

# SUMO System, a Universal Platform for Protein Expression, Purification and Function.

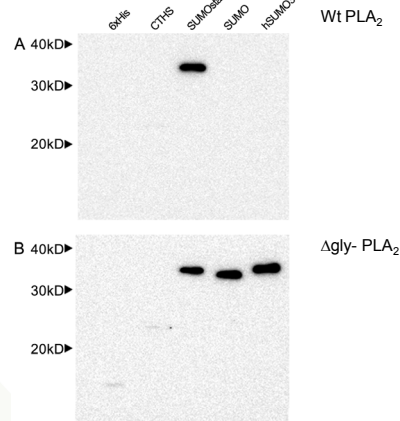
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LifeSensors  
from genomics to proteomics

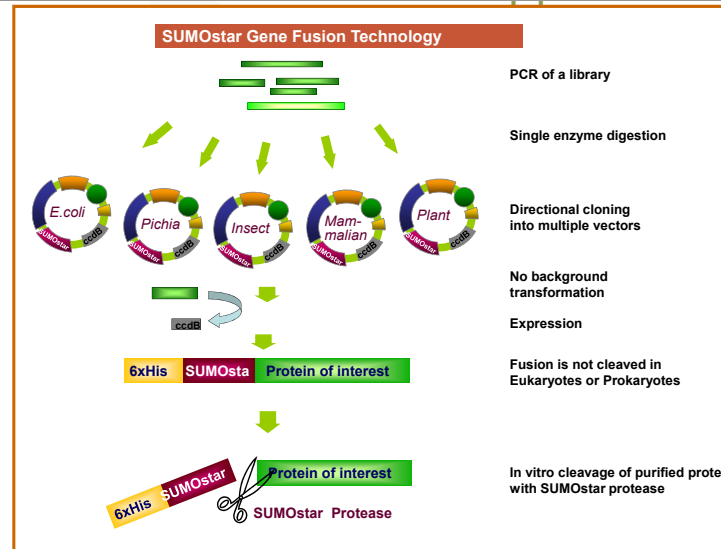
## Abstract:

The SUMO-fusion system for recombinant protein expression was developed seven years ago and continues to gain prominence in *E. coli*. Fusion of proteins to SUMO dramatically enhances the expression of many difficult-to-express proteins in *E. coli*. A novel SUMO system has been developed, "SUMO Star," that enhances protein expression and secretion of challenging proteins in eukaryotes by 5-80 fold compared to un-fused proteins. In eukaryotic cells, SUMOylation is a dynamic process involved in compartmentalization of tagged proteins. LifeSensors has exploited this natural property to screen SUMO tags that work universally in *E. coli*, yeast, (*S. cerevisiae* and *P. pastoris*), insect and mammalian cells. Different SUMOstar tags have been developed that are not cleaved in eukaryotic cells, facilitate expression and increase half-life and secretion of proteins. Respective SUMOstar proteases have been engineered that efficiently cleave SUMOstar-fusion proteins to generate native N-termini. SUMOstar has permitted expression of proteins that were previously not expressed or were poorly expressed. The development of SUMOstar tags that work in a variety of cells allows different hosts to be tested for expression. Examples of successfully expressed and secreted proteins include growth factors, chemokines, proteases, membrane proteins, and anti-bacterial peptides among others. In many cases, biological activity has been restored following fusion to SUMO tags. By improving the yield and biological activity, the SUMO system can reduce the cost of goods for therapeutic proteins that could not be developed otherwise. Development of tags that work in a variety of hosts will simplify expression of difficult to express proteins

## Western blot analyses of sPLA<sub>2</sub>-X expression



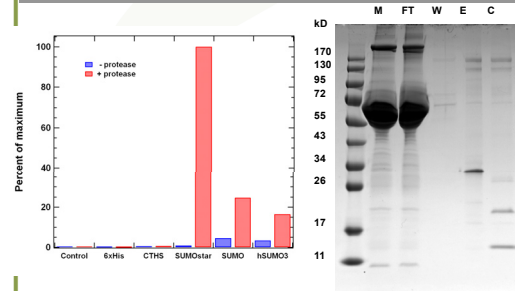
## Broad application of SUMOstar technology for eukaryotic and prokaryotic systems.



## Key features of SUMOstar-Fusion systems:

- Attachment of SUMOstar to the amino-terminus of candidate proteins enhances expression of proteins in eukaryotes and prokaryotes,
- Introduction of amino-terminal SUMOstar enhances the solubility of target proteins,
- Robust properties of SUMOstar protease allows generation of native amino-termini in vitro.

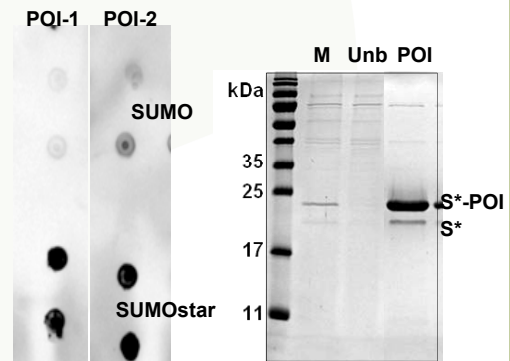
## Purification and determination of PLA<sub>2</sub> enzymatic activity



## Conclusion:

Fusion of PLA<sub>2</sub> to SUMOstar led to higher levels of expression in transiently transfected HEK293 cells. Improved expression could be due to better folding or reduced toxicity or both. The protein could be readily purified and specifically activated by removal of the SUMOstar tag using SUMOstar protease.

## Expression of secreted proteins of interest (POI) in *P. pastoris*



## Conclusion:

Fusion of SUMOstar led to enhanced protein expression and secretion in *Pichia pastoris*. The protein could be readily purified from conditioned medium and cleaved with SUMOstar Protease 1 (not shown).