

Development of Antibiotic-Independent Expression System, HCE-AMPFREE system, for the Industrial Production of Recombinant Enzymes and Proteins

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We have attempted to develop efficient and economical expression system for the industrial production of useful enzyme and protein. The HCE-AMPFREE system using the constitutively expressing HCE promoter and amino acid auxotrophy is not required to add IPTG for induction of protein expression and antibiotics for the selection of recombinants and the maintenance of plasmids. In this HCE-AMPFREE system, the stability of plasmids was maintained by an enzyme gene related to D-amino acid synthesis for the biosynthesis of peptidoglycan, which is an essential gene for growth, as a selective marker instead of a antibiotic-resistant gene. In fact, the plasmids harbored a D-amino acid synthetic enzyme gene without antibiotic-resistant gene was maintained constantly through 9 generation in the host strain, *Escherichia coli* WM335. As culture scale is increased, the use of antibiotics and inducers leads to the higher cost and the environmental pollution. Thus HCE-AMPFREE system may be the most suitable for the mass production of protein. The expression of recombinant human tumor necrosis factor- α (rhTNF- α), tyrosine phenol-lyase (TPL) and tryptophan indole-lyase (TNA) using the HCE-AMPFREE expression system was performed and its convenience and economical advantages were conformed.

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