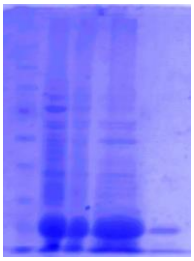

5 43 2 1

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

1: CREB protein expressed in E. coli without N-terminal tag
2-4: CREB protein expressed in presence of N-terminal tag.
5: Protein size marker

Name of the Institute: National Agri-food Biotechnology Institute, Mohali, Punjab

Stage of Development: Plasmid is ready to be used for cloning and expression of any recombinant protein

Patent/IPR status: Patent pending

Scientific Expert: Dr. Vikas Rishi

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

Technology description: We have developed a modified plasmid vector system for enhanced expression of any gene using E. coli as host. We used a novel tag at the N-terminal of recombinant protein that when present manifold enhances the expression of any cloned gene in *Escherichia coli* (but not restricted to) host. Expression of eukaryotic recombinant proteins as c-terminal fusion to the novel N-terminal tag that have 31-amino acids has been found to significantly increase the yield of moderate 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 one to remove 31-AA tag from the 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 chromatography 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 twenty-fold over the expression of the proteins from different organisms. This modified vector system will be suitable for the expression and rapid purification of a broad range of proteins and peptides, and should be amenable to high-throughput applications.

Background: Industrial utilization of the recombinant enzymes is hampered by their low-expression in *E. coli* host. Usually presence of a tag helps in stability of expressed protein or its presence helps in the affinity purification (e.g., His-tag, T7 tag). Other tags like GST or MBP (maltose binding protein) improves the solubility of expressed proteins. We have designed a novel tag which when placed at N-terminal of expressed protein enhances its expression. To best of our knowledge this is the first report where expression of an inserted gene in pET plasmid depends on the presence of N-terminal tag. Furthermore, this novel tag can be utilized for affinity purification as well as immune-detection of expressed protein. We have successfully used this plasmid to expressed numbers of transcription factors from different organisms. We are using above mentioned plasmid to express proteins that are hard to express and isolate in native/active conformation (e.g., membrane proteins, kinases and phosphatases)

Benefits and Utility: Our pET plasmids will be a boon for the expression of proteins at industrial scale. Simplicity of technology will make it pliable to express and purify proteins of different origin and amino acids composition.

Country context: The expression plasmid construct is ready for recombinant protein in any country

Scalability: Plasmid construct is ready and can be used to expresses any protein in E. coli

Business and Commercial Potential: With the advent in *ex vivo* production of enzymes both for medical and agriculture purposes our novel expression plasmids will generate interest from industry as well as scientific community

Potential Investors to this technical innovation: Businesses working in the field of industrial production of therapeutic as well as agriculture enzymes.