Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	015
Title	Structural Genomics Methods Applied to Production of TEV Protease
Research Unit	Protein Purification
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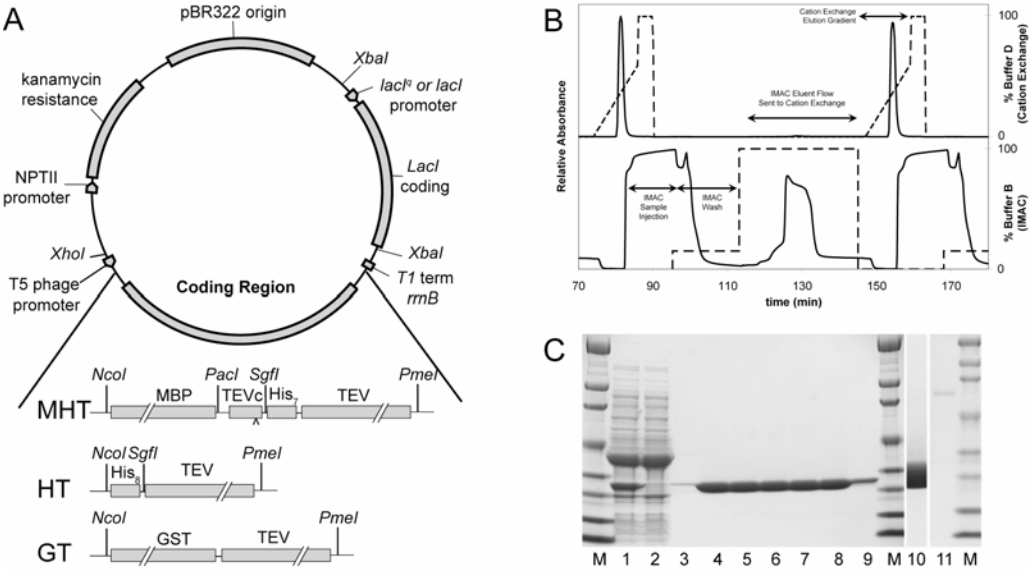

Summary

Tobacco etch virus NIa proteinase (TEV protease) is an important tool for the removal of fusion tags from recombinant proteins. Production of TEV protease in *E. coli* has been hampered by insolubility and addressed by many different strategies. However, the best previous results and newer approaches for protein expression have not been combined to test whether further improvements are possible. Here we use a quantitative, high throughput assay for TEV protease activity in cell lysates to evaluate the efficacy of combining several previous modifications with new expression hosts and induction methods [1]. Small-scale screening, purification and mass spectral analysis showed that TEV protease with a C-terminal poly-Arg tag was proteolysed in the cell to remove 4 of the 5 arginine residues. The truncated form was active and soluble but in contrast, the tagged version was also active but considerably less soluble. An engineered TEV protease lacking the C-terminal residues 238−242 was then used for further expression optimization. From this work, expression of TEV protease at high levels and with high solubility was obtained by using auto-induction medium at 37˚C. In combination with the expression work, an automated two-step purification protocol was developed that yielded His-tagged TEV protease with >99% purity, high catalytic activity and purified yields of ~400 mg/L of expression culture (~15 mg pure TEV protease per g of *E. coli* cell paste). Methods for producing glutathione S-transferase tagged TEV with similar yields (~12 mg pure protease fusion per g of *E. coli* cell paste) are also reported. These vectors are available by completion of standard biological materials transfer agreement, and will be deposited in the NIH PSI-Materials Repository.

Publication:

[1] Blommel, P.G. and Fox, B.G., A combined approach to improving large-scale production of tobacco etch virus protease. *Protein Expr Purif* 55(1):53-68.

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