# Center for Eukaryotic Structural Genomics

## Technology Dissemination Report

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| **CESG Tech Report No.** | 025 |
| **Title** | Protease Based Salvage Pathways |
| **Research Unit** | Protein Purification |
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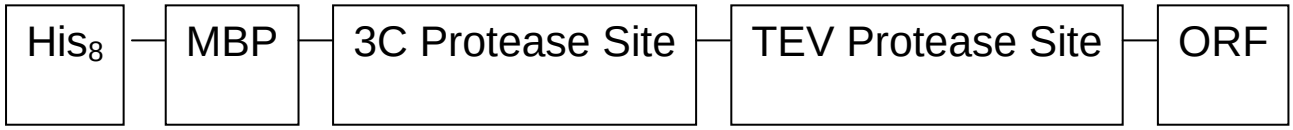
**A:**

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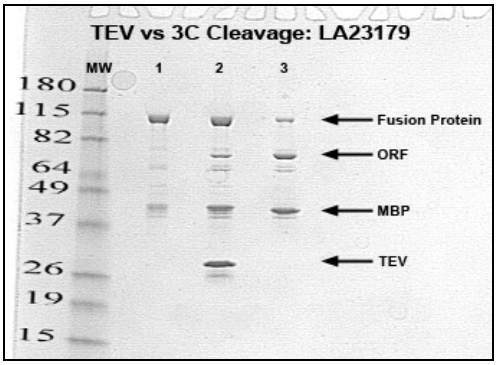
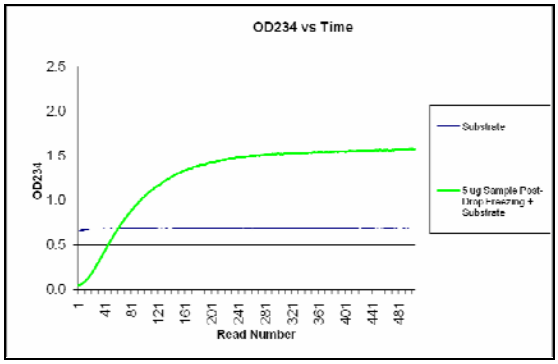



**C:**





## Summary

The majority of open reading frames (ORFs) over-expressed at CESG begin as fusion proteins containing added tags to aid in purification. During the purification process, these tags are removed by protease cleavage, principally by Tobacco Etch Virus (TEV) protease. Occasionally, TEV is unable to cleave the fusion protein. By designing vectors with more than one protease site, CESG's vectors provide a possible salvage pathway in the event that TEV is unable to cleave.

**Panel A** shows a schematic of a common vector designed and used by CESG known as pVP68K. It consists of a $His_8$ sequence fused to maltose binding protein (MBP) followed by a 3C protease cleavage site, a TEV protease cleavage site, and then the ORF.

**Panel B** is a gel showing an example of a fusion protein that exhibited poor TEV cleavage; however, this sample cleaved well with 3C protease. Lane 1 is uncut fusion protein, lane 2 is fusion protein treated with TEV protease, lane 3 is fusion protein treated with 3C protease (note: 3C protease is not visible on the gel due to the lesser amount used). One can see the slightly different sizes of MBP depending on whether TEV or 3C protease is used. Twice as much sample, by volume, is loaded in lanes 2 and 3.

**Panel C** shows an enzymatic assay performed on this sample. It is a pure, functionally active protein that would not have been able to be purified if not for the option of other protease cleavage sites that were engineered into the over expression construct. This enzymatic activity also demonstrates that the lack of TEV cleavage is not simply due to some generic mis-folding issue with this sample.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| **Acquiring the Technology** | Contact Brian Fox bgfox@biochem.wisc.edu. |
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