# Center for Eukaryotic Structural Genomics

## Technology Dissemination Report

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| **CESG Tech Report No.** | 026 |
| **Title** | Protease Based Salvage Pathways: Screening Prior to Large Volume Cleavage |
| **Research Unit** | Protein Purification |
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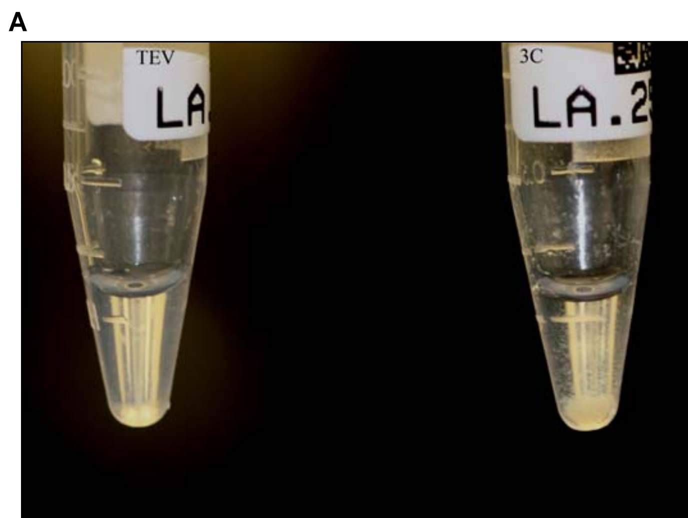
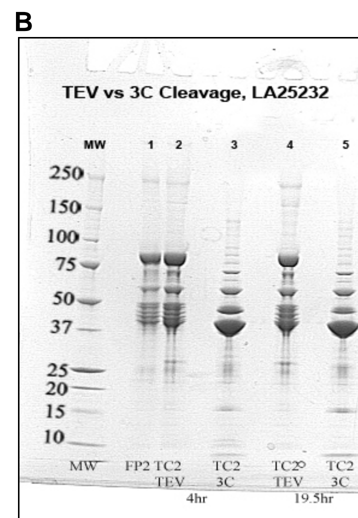
**A**




**B**





## Summary

The majority of open reading frames (ORFs) over-expressed at CESG begin as fusion proteins containing tags to aid in the purification process, usually a $His_8$ tag and a maltose binding protein (MBP) tag. These tags are then removed by treatment with either TEV or 3C protease. By incorporating a small-scale cleavage screening method into the purification process, sample characteristics such as percent cleavage with a particular protease and precipitation can be noted. Methods can then be adjusted prior to cleaving the larger batch of sample, increasing the likelihood of a successful purification.

In order for this screening method to mesh quickly and easily with the purification process, the assay is based simply on volume and uses an excess of protease compared to the more defined conditions used for cleavage of the larger batch of material. Two 100 µl aliquots of fusion protein from the 1$^{st}$ IMAC are removed. 5 µl of either TEV or 3C protease (both at 1 mg/ml) is added to the sample and incubated at room temperature. 20 µl aliquots are removed for gel analysis at 4 hr and overnight time points.

The sample in **Panel A** shows evidence of precipitation upon 3C protease cleavage. Knowing this in advance, one can take preemptive measures in order to keep the sample in solution, such as increasing the amount of sodium chloride prior to cleaving a larger amount of sample. **Panel B** is a gel of this cleavage reaction. Lane 1 is fusion protein; lane 2 is fusion treated with TEV protease, 4 hr time point; lane 3 is fusion treated with 3C protease, 4 hr time point; lane 4 is fusion treated with TEV protease, overnight time point; lane 5 is fusion treated with 3C protease, overnight time point. Including gel analysis gives a more complete picture, in this case showing that only 3C protease cleaves the fusion protein.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| **Acquiring the Technology** | Contact Brian Fox bgfox@biochem.wisc.edu. |
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