CLAIMS

What is claimed is:

1. A method of inserting a large sequence of heterologous insert DNA into a genome of a host cell using (i) a donor plasmid comprising from 5' to 3' (1) a recognition sequence of a restriction endonuclease encoded by the helper plasmid, (2) a first homology region having a size of 0.4-2.0kb , (3) a heterologous insert DNA of at least 8kb, and (4) a second homology region having a size of 0.4-2.0kb and b) a counter selection marker and ii) a helper plasmid comprising under control of a first promoter, an open reading frame encoding lambda red recombinase and under control of a second promoter, an open reading frame encoding a restriction endonuclease that has a recognition sequence that is not present in the host cell genome comprising the steps of:
   a) introducing the donor plasmid and the helper plasmid into the host cell; b) culturing under conditions which allow the expression of the restriction endonuclease and the lambda red recombinase; and c) selecting recombined clones showing an antibiotic resistance phenotype consistent with (i) loss of the helper and donor plasmids and (ii) presence of the heterologous DNA insert.

2. The method of claim 1 wherein the counter selection marker is flanked by FRT sites or dif sites.

3. The method of claim 1 or 2 wherein the donor plasmid comprises an origin of replication.

4. The method of any one of claims 1-3 wherein the helper plasmid comprises a selection cassette.

5. The method of any one of claims 1-4 wherein the helper plasmid comprises a regulon for the expression of a RecA homolog.

6. The method of any one of claims 1-5 wherein the helper plasmid comprises a conditional origin of replication.

7. The method of any one of claims 1-6 wherein the heterologous insert DNA is at least 10kb in size.

8. The method of any one of claims 1-7 wherein host cell genomic DNA is removed and is directly replaced with the heterologous insert DNA.

9. The method of claim 8 wherein the waaL gene of the host cell genomic DNA is removed.

10. The method of claim 8 wherein genes encoding glycosyltransferases are removed from the host cell genomic DNA.

11. The method of claim 8 wherein genes encoding nucleotide sugar biosynthesis or genes encoding periplasmic proteases are removed from the host cell genomic DNA.

12. The method of any one of claims 1-11 wherein the heterologous insert DNA encodes an oligosaccharyl transferase and/or glycosyltransferases.

13. The method of any one of claims 1-12 wherein the heterologous insert DNA encodes a carrier protein modified to include at least one glycosylation site.

14. The method of any one of claims 1-13 wherein the first and second homology regions are homologous to regions of DNA present on the genome of the host cell.