Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	027
Title	New Expression Vectors for Small-Scale Cell-Based Protein Production and Purification
Research Unit	Protein Production (Small-Scale Expression Testing)
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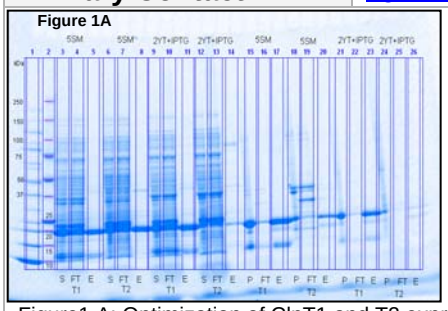

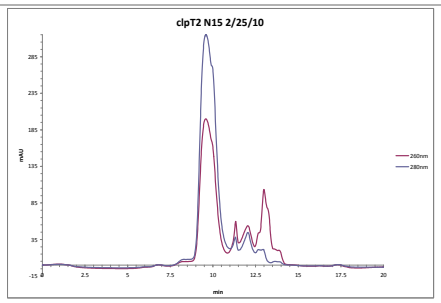

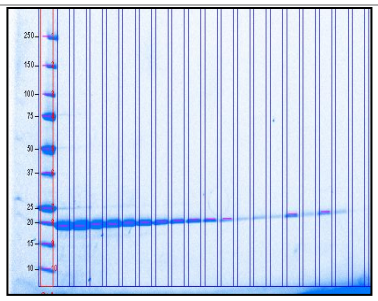

Figure1 A: Optimization of ClpT1 and T2 expression and Maxwell purification (Seleno-methionine labeling in B834-pRARE2. Figure 1 B) shows the HPLC gel filtration, and Figure 1 C) shows SDS-PAGE of gel filtration fractions.

Summary

Difficult eukaryotic targets such as membrane and disulfide bonded proteins are important classes of proteins that require new technologies to improve their production and purification. We present examples for new small-scale cell-based strategies that have been developed for optimizing and improving the overexpression of these types of proteins. *E. coli* expression host screens have been used to improve the yield of outside requests and membrane proteins, and new methods have been designed that allow the successful growth of *E. coli* Origami strains (trxB- and gor-) used to allow the formation of disulfide bonds in the cytoplasm. We have developed fast and economical methods to speed-up and improve the delivery of isotopically labeled proteins (^{15}N or seleno-methionine enriched) directly from meso- and small-scale cultures to support structural and biophysical analysis. Sufficient isotopically-labeled protein can be produced using multiple wells of 0.5 mL cell cultures (in 96-well growth blocks), which can then utilize the Maxwell 16 bench top robot[1, 2], for purification. Protein purification has been improved to increase yields from 300μg to 500μg of protein (per cartridge) in ~45 minutes, by adding more His-tag binding resin [2]. Results for ClpT1 and ClpT2, various membrane proteins, are shown above. In addition, examples of new vectors include; periplamic targeted OmpA leader vector (pVP89A), N-terminal thioredoxin fusion, and yeast expression vectors based on ADH2 promoter, Gal-promoter, and a hybrid UAS-GDP promoter (based on Rochester University Saccharomyces cerevisiae vectors [3]). The periplasmic targeting vectors were designed to improve the production of membrane proteins and those with predicted disulfide bonds. The ADH2 and UAS-GDP vectors are designed to be auto-inducible (by glucose depletion), and employ modular Flexi-cloning strategy [4]. About 0.4mg of eGFP per ml of cell culture (n=4 S.D.=0.05) for the ADH2-plasmids.

Publication(s):

[1] Frederick, R.O., Bergeman, L., Blommel, P.G., Bailey, L.J., Song, J., Meske, L., Bingman, C.A., Riters, M.. Dillon, N., Kunert, J., Yoon, J., Lim, A., Cassidy, M., Bunge, J., Aceti, D.J., Primm, J.P., Markley, J.L., Phillips, G.N., Jr., and Fox, B.G. (2007) Small-scale, semi-automated purification of eukaryotic proteins for structure determination. *JSFG* 8(4):153-66.

[2] Jensen DR, Woytovich C, Li M, Duvnjak P, Cassidy MS, Frederick RO, Bergeman LF, Peterson FC, Volkman BF., (2010) Rapid, robotic, small-scale protein production for NMR screening and structure determination. Protein Sci. 2010 Mar;19(3):570-8.

[3] Malkowski MG, Quartley E, Friedman AE, Babulski J, Kon Y, Wolfley J, Said M, Luft JR, Phizicky EM, DeTitta GT, Grayhack EJ.(2007) Blocking S-adenosylmethionine synthesis in yeast allows selenomethionine incorporation and multiwavelength anomalous dispersion phasing. Proc Natl Acad Sci U S A. 2007 Apr 17;104(16):6678-83. Epub 2007 Apr 10.

[4] Blommel, P.G., Martin, P.A., Wrobel, R.L., Steffen, E., and Fox, B.G. (2006) High efficiency single step production of expression plasmids from cDNA clones using the Flexi Vector cloning system. *Protein Expr Purif* 47(2):562-70.

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