Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	032
Title	Optimization of Membrane Protein Production in *E. coli* and Robotic Purification
Research Unit	Protein Production (Small-Scale Expression Testing)
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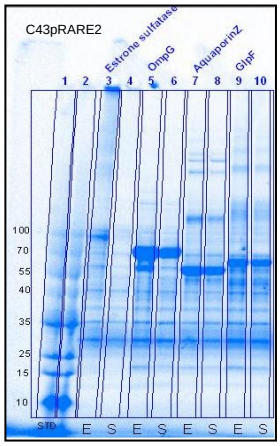

Figure1a. Membrane protein *E. coli* cell line trial C43pRARE2

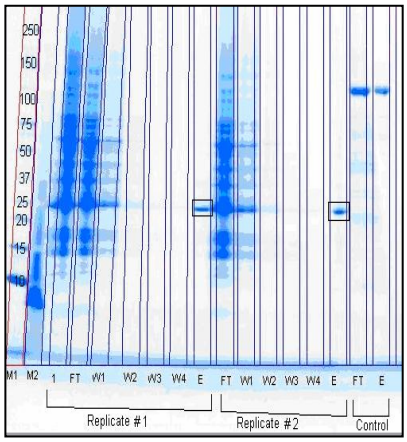

Figure 1b. Small-scale Maxwell purification of Bacteriorhodopsin.

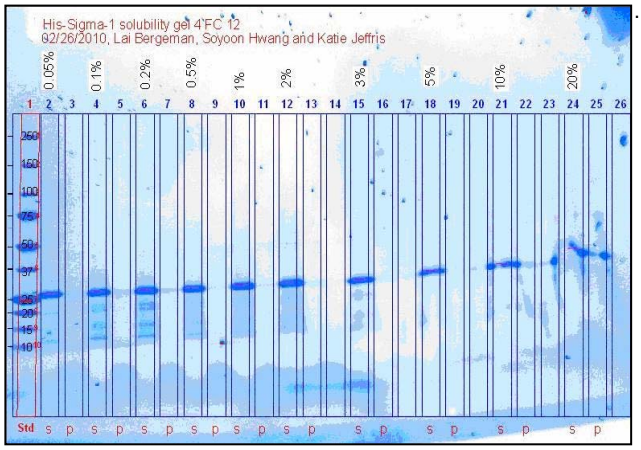

Figure 1c. Sigma 1 receptor detergent solubilization screen with FC-12

Summary

Several membrane proteins have been successfully expressed in *E. coli* and purified using Maxwell robotic protein purification systems. The expression of three homologs of Sigma-1 (human, rat, and Guinea pig), OmpG, Estrone sulfatase, AquaporinZ and β2-adrenergic receptor have been optimized by screening for expression in six different *E. coli* strains (Figure 1A) , and purified by an automated immobilized metal affinity chromatography (IMAC) system (Maxwell 16) [2], and a final clean-up step using HPLC gel filtration. In the case of sigma-1 receptor, the protein was expressed at the small-scale using single colony transformants grown overnight in 0.4 mL of auto-induction medium [1]. The expressed protein was found in the membrane fraction of lysed and sonicated *E. coli* cells, and was resolubilized in detergent using a high-throughput screen. Finally, sigma-1 was purified using 0.05% fos-choline 12 detergent (FC-12) and IMAC Maxwell system, and polished using a HPLC gel filtration. The Maxwell purification system has also been used for rapid purification of Cell-Free produced bacteriorhodopsin (bR). By combining small-scale *E. coli* expression strain optimization and robotic automated protein purification using the Maxwell system, we employ relatively simple automated methods for cost-effective isolation of isotopically labeled recombinant membrane proteins at levels sufficient for either functional characterization or structural studies.

Publication(s):

[1] Blommel, P.G., Becker, K.J., Duvnjak, P., and Fox, B.G. (2007) Enhanced bacterial protein expression during auto-induction obtained by alteration of lac repressor dosage and medium composition. *Biotechnol Prog* 23(3):585-98.

[2] 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.-Y., 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.

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