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 comprises a selection marker.

3. The host cell of claim 2, wherein the selection marker is flanked by flippase recognition target (FRT) sites.

4. The host cell of any one of claims 1-3, wherein the first and second homology regions are homologous to adjacent regions of the host cell genome.

5. The host cell of any one of claims 1-4, wherein the first homology region is at least 2 kb.

6. The host cell of any one of claims 1-5, wherein the second homology region is at least 2 kb.

7. The host cell of any one of claims 1-6, wherein the heterologous insert DNA is at least 20 kb.

8. The host cell of any one of claims 1-7, wherein the recognition sequence comprises at least 18 base pairs.

9. The host cell of claim 1, wherein the restriction endonuclease is *Sce*I.

10. The host cell of claim 1, wherein the counterselection marker is *sacB*.

11. The host cell of any one of claims 1-10, further comprising an oligosaccharyl transferase.

12. The host cell of claim 11, wherein said oligosaccharyl transferase is heterologous to the host cell.

13. The host cell of claim 11 or 12, wherein said oligosaccharyl transferase is a prokaryotic oligosaccharyl transferase.

14. The host cell of any one of claims 1-13, further comprising at least one glycosyltransferase.

15. The host cell of claim 14, wherein said glycosyltransferase is heterologous to the host cell.

16. The host cell of claim 15, wherein said heterologous glycosyltransferase is a prokaryotic glycosyltransferase.

17. The host cell of any one of claims 1-16, wherein one or more genes native to the host cell have been deleted or inactivated.

18. The host cell of any one of claims 1-17, wherein said heterologous insert DNA comprises an *rfb* cluster of a prokaryotic organism.

19. The host cell of claim 18, wherein said *rfb* cluster is an *E. coli rfb* cluster, a *Pseudomonas rfb* cluster, a *Salmonella rfb* cluster, a *Yersinia rfb* cluster, a *Francisella rfb* cluster, a *Klebsiella rfb* cluster, an *rfb* cluster from an *Acinetobacter baumannii* strain, a *Shigella rfb* cluster, or a *Burkholderia rfb* cluster.

20. The host cell of claim 19, wherein said *rfb* cluster is an *E. coli rfb* cluster.

21. The host cell of claim 20, wherein said *E. coli rfb* cluster is of serotype O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O11, O12, O13, O14, O15, O16, O17, O18, O19, O20, O21, O22, O23, O24, O25, O26, O27, O28, O29, O30, O32, O33, O34, O35, O36, O37, O38, O39, O40, O41, O42, O43, O44, O45, O46, O48, O49, O50, O51, O52, O53, O54, O55, O56, O57, O58, O59, O60, O61, O62, O63, O64, O65, O66, O68, O69, O70, O71, O73, O74, O75, O76, O77, O78, O79, O80, O81, O82, O83, O84, O85, O86, O87, O88, O89, O90, O91, O92, O93, O95, O96, O97, O98, O99, O100, O101, O102, O103, O104, O105, O106, O107, O108, O109, O110, O111, O112, O113, O114, O115, O116, O117, O118, O119, O120, O121, O123, O124, O125, O126, O127, O128, O129, O130, O131, O132, O133, O134, O135, O136, O137, O138, O139, O140, O141, O142, O143, O144, O145, O146, O147, O148, O149, O150, O151, O152, O153, O154, O155, O156, O157, O158, O159, O160, O161, O162, O163, O164, O165, O166, O167, O168, O169, O170, O171, O172, O173, O174, O175, O176, O177, O178, O179, O180, O181, O182, O183, O184, O185, O186, or O187.

22. The host cell of any one of claims 1-17, wherein said heterologous insert DNA comprises a capsular polysaccharide gene cluster of a prokaryotic organism.

23. The host cell of claim 22, wherein said polysaccharide gene cluster is from an *E. coli* strain, a *Streptococcus* strain, a *Staphylococcus* strain, or a *Burkholderia* strain.

24. The host cell of any one of claims 1-17, wherein said heterologous insert DNA encodes an O antigen of *E. coli*, *Salmonella*, *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Chlamydia trachomatis*, *Vibrio cholera*, *Listeria*, *Legionella pneumophila*, *Bordetella parapertussis*, *Burkholderia mallei* and *pseudomallei*, *Francisella tularensis*, or *Campylobacter*.

25. The host cell of claim 24, wherein said O antigen of *E. coli* is O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O11, O12, O13, O14, O15, O16, O17, O18, O19, O20, O21, O22, O23, O24, O25, O26, O27, O28, O29, O30, O32, O33, O34, O35, O36, O37, O38, O39, O40, O41, O42, O43, O44, O45, O46, O48, O49, O50, O51, O52, O53, O54, O55, O56, O57, O58, O59, O60, O61, O62, O63, O64, O65, O66, O68, O69, O70, O71, O73, O74, O75, O76, O77, O78, O79, O80, O81, O82, O83, O84, O85, O86, O87, O88, O89, O90, O91, O92, O93, O95, O96, O97, O98, O99, O100, O101, O102, O103, O104, O105, O106, O107, O108, O109, O110, O111, O112, O113, O114, O115, O116, O117, O118, O119, O120, O121, O123, O124, O125, O126, O127, O128, O129, O130, O131, O132, O133, O134, O135, O136, O137, O138, O139, O140, O141, O142, O143, O144,O145, O146, O147, O148, O149, O150, O151, O152, O153, O154, O155, O156, O157, O158, O159, O160, O161, O162, O163, O164, O165, O166, O167, O168, O169, O170, O171, O172, O173, O174, O175, O176, O177, O178, O179, O180, O181, O182, O183, O184, O185, O186, or O187.

26. The host cell of claim 24, wherein said O antigen of *Klebsiella* is *K. pneumonia* serotype O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O11, or O12.

27. The host cell of any one of claims 1-17, wherein said heterologous insert DNA encodes a *Borrelia burgdorferi* glycolipid, a *Neisseria meningitidis* pilin O glycan or lipooligosaccharide (LOS), a *Haemophilus influenza* LOS, a *Leishmania major* lipophosphoglycan, or a tumor associated carbohydrate antigen.

28. The host cell of any one of claims 1-27, wherein said host cell further comprises a nucleic acid encoding a carrier protein comprising a consensus sequence for N-glycosylation.

29. The host cell of claim 28, wherein the nucleic acid encoding the carrier protein is heterologous to the host cell.

30. The host cell of claim 28 or 29, wherein said carrier protein is detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM197, Diphtheria toxoid, tetanus toxoid, detoxified hemolysin A of *S.*

aureus, clumping factor A, clumping factor B, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* sat protein, the passenger domain of *E. coli* sat protein, *C. jejuni* AcrA, or a *C. jejuni* natural glycoprotein.

31. The host cell of claim 30, wherein said carrier protein is detoxified Exotoxin A of *P. aeruginosa* (EPA).

32. The host cell of any one of claims 1-31, wherein said host cell is an *Escherichia* species, *Shigella* species, *Klebsiella* species, *Xhantomonas* species, *Salmonella* species, *Yersinia* species, *Lactococcus* species, *Lactobacillus* species, *Pseudomonas* species, *Corynebacterium* species, *Streptomyces* species, *Streptococcus* species, *Staphylococcus* species, *Bacillus* species, or a *Clostridium* species.

33. The host cell of claim 32, wherein said host cell is an *E. coli* species.

34. A method of producing a glycoconjugate comprising a carrier protein and an antigen, wherein said method comprises culturing the host cell of any one of claims 28-33 under conditions suitable for the production of proteins.

35. A glycoconjugate produced by the method of claim 34.

36. The glyconjugate of claim 35, wherein said glyconjugate comprises a carrier protein and an antigen.

37. The glyconjugate of claim 36, wherein said antigen is (i) an O antigen of *E. coli*, *Salmonella*, *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Chlamydia trachomatis*, *Vibrio cholera*, *Listeria*, *Legionella pneumophila*, *Bordetella parapertussis*, *Burkholderia mallei* and *pseudomallei*, *Francisella tularensis*, or *Campylobacter*; (ii) a capsular polysaccharide of *Clostridium difficile*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, *E. coli*, *Streptococcus agalacticae*, *Neisseria meningitidis*, *Candida albicans*, *Haemophilus influenza*, *Enterococcus faecalis*; or (iii) a *Borrelia burgdorferi* glycolipid, a *Neisseria meningitidis* pilin O glycan or lipooligosaccharide (LOS), a *Haemophilus influenza* LOS, a *Leishmania major* lipophosphoglycan, or a tumor associated carbohydrate antigen.

38. The glyconjugate of claim 37, wherein said O antigen of *E. coli* is O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O11, O12, O13, O14, O15, O16, O17, O18, O19, O20, O21, O22, O23, O24, O25, O26, O27, O28, O29, O30, O32, O33, O34, O35, O36, O37, O38, O39, O40, O41, O42, O43, O44, O45, O46, O48, O49, O50, O51, O52, O53, O54, O55, O56, O57, O58, O59,

O60, O61, O62, O63, O64, O65, O66, O68, O69, O70, O71, O73, O74, O75, O76, O77, O78, O79, O80, O81, O82, O83, O84, O85, O86, O87, O88, O89, O90, O91, O92, O93, O95, O96, O97, O98, O99, O100, O101, O102, O103, O104, O105, O106, O107, O108, O109, O110, O111, O112, O113, O114, O115, O116, O117, O118, O119, O120, O121, O123, O124, O125, O126, O127, O128, O129, O130, O131, O132, O133, O134, O135, O136, O137, O138, O139, O140, O141, O142, O143, O144,O145, O146, O147, O148, O149, O150, O151, O152, O153, O154, O155, O156, O157, O158, O159, O160, O161, O162, O163, O164, O165, O166, O167, O168, O169, O170, O171, O172, O173, O174, O175, O176, O177, O178, O179, O180, O181, O182, O183, O184, O185, O186, or O187.

39. The glyconjugate of claim 37, wherein said O antigen of *Klebsiella* is *K. pneumonia* serotype O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O11, or O12.

40. The glyconjugate of any one of claims 36-39, wherein said carrier protein is detoxified EPA.

41. An immunogenic composition comprising the glyconjugate of any one of claims 35-40.

42. A method of treating or preventing an infection in a subject comprising administering the immunogenic composition of claim 41 to the subject.

43. The method of claim 42, wherein said infection is an infection by uropathogenic *E. coli*.

44. A method of inducing an immune response in a subject comprising administering the immunogenic composition of claim 41 to the subject.

45. The method of claim 44, wherein said immune response is an immune response against a pathogen.

46. The method of claim 45, wherein said pathogen is *E. coli*, *Salmonella*, *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Chlamydia trachomatis*, *Vibrio cholera*, *Listeria*, *Legionella pneumophila*, *Bordetella parapertussis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Francisella tularensis*, *Campylobacter*; *Clostridium*, *Staphylococcus*, *Streptococcus*, *Neisseria meningitidis*, *Candida albicans*, *Haemophilus influenza*, *Enterococcus faecalis*; *Borrelia burgdorferi* or *Leishmania major*.

47. The method of any one of claims 42-46, wherein the subject is a human.

48. A kit 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.

49. An isolated plasmid comprising (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.

50. An isolated plasmid comprising (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.

51. A method of making a host cell, wherein said host cell comprises a donor plasmid and a helper plasmid, comprising introducing the plasmids of claims 49 and 50 into the host cell.

52. The method of claim 51, wherein said introduction comprises transformation.

53. An isolated host cell, wherein said host cell comprises a donor plasmid and a helper plasmid, wherein said host cell is produced according to the following method: (i) introducing a donor plasmid into the host cell and (ii) introducing a helper plasmid into the host cell.

54. The host cell of claim 53, 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.

55. The host cell of claim 53 or 54, 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.

56. The host cell of any one of claims 53-55, wherein said introduction comprises transformation.

57. The host cell of any one of claims 53-56, wherein said donor plasmid and said helper plasmid are introduced separately into the host cell.

58. The host cell of any one of claims 53-56, wherein said donor plasmid and said helper plasmid are introduced into the host cell simultaneously.

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

60. The isolated host cell of claim 59, wherein said host cell is *E. coli*.

61. The isolated host cell of claim 59 or 60, wherein said gene is the *C. jejuni pglB* gene.

62. The isolated host cell of any one of claims 59-61, wherein the copy number of the heterologous gene in the host cell is 1, 2, 3, 4, or 5.

63. The isolated host cell of claim 62, wherein the copy number of the heterologous gene in the host cell is 1.

64. The isolated host cell of any one of claims 59-63, 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.

65. The isolated host cell of claim 64, wherein said carrier protein is heterologous to said host cell.

66. The isolated host cell of claim 64 or 65, wherein said carrier protein is *Pseudomonas* exotoxin A, cholera toxin B, AcrA, HlA or ClfA.

67. A method of producing an N-glycosylated protein, wherein said method comprises culturing the host cell of any one of claims 59-66 under conditions suitable for the production of proteins.

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

9. A glycoconjugate produced by the method of claim 8.

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

11. The isolated host cell of claim 10, wherein said host cell is *E. coli*.

12. The isolated host cell of claim 10 or 11, wherein said gene is the *C. jejuni pglB* gene.

13. The isolated host cell of any one of claims 10-12, wherein the copy number of the heterologous gene in the host cell is 1, 2, 3, 4, or 5.

14. The isolated host cell of any one of claims 10-13, 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.

15. A method of producing an N-glycosylated protein, wherein said method comprises culturing the host cell of any one of claims 10-14 under conditions suitable for the production of proteins.