# Project feasibility analysis

## 1.feasibility

This project used the fusion protein genetic engineering technique to recombinantly construct three enzymes, IsPETase, TPADO, and DCD dehydrogenase, to ultimately form a multi-enzyme system. The fusion protein genetic engineering technique used in this project has been around for more than 60 years and has been used in many articles, for example, Brandon C. Knott et al. constructed MHETase:PETase chimeric proteins and found that the chimeric proteins have better PET and MHET degradation ability compared to the free enzymes. This marks that the technology has gradually matured. Meanwhile, this project used SOE PCR for gene amplification. Unlike traditional PCR, the primers of SOE PCR need to be designed with overlapping regions so that two different genes can be linked together. For primer design, we used SnapGene software to design specific primers for the desired genes to be expressed (Fig. 1), which were Is-F, Is-R, E6-F, and E6-R.

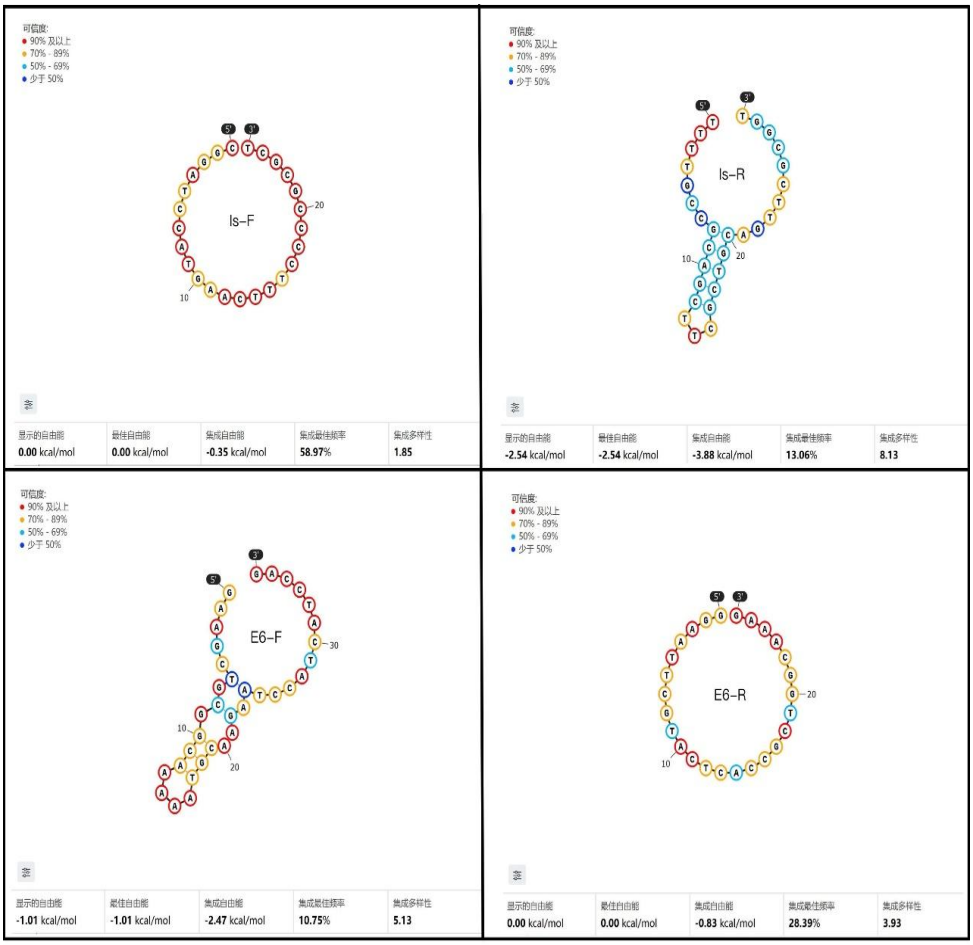


Figure 1 4 primer structures

The primers for Is-F and E6-R were found to be free of hairpin structure and within the range of Tm and GC values, which indicated that the primers were good. their primers are good. However, although the Tm and GC values of s-R and E6-F were within the range, the primers had hairpin structures due to the addition of the rigid peptide gene, but the free energy of Is-F and E6-R was less than 4.5 kcal/mol, which made them less likely to produce primer dimers, and the PCR amplification products were complete, and a recombinant plasmid was constructed with the SnapGene software (Figure 2), which indicated that the four primers were better designed than those of the other primers.

This indicates that the four primers were designed reasonably.

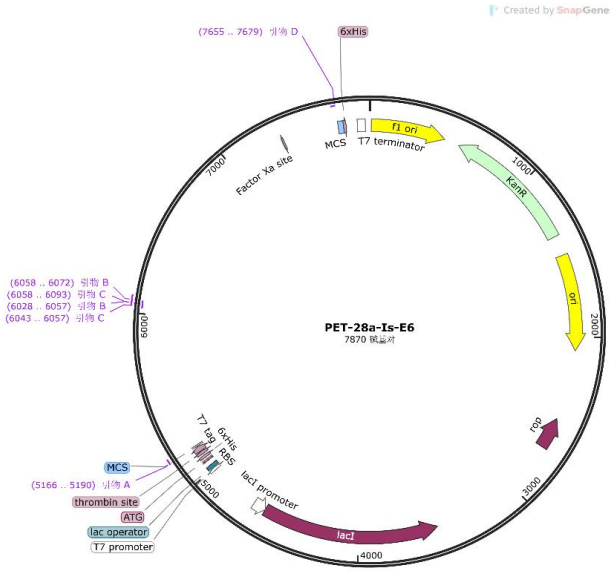


Figure 2 pET-28a-Is-E6 plasmid constructed in SnapGene software

## 2. Innovations and features

Our team searched the relevant literature on plastic upcycling through PubMed, Web of science and other websites, and found that there is no multi-enzyme system using three enzymes in plastic upcycling, and we have not found the recombination of these three enzymes, IsPETase, TPADO and DCD dehydrogenase.Therefore, in this project, the three enzymes required for PET degradation were fused using fusion protein genetic engineering technology, and a multi-enzyme system was constructed to directly decompose PET into protocatechuic acid. At the same time, we used a rigid peptide linkage to connect the three enzymes, which is beneficial to the separation of the functional domains of the three enzymes and maintains their independent functions.The construction of the multi-enzyme system of IsPETase, TPADO, and DCD dehydrogenase enables PET to be converted into protocatechuic acid (PCA) directly without intermediates.