~~CLAIMS~~

~~What is claimed is:~~

~~1. A host cell comprising a donor plasmid and a helper plasmid,~~

~~(a) wherein the helper plasmid comprises: (i) under control of a first promoter, an open reading frame encoding lambda red recombinase; and (ii) 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; and~~

~~(b) wherein the donor plasmid comprises: (i) from 5' to 3': (1) the recognition sequence of the restriction endonuclease; (2) a first homology region of at least 0.5 kilobases (kb), (3) a heterologous insert DNA of at least 8 kb; and (4) a second homology region of at least 0.5 kb; and (ii) a counterselection marker.~~

~~2. The host cell of claim 1, wherein the heterologous insert DNA is at least 20 kb.~~

~~3. The host cell of any one of claims 1-2, further comprising an oligosaccharyl transferase.~~

~~4. The host cell of any one of claims 1-3, further comprising at least one glycosyltransferase.~~

~~5. The host cell of any one of claims 1-4, wherein one or more genes native to the host cell have been deleted or inactivated.~~

~~6. The host cell of any one of claims 1-5, wherein said heterologous insert DNA comprises an *rfb* cluster of a prokaryotic organism.~~

~~7. The host cell of any one of claims 1-6, wherein said host cell further comprises a nucleic acid encoding a carrier protein comprising a consensus sequence for N-glycosylation.~~

8. A method for inserting a large sequence of DNA into a genome of a host cell comprising the use of a donor plasmid and a helper plasmid, comprising the steps of A) introducing the donor plasmid and the helper plasmid into the same host cell and B) initiating the insertion procedure;

(a) wherein the helper plasmid comprises: (i) under control of a first promoter, an open reading frame encoding lambda red recombinase; and (ii) 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; and

(b) wherein the donor plasmid comprises: (i) from 5' to 3': (1) the recognition sequence of the restriction endonuclease; (2) a first homology region of at least 0.5 kilobases (kb), (3) a heterologous insert DNA of at least 8 kb; and (4) a second homology region of at least 0.5 kb; and (ii) a counterselection marker.

9. A host cell produced by the method of claim 8 wherein said host cell comprises one or more genes that encode a protein of interest.

10. A method of producing a glycoconjugate comprising a carrier protein and an antigen, wherein said method comprises culturing the host cell of claim 7 or 9 under conditions suitable for the production of proteins.

11. An isolated host cell, wherein a heterologous gene that encodes an oligosaccharyl transferase has been inserted into the genome of the host cell.

12. The isolated host cell of claim 11, wherein said host cell is *E. coli*.

13. The isolated host cell of claim 11 or 12, wherein said gene is the *C. jejuni pglB* gene.

14. The isolated host cell of any one of claims 11-13, wherein the copy number of the heterologous gene in the host cell is 1, 2, 3, 4, or 5.

~~15. The isolated host cell of any one of claims 11-14, wherein said host cell comprises a carrier protein comprising a consensus sequence Asn-X-Ser(Thr), wherein X can be any amino acid except Pro.~~

~~16. A method of producing an N-glycosylated protein, wherein said method comprises culturing the host cell of any one of claims 11-15 under conditions suitable for the production of proteins.~~

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.