CLAIMS

1. A method for preparing a fucosylated oligosaccharide, wherein the method comprises: transferring a fucosyl group of a donor to an oligosaccharide receptor by a fucosyltransferase heterologously expressed in a genetically engineered bacterium; wherein the donor is a nucleotide-activated donor, and the fucosyltransferase has α-1,2-fucosyltransferase activity;

wherein the fucosyltransferase is an enzyme corresponding to NCBI Accession Number RTL12957.1 or WP_120175093.1;

wherein the genetically engineered bacterium further expresses a bifunctional enzyme with both L-fucokinase and fucose-1-phosphate guanyltransferase activities and the bifunctional enzyme is an enzyme corresponding to NCBI Accession Number WP_010993080.1.

2. The method of claim 1, wherein the oligosaccharide receptor is selected from the group consisting of lactose, 3-fucosyllactose, lacto-N-tetraose, lacto-N-neotetraose, lacto-N-fucosylpentose II, lacto-N-hexose and sialyllacto-N-tetraose b;

and/or, the fucosylated oligosaccharide is selected from the group consisting of 2'-fucosyllactose, 2',3-difucosyllactose, lacto-N-fucosylpentose I, lacto-N-neofucosylpentose I, lacto-N-difucosylhexose I, lacto-N-fucosylheptose I and fucosyllacto-N-sialylpentose b;

and/or, the donor is guanosine diphospho-fucose;

and/or, the genetically engineered bacterium is an engineered *Escherichia coli* (*E. coli*) or yeast; preferably, the genetically engineered bacterium is an engineered *E. coli* BL21 (DE3) strain.

3. The method of claim 1 or 2, wherein in the genetically engineered bacterium, a bypass metabolic pathway of the oligosaccharide receptor is inhibited; preferably, the bypass metabolic pathway of the oligosaccharide receptor is inhibited by knocking out or mutating a gene; more preferably, when the oligosaccharide receptor is lactose, a gene encoding β-

galactosidase in the genetically engineered bacterium, such as *lacZ* gene, is knocked out and inactivated, and a metabolic pathway of lactose degradation to galactose is inhibited;

and/or, in the genetically engineered bacterium, a bypass metabolic pathway of a precursor of the donor is inhibited; preferably, the bypass metabolic pathway of the precursor is inhibited by knocking out or mutating a gene; more preferably, when the donor is guanosine diphospho-fucose, the precursor is L-fucose, and genes encoding L-fucose isomerase and/or L-fuculokinase in the genetically engineered bacterium, such as *FucI* and/or *FucK*, are knocked out and inactivated, and the bypass metabolic pathway of L-fucose is inhibited;

and/or, in the genetically engineered bacterium, a bypass metabolic pathway of the donor is inhibited; preferably, the bypass metabolic pathway of the donor is inhibited by knocking out or mutating a gene; more preferably, when the donor is guanosine diphospho-fucose, a gene encoding UDP-glucose lipid carrier transferase in the genetically engineered bacterium, such as *wacJ*, is knocked out and inactivated, and the competitive utilization pathway of guanosine diphospho-fucose degradation to colanic acid is blocked.

4. The method of any one of claims 1 to 3, wherein the method further comprises the fermentation culture of the genetically engineered bacterium in a fermentation medium;

preferably, the fermentation medium comprises: 20-25 g/L of glycerol, 10-12 g/L of peptone, 5-6 g/L of yeast powder, 10-12 g/L of NaCl, as well as 0.1-0.2 mM of IPTG, 5-6 g/L of a precursor molecule for synthesizing the donor such as L-fucose, and 10-15 g/L of oligosaccharide such as lactose which are added when the OD_{600} of the fermentation medium is 0.6-0.8; and/or, the condition of the fermentation culture is: 25-27°C and 220 r/min.

5. The method of any one of claims 1 to 4, wherein the genetically

engineered bacterium is an engineered *E. coli* BL21 (DE3) strain, in which *lacZ* gene, *FucI FucK,* and *wacJ* are knocked out.

6. A genetically engineered bacterium heterologously expressing a fucosyltransferase, wherein the fucosyltransferase has α-1,2-fucosyltransferase activity; the fucosyltransferase transfers a fucosyl group of a donor to an oligosaccharide receptor, and the donor is a nucleotide-activated donor;

wherein, the fucosyltransferase is one or more of enzymes corresponding to NCBI Accession Numbers RTL12957.1 or WP_120175093.1;

wherein the genetically engineered bacterium expresses a bifunctional enzyme with both L-fucokinase and fucose-1-phosphate guanyltransferase activities and the bifunctional enzyme is an enzyme corresponding to NCBI Accession Number WP_010993080.1.

7. The genetically engineered bacterium of claim 6, wherein the oligosaccharide receptor is selected from the group consisting of lactose, 3-fucosyllactose, lacto-N-tetraose, lacto-N-neotetraose, lacto-N-fucosylpentose II, lacto-N-hexose and sialyllacto-N-tetraose b;

and/or, the fucosylated oligosaccharide is selected from the group consisting of 2'-fucosyllactose, 2',3-difucosyllactose, lacto-N-fucosylpentose I, lacto-N-neofucosylpentose I, lacto-N-difucosylhexose I, lacto-N-fucosylheptose I and fucosyllacto-N-sialylpentose b;

and/or, the donor is guanosine diphospho-fucose;

and/or, the genetically engineered bacterium is an engineered *E. coli* or yeast; preferably, the genetically engineered bacterium is an engineered *E. coli* BL21 (DE3) strain.

8. The genetically engineered bacterium of claim 6, wherein the nucleotide sequence encoding the fucosyltransferase is set forth in any one of SEQ ID NOs: 2 and 5 and/or the nucleotide sequence encoding the bifunctional enzyme is set forth in SEQ ID NO: 10;

and/or, in the genetically engineered bacterium, a bypass metabolic pathway of the oligosaccharide receptor is inhibited; preferably, the bypass metabolic pathway of the oligosaccharide receptor is inhibited by knocking out or mutating a gene; more preferably, when the oligosaccharide receptor is lactose, a gene encoding β-galactosidase in the genetically engineered bacterium, such as *lacZ* gene, is knocked out and inactivated, and the metabolic pathway of lactose degradation to galactose is inhibited;

and/or, in the genetically engineered bacterium, a bypass metabolic pathway of a precursor of the donor is inhibited; preferably, the bypass metabolic pathway of the precursor is inhibited by knocking out or mutating a gene; more preferably, when the donor is guanosine diphospho-fucose, the precursor is L-fucose, and genes encoding L-fucose isomerase and/or L-fuculokinase in the genetically engineered bacterium, such as *FucI* and/or *FucK*, are knocked out and inactivated, and the bypass metabolic pathway of L-fucose is inhibited;

and/or, in the genetically engineered bacterium, a bypass metabolic pathway of the donor is inhibited; preferably, the bypass metabolic pathway of the donor is inhibited by knocking out or mutating a gene; more preferably, when the donor is guanosine diphospho-fucose, a gene encoding UDP-glucose lipid carrier transferase in the genetically engineered bacterium, such as *wacJ*, is knocked out and inactivated, and the competitive utilization pathway of guanosine diphospho-fucose degradation to colanic acid is blocked.

9. The method of any one of claims 1 to 4, wherein the genetically engineered bacterium is an engineered *E. coli* BL21 (DE3) strain, in which *lacZ* gene, *FucI FucK,* and *wacJ* are knocked out.

10. A method for preparing a fucosylated oligosaccharide, wherein the method comprises:

providing a fucosyltransferase having α-1,2-fucosyltransferase activity in a reaction system, the fucosyltransferase transfers a fucosyl

group of a nucleotide-activated donor to an oligosaccharide receptor;

wherein, the fucosyltransferase is selected from one or more of enzymes corresponding to NCBI Accession Number RTL12957.1 or WP_120175093.1; and

further providing a bifunctional enzyme having both L-fucokinase and fucose-1-phosphate guanyltransferase activities, in the reaction system, wherein the bifunctional enzyme corresponds to NCBI Accession Number WP_010993080.1.

11. A combination of enzymes, wherein the combination of enzymes comprises a fucosyltransferase corresponding to NCBI Accession Number RTL12957.1 or WP_120175093.1 and a bifunctional enzyme with both L-fucokinase and fucose-1-phosphate guanyltransferase activities, wherein the bifunctional enzyme corresponds to NCBI Accession Number WP_010993080.1.

12. Use of a combination of enzymes in the preparation of a fucosylated oligosaccharide, wherein the combination of enzymes is as defined in claim 11.