CLAIMS

1. A method for highly expressing recombinant protein of engineering bacteria, wherein the end with hydrophilicity of the recombinant protein is colicin polypeptide and the other end with hydrophobic nature is polypeptide of target moiety which is capable of binding target, the method comprising:

(1) transfecting recombinant plasmid of expressing the recombinant protein into E.coli engineering bacteria with pET system to obtain positive monoclonal colonies,

(2) producing seed bacteria solution of the positive monoclonal colonies, and inducing protein expression and enlargement culturing of the seed bacteria solution; the supernatant of the enlargement cultured solution contains expressed recombinant protein,

(3) extracting and purifying the recombinant protein from the supernatant, wherein the *E.coli* engineering bacteria with pET system is E.coliB834 (DE3).

2. According to the method of claim 1, enlargement culturing medium used for said enlargement culturing of the seed bacteria solution has water as solvent and comprises following components: NaCl 6.0~6.7g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 0.6~2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8～18.3g/L, KH_2PO_4 3.0～4.3g/L, NH_4Cl 1.0~1.4g/L, $MgSO_4$ 0.2~0.4g/L, $CaCl_2$0.01g/L, methionine 0~40mg/L.

3. According to the method of claim 2, the enlargement culturing medium has

water as solvent and comprises following components: NaCl 6.0g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8g/L, KH_2PO_4 3.0g/L, NH_4Cl 1.0g/L, $MgSO_4$ 0.2g/L, $CaCl_2$0.01g/L, methionine 0~40mg/L .

4. According to any method of claim 1~3, wherein said enlargement culturing of the seed bacteria solution comprises following steps: the seed bacteria liquid was added into a container and started growth for 2 to 3 hours at 30℃, when the OD value reached 0.4-0.6, the seed bacteria solution was conducted heat shock at 42℃ for 30 minutes, and then cooled down to 37 ℃and kept growth for 1.5 to 2 hours before being collected.

5. According to the method of claim 4, in conducting heat shocks, the IPTG with final concentration 0.5mmol/L was added into said enlargement culturing medium.

6. According to the method of claim 1, said extracting and purifying the recombinant protein from the supernatant was by CM ion exchange column, and the loading quantity of the supernatant samples depends on the ratio value which is 2.5mg/ml between the weight of the recombinant protein in the supernatant and the volume of Gel particles used in the CM ion exchange column.

7. According to the method of claim 6, the eluent solution used for said extracting and purifying in CM ion exchange column is boric acid buffer solution with 0.2mol/L NaCl.

8. According to the method of claim 1, the recombinant plasmid of expressing the recombinant protein is selected from the group consisted of pBHC-SA1、pBHC-SA2、pBHC-SA3 pBHC-SA4、pBHC-SE, pBHC-PA and pBHC-PorA1.

9. The application of any method of claims 1~8 in preparing the recombinant peptides PMC-SA1, PMC-SA2, PMC -SA3, PMC-SA4, PMC-SE, PMC-PA or PMC-AM.

10. A medium used for *E.coli* engineering bacteria with pET system, the medium has water as solvent and comprises following components: NaCl 6.0~6.7g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 0.6~2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8 ~ 18.3g/L, KH_2PO_4 3.0 ~ 4.3g/L, NH_4Cl 1.0~1.4g/L, $MgSO_4$ 0.2~0.4g/L, $CaCl_2$0.01g/L, methionine 0~40mg/L.

11. According to the medium of claim 10, wherein said *E.coli* engineering bacteria with pET system is *E.coli* B834 (DE3), and the medium has water as solvent and comprises following components: NaCl 6.0g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8g/L, KH_2PO_4 3.0g/L, NH_4Cl 1.0g/L, $MgSO_4$ 0.2g/L, $CaCl_2$ 0.01g/L, methionine 0~40mg/L.

CLAIMS

1. A method for highly expressing recombinant protein of engineering bacteria, wherein the end with hydrophilicity of the recombinant protein is colicin polypeptide and the other end with hydrophobic nature is polypeptide of target moiety which is capable of binding target, the method comprising:

(1) transfecting recombinant plasmid of expressing the recombinant protein into E.coli engineering bacteria with pET system to obtain positive monoclonal colonies,

(2) producing seed bacteria solution of the positive monoclonal colonies, and inducing protein expression and enlargement culturing of the seed bacteria solution; the supernatant of the enlargement cultured solution contains expressed recombinant protein,

(3) extracting and purifying the recombinant protein from the supernatant, wherein the *E.coli* engineering bacteria with pET system is E.coliB834 (DE3); enlargement culturing medium used for said enlargement culturing of the seed bacteria solution has water as solvent and comprises following components: NaCl 6.0~6.7g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 0.6~2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8 ~ 18.3g/L, KH_2PO_4 3.0 ~ 4.3g/L, NH_4Cl 1.0~1.4g/L, $MgSO_4$ 0.2~0.4g/L, $CaCl_2$0.01g/L, methionine 0~40mg/L.

2. The method according to claim 1, the enlargement culturing medium has water as solvent and comprises following components: NaCl 6.0g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8g/L, KH_2PO_4

3.0g/L, NH_4Cl 1.0g/L, $MgSO_4$ 0.2g/L, $CaCl_2$0.01g/L, methionine 0~40mg/L .

3. The method according to claim 1 or 2, wherein said enlargement culturing of the seed bacteria solution comprises following steps: the seed bacteria liquid was added into a container and started growth for 2 to 3 hours at 30℃, when the OD value reached 0.4-0.6, the seed bacteria solution was conducted heat shock at 42℃ for 30 minutes, and then cooled down to 37 ℃and kept growth for 1.5 to 2 hours before being collected.

4. The method according to claim 3, in conducting heat shocks, the IPTG with final concentration 0.5mmol/L was added into said enlargement culturing medium.

5. The method according to claim 1, said extracting and purifying the recombinant protein from the supernatant was by CM ion exchange column, and the loading quantity of the supernatant samples depends on the ratio value which is 2.5mg/ml between the weight of the recombinant protein in the supernatant and the volume of Gel particles used in the CM ion exchange column.

6. The method according to claim 5, the eluent solution used for said extracting and purifying in CM ion exchange column is boric acid buffer solution with 0.2mol/L NaCl.

7. The method according to claim 1, the recombinant plasmid of expressing the recombinant protein is selected from the group consisted of pBHC-SA1、pBHC-SA2、pBHC-SA3 pBHC-SA4、pBHC-SE, pBHC-PA and pBHC-PorA1.

8. The application of any method of claims 1~7 in preparing the recombinant

peptides PMC-SA1, PMC-SA2, PMC -SA3, PMC-SA4, PMC-SE, PMC-PA or PMC-AM.

9. A medium used for *E.coli* engineering bacteria with pET system, the medium has water as solvent and comprises following components: NaCl 6.0~6.7g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 0.6~2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8～18.3g/L, KH_2PO_4 3.0～4.3g/L, NH_4Cl 1.0~1.4g/L, $MgSO_4$ 0.2~0.4g/L, $CaCl_2$0.01g/L, methionine 0~40mg/L.

10. According to the medium of claim 9, wherein said *E.coli* engineering bacteria with pET system is *E.coli* B834 (DE3), and the medium has water as solvent and comprises following components: NaCl 6.0g/L, peptone 25.0g/L, yeast powder 7.5g/L, glucose 2.0g/L, $Na_2HPO_4 \cdot 7H_2O$ 6.8g/L, KH_2PO_4 3.0g/L, NH_4Cl 1.0g/L, $MgSO_4$ 0.2g/L, $CaCl_2$ 0.01g/L, methionine 0~40mg/L.