Development of a modified plasmid vector system with a novel N-terminal tag that manifold enhances the expression of recombinant proteins in *Escherichia coli*

Industrial utilization of recombinant enzymes is handicapped by their low-expression in *Escherichia coli* host. We used a novel tag at the N-terminal of recombinant protein that when present manifold enhances the expression of cloned gene in *Escherichia coli* (but not restricted to) host. Expression of eukaryotic recombinant proteins as fusions to the novel N-terminal tag having 31-amino acids has been found to significantly increase the yield of moderately to poorly expressed proteins. The modules for N-terminal tagging with 31-amino acids peptide were constructed by the modification of the pET vector. DNA sequence coding 31-amino acids tag was cloned at N-terminal as NdeI-31-AA tag-thrombin protease site-T7 peptide-BamHI fragment. The presence of thrombin protease site enabled us to remove 31-AA tag from the final expressed protein. The presence of T7 tag in the final protein product with or without novel N-terminal 31 AA tag will enable the downstream processing of expressed protein and may be used for T7 affinity chromatographic purification and Western blotting. A gene can be cloned in the vector as BamHI-HindIII (but not restricted to) fragment. The expression system has increased twentyfold over the expression of the proteins from different organism. This modified vector system would be suitable for the expression and rapid purification of a broad range of proteins and peptides, and should be amenable to high-throughput applications.

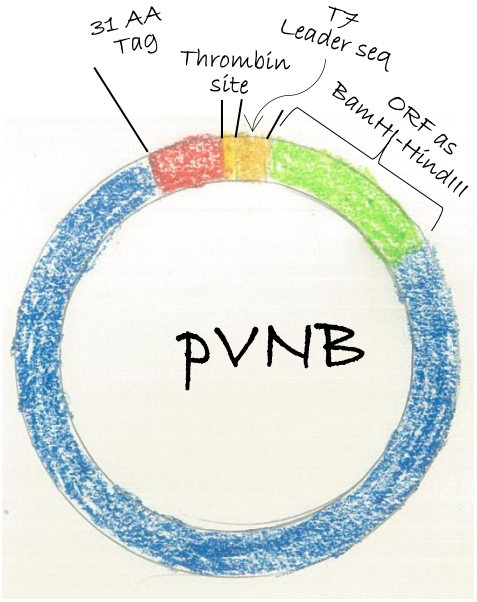
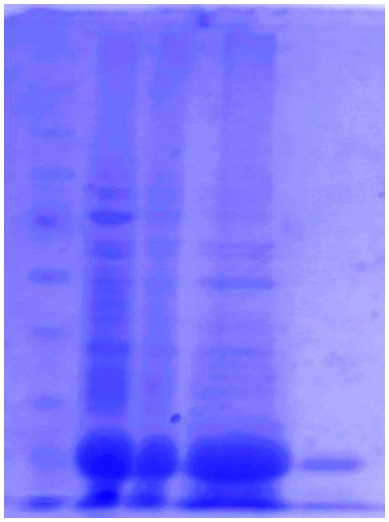


Figure 1: Plasmid map with novel 31-amino acids tag at N-terminal

Figure 2: SDS PAGE showing CREB protein expression with and without N-terminal tag



5 4 3 2 1

1 CREB protein expressed without tag
2-4 CREB protein with tag
5 Protein marker