Overview

During the course of our project development, we conducted multiple engineering cycles to enhance our design and experimental implementation. We present our engineering progress in two sections: engineering of the expression system and engineering of the hardware. For each engineering process, we documented the four general stages (design, build, test, and learn) to demonstrate our improvement.

Engineering of the expression system

Design：After conducting preliminary research, we found that cytochrome cd1-type nitrite reductase is more suitable for food applications and has also been studied in terms of electrode loading. We first tried to choose the more mature expression system: pET-22b and E.coli (DH5α).

Build

The construction of the recombinant plasmid was completed by Beijing Tsingke Biotech Co., Ltd.

Test

During the induction expression process, as detailed in the protocol, we tried many times but did not obtain the target protein.

Learn

According to the experimental results, it is speculated that there is no pathway for expressing cytochrome d1 in E.coli. Therefore, we changed the expression system and selected genes related from Bacillus cereus to connect to the pET-28a, and finally successfully obtained the target protein.

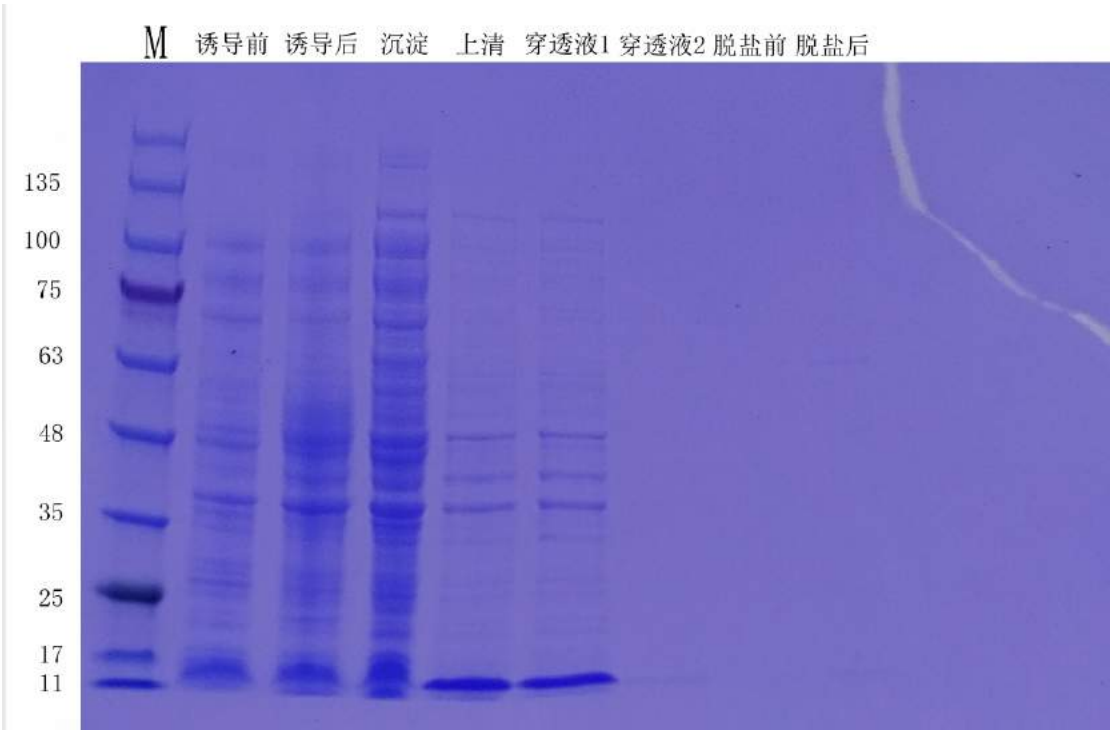

Figure 1Successful protein expression bands after plasmid replacement

Engineering of the hardware

Design

The electrode load part, we chose to construct a Au NPs-modified enzyme bioelectrode. The first thing we need to do is to prepare Au NPs as the electrode substrate loading material. The purity and particle size of Au NPs will affect the enzyme loading.

Build

We first used a conventional method to thoroughly clean all glass instruments in aqua regia, rinse them thoroughly with ultrapure water, and dry them in an oven at 40 ℃ for standby. Heat 100 mL of water containing 0.117 g/L HAuCl4 to boiling, quickly add 1.173 mL of a 1% sodium citrate solution that has been prepared and stir continuously. When the color of the solution changes from light yellow to wine red, continue boiling for 10 min, then stop heating and continue stirring for 15 min.

Test

The Au NPs prepared by the above method did not form a colloid but instead underwent agglomeration. After multiple experiments, we found that this was related to the amount of sodium citrate added. When the amount of sodium citrate added in the above experiment was changed to 3 ml, we successfully obtained a purple colloid.

Figure 2Figure 2: Preparation failure of Au NPs

Figure 3: Preparation success of Au NPs

Learn

The conditions and methods for preparing Au NPs will affect the properties such as particle size, shape, and dispersion. Therefore, the preparation of Au NPs needs to be optimized and adjusted according to specific situations.