**Basic part**

**Her2-affibody(ZHer2:342**)

Sequence: gtggataacaaatttaacaaagaaatgcgcaacgcgtattgggaaattgcgctgctgccg  
aacctgaacaaccagcagaaacgcgcgtttattcgcagcctgtatgatgatccgagccag  
agcgcgaacctgctggcggaagcgaaaaaactgaacgatgcgcaggcgccgaaa

Source: Search a lot of literature, finally obtain the required literature at NCBI, for codon optimization.

Brief description: Her2 is a transmembrane receptor with tyrosine kinase activity and is important in cell growth regulation, survival and differentiation. Meanwhile Her2 is overexpressed in breast cancer, ovarian cancer, gastric cancer and salivary gland tumors and represents an attractive target for cancer diagnosis and therapy. Therefore, Her2 was selected as the target and anti-HER2 antibody was used for tumor-specific targeting due to its high affinity for HER2 (Kd = 22 pmol / L) and its small size (58 amino acids). We introduced the anti-HER2 antibody gene into E.coli Nissle 1917 (EcN), and when successfully expressed, it had the effect of dual targeting, improving the accuracy of treatment.

Part type:

Design considerations: Removal of restriction site mutations and codon optimization

**pelB:**

Sequence：atgaaatacctgctgccgaccgctgctgctggtctgctgctcctcgctgcccagccggcgatggcc

(aa. MKYLLPTAAAGLLLLAAQPAMA)

Source: https://www.ncbi.nlm.nih.gov

Brief description: pelb is a secretory signal peptide that is widely used to export foreign proteins into the E. coli cytoplasmic space or secrete them directly into the extracellular medium. When attached to one protein, the protein is directed to the periplasmic membrane of E. coli, where the sequence is removed by the pelB peptidase. It is used to encapsulate protein antigen fusions directly onto the cell surface.

Part type:

Design considerations: Removal of restriction site mutations and codon optimization

**6xHis-tag**

Sequence: catcaccatcaccatcat

Source: reference

Brief description: 6xHis-tag is a recombinant protein fusion peptide tag that can be detected by rapid and sensitive homogeneous analysis or colorimetric detection in Western blot.

Proteins can also be rapidly purified from crude extracts using a one-step affinity isolation method.

This part was used to detect whether the constructed fusion protein was successfully expressed.

Part type:

Design consideration: The codon is optimized and needs to be easy to check.

isoleucine zipper：

Adding artificial trimer motifs into TRAIL proteins enhances biological activity. Here, we show that ligation of an isoleucine zipper hexamerization motif to the N terminus of TRAIL leads to multimerization of its trimeric form, resulting in higher cytotoxic activity compared to its native state.[1]

TAAACAGATCGAGGACAAAATCGAAGAAATTCTGAGCAAAATCTACCACATCGAGAACGAGATCGCGCGCATCAAAAAACTGATCGGCGAACGCGA

[1] Han J H, Moon A R, Chang J H, et al. Potentiation of TRAIL killing activity by multimerization through isoleucine zipper hexamerization motif[J]. BMB reports, 2016, 49(5): 282.

sTRAIL:

Tumor necrosis factor (TNF) - related apoptosis inducing ligand (TRAIL), originally identified as a member of the TNF family, can induce apoptosis in various cancer cells. TRAIL mediated induction of apoptosis in most transformed tumour cells occurs through stimulation of their cognate receptors DR4 (or TRAIL1) and DR5 (or TRAIL2). TRAIL is a type transmembrane protein that can be produced in a soluble form by protease mediated cleavage of its extracellular region or by bacterial expression of this recombinant form containing its extracellular region. Structural studies of TRAIL reveal that it forms a trimer, which further binds to the trimeric protein DR5. TRAIL can form, in its native soluble form, trimers that induce apoptosis.[1]

As a type II transmembrane protein, traces can be cleaved by specific proteases to form a soluble molecule in the extracellular region. The study of protein crystal structure shows that soluble TRAIL (sTRAIL) forms a homotrimer, which is the key structure of receptor recognition and apoptosis.[2] We improved the sTRAIL sequence, deleted several amino acids and optimized the codon to make it easier to express in E. coli Nissle 1917 (ECN). By connecting an isoleucine zipper at the N end of sTRAIL, it leads to its trimerization and has higher cytotoxic activity than its natural state.

[1] Han J H, Moon A R, Chang J H, et al. Potentiation of TRAIL killing activity by multimerization through isoleucine zipper hexamerization motif[J]. BMB reports, 2016, 49(5): 282.

[2] Yan C, Li S, Li Z, et al. Human umbilical cord mesenchymal stem cells as vehicles of CD20-specific TRAIL fusion protein delivery: a double-target therapy against non-Hodgkin’s lymphoma[J]. Molecular pharmaceutics, 2013, 10(1): 142-151.

AGTTCGCGAACGCGGTCCGCAACGTGTTGCAGCACATATTACCGGTACCCGTGGTCGTAGTAATACCCTGAGTAGTCCGAACAGCAAAAACGAGAAAGCGCTGGGTCGTAAAATCAACAGCTGGGAAAGCAGCCGTAGCGGTCATAGCTTTCTGAGCAACCTGCATCTGCGTAACGGCGAACTGGTTATCCACGAGAAAGGCTTCTACTACATCTACAGCCAGACCTACTTCCGCTTCCAGGAAGAAATTAAAGAGAACACCAAAAACGACAAACAGATGGTCCAGTACATCTACAAATACACCAGCTACCCGGATCCGATTCTGCTGATGAAAAGCGCGCGTAACAGCTGTTGGAGTAAAGACGCGGAATACGGTCTGTACAGCATCTATCAGGGCGGCATTTTCGAGCTGAAAGAGAACGATCGCATCTTCGTCAGCGTTACCAACGAGCATCTGATCGACATGGACCACGAAGCAAGCTTTTTCGGCGCGTTTCTGGTTGGTTAACT

Vgb

Vireoscilla hemoglobin (VHb) of Gram-negative aerobic bacterium vireoscilla is the first bacterial hemoglobin found. Its function is to isolate oxygen, especially when it is at low concentration, and transport it to terminal respiratory oxidase, so as to enhance oxidative phosphorylation.[1]

Vitreoscilla hemoglobin gene (vgb) was introduced into E. coli, cell growth and target protein production increased significantly. The vgb promoter is regulated by oxygen at the transcriptional level, and hypoventilation is sufficient to induce VHb gene expression.[2] We used vgb promoter to replace the T7 promoter of pET-28A (+) to enable E. coli Nissle 1917 (ECN) to transcribe under hypoxic conditions.

[1] Kim Y, Webster D A, Stark B C. Improvement of bioremediation by Pseudomonas and Burkholderia by mutants of the Vitreoscilla hemoglobin gene (vgb) integrated into their chromosomes[J]. Journal of Industrial Microbiology and Biotechnology, 2005, 32(4): 148-154.

[2] Liu T, Chen J-Y, Zheng Z, et al. Construction of highly efficient E. coli expression systems containing low oxygen induced promoter and partition region[J]. Applied microbiology and biotechnology, 2005, 68(3): 346-354.

acaggacgctggggttaaaagtatttgagttttgatgtggattaagttttaagaggcaataaagattataataagtgctgctacaccatactgatgtatggcaaaaccataataatgaacttaaggaagaccctc

Q:Enter any design considerations you had to deal with during the detailed design of the sequence.

The hypoxic environment is not well controlled and cannot be transcribed.