

## Preparation of human beta-defensin 2 in a cell-free protein synthesis system

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Human beta-defensin 2 (hBD2), an antimicrobial peptide that was first discovered in human skin, is an essential peptide which protects human body from inflammatory stimulation. Because of the small size (4~5 kDa), it is easily digested by proteases and in vivo expression is problematic. In this study, we have attempted the expression of hBD2 in a cell-free protein synthesis system. First, we optimized the codon usage of hBD2 replacing the rare codons with more common ones. We also examined the effect of the presence of N-terminal fusion partners on the efficiency of expression. The time and labor for the construction of the different plasmid constructs were saved by directly using the PCR-amplified DNAs as the reaction templates. With all of the fusion partners examined (CAT-DB, ubiquitin and GST-ubiquitin), substantial increase of productivity was observed. In particular, the use of ubiquitin sequence gave the highest enhancement of the peptide expression and solubility. The high solubility of the fusion protein enabled efficient cleavage of the fusion partner by using appropriate proteases. Our results demonstrate the potential of cell-free protein synthesis in the expression of antimicrobial peptides.