Small-Scale Screening of Eukaryotic Protein Expression, Solubility, and TEV Protease Cleavage

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Introduction
One of the main goals of the PSI Protein Structure Initiative (PSI-1 & 2) is to develop high-throughput technologies to assist protein structure determination. The University of Wisconsin-Madison Center for Eukaryotic Structural Genomics (CESG) has developed a rapid, small-scale, high-throughput screening method for identifying promising targets. Targets are selected on the basis of expression, solubility, and protein purification efforts.

Expression Plasmid Design
The cloned eukaryotic genes are expressed under control of either a T7 or T3 plasmid-based promoter that is isoelectric inducible. The expressed proteins are fused to an N-terminal (t-pi tagged) or C-terminal maltose-binding protein (MBP), which enhances solubility and expression. The expression medium contains both M9 (defined) and M63 (defined) media as well as the appropriate amino acids (in the case of MBP and target protein). The modular architecture of the expression plasmid allows for flexibility to future design modifications. For example, the MBP segment can be readily replaced with many other solubility enhancers such as glutathione-S-transferase (GST), His6 or NusA. The E. coli host strain, B834-pRARE2 (supplemented with seven rare amino acids) is used for both small-scale expression screening and for large-scale protein production (2L cell growths). Displays 1 shows an overview of the CESG protein production pipeline.

Expression Analysis WG1323

Total LSPP growths = 66
Total LSPP failures = 10
Unsuitable in LSPP = 6
Unsuitable (small-scale error) = 4

Small-scale predicted 49 (suitable) + 6 (unsuitable)

(True positive + true negative), therefore success rate = 49/56 = 87.1%
False negative rate = 3/40 = 7.5%
False positive rate = 6/10 = 60%

Conclusion
For PSI-2, small-scale screening at CESG has achieved an 86% success rate for predicting whether a protein fusion target is suitable for large-scale protein production. These results are based on the combined use of both small-scale expression screening methods. Current work at CESG is focused on improving the success rate of the small-scale expression screening methods. This includes identifying the optimal expression medium, host strain, and protein fusion target for each unique protein.

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