of proteins, wherein each protein is expressed from a separate genetic locus of the baculovirus.

14. The recombinant bacmid of claim 12 or the recombinant baculovirus of claim 13, wherein the plurality of proteins interact to form protein complex.

15. The recombinant bacmid or the recombinant baculovirus of claim 14, wherein the protein complex is a virus like particle.

16. The recombinant bacmid of claim 12 or the recombinant baculovirus of claim 13, wherein the separate genetic loci are selected from *ctx*, *egt*, *39k*, *orf51*, *gp37*, *iap2*, and *odv-e56*.

17. A cell containing a transfer vector according to any one of claims 1 to 6, a bacmid according to any one of claims 10, 12, 14 to 16, or a baculovirus according to any one of claims 11, 13 to 16.

18. A method for producing one or more proteins comprising culturing the recombinant bacmid according to any one of claims 10, 12, 14 to 16, or a baculovirus according to any one of claims 11, 13 to 16 under suitable conditions.