

## Construction of a Shuttle Vector for the Overexpression of Recombinant Proteins in Insect Cell Lines

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In previous experiment, we reported that the expression rates of the secretory protein by baculovirus expression vector system containing the polyhedrin promoter were very poor, because the molecular chaperone was rate-limited expression at the time of the maximum expression of the heterologous protein. To overcome the disadvantage of polyhedrin promoter-driven vector, we constructed a shuttle vector constructed based on a insect cell line-*Escherichia coli* shuttle vector, pIZT-V5-His. Both the OpIE2 and OpIE1 promoters are from the baculovirus *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus (OpMNPV). The virus natural host is the Douglas fir tussock moth; however, the promoters allow protein expression in *Lymantria dispar* (LD652Y), *Spodoptera frugiperda* cells (Sf9), Sf21, *Trichoplusia ni* (High Five), *Drosophila* (Kc1, SL2) and mosquito cell lines. The OpIE2 promoter has been shown to be about 5-10-fold stronger than the OpIE1 promoter, and provides relatively high levels of constitutive expression, although not as high as might be expected from baculovirus late promoters such as polyhedrin or very late promoters such as p10. To enhance the active protein production, we inserted the cis-acting unfolded protein response element (UPRE) into the upstream of OpIE2 promoter and the *Bombyx mori* molecular chaperone, protein disulfide isomerase (bPDI) into the downstream of promoter for PDI-fusion protein expression and constructed the novel vector, pUIZ-PDI. The target gene expression using pUIZ-PDI vector can be executed in stably transfected cell lines with transcription factor HAC1 homologue, binds directly to the cis-acting UPRE.