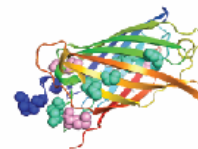
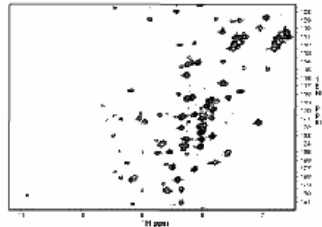
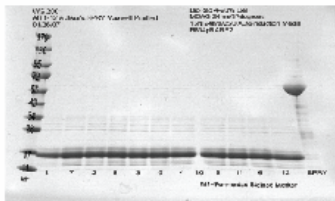


# Center for Eukaryotic Structural Genomics

## Technology Dissemination Report

<b>CESG Tech Report No.</b>	008
<b>Title</b>	Small-Scale Semi-Automated Purification of Eukaryotic Proteins for Structure Determination
<b>Research Unit</b>	Small-Scale Expression Testing
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A. Maxwell 16 purification of  $^{15}\text{N}$  labeled Human embryonic stem cell protein, Tcl-1    B.  $^{15}\text{N}$  HSQC NMR spectrum of HumanTcl-1 embryonic stem protein at 750 MHz (cryoprobe)    C. X-ray structure at 1.7Å resolution of an engineered synthetic GFP



### Summary

A simple approach that allows cost-effective automated purification of recombinant proteins in levels sufficient for functional characterization or structural studies is described. Studies with four human stem cell proteins, an engineered version of green fluorescent protein, and other proteins are included. The method combines an expression vector (pVP62K) that provides *in vivo* cleavage of an initial fusion protein, a factorial designed auto-induction medium that improves the performance of small-scale expression, and rapid, automated metal affinity purification of His8-tagged proteins. For initial small-scale expression screening, single colony transformants were grown overnight in 0.4 mL of auto-induction medium, expressed proteins were purified using the Promega Maxwell 16, and purification results were analyzed by Caliper LC90 capillary electrophoresis. The yield of purified [ $^{15}\text{N}$ ]-His8-Tcl-1 was 7.5  $\mu\text{g}$  per mL of culture medium, of purified [ $^{15}\text{N}$ ]-His8-GFP was 68  $\mu\text{g}$  per mL, and of purified selenomethionine-labeled AIA-GFP (His8 removed by treatment with TEV protease) was 345  $\mu\text{g}$  per mL. The yield information obtained from a successful automated purification from 0.4 mL was used to inform the decision to scale-up for a second meso-scale (10-50 mL) cell growth and automated purification.  $^1\text{H}$ - $^{15}\text{N}$  NMR HSQC spectra of His8-Tcl-1 and of His8-GFP prepared from 50 mL cultures showed excellent chemical shift dispersion, consistent with well-folded states in solution suitable for structure determination. Moreover, AIA-GFP obtained by proteolytic removal of the His8 tag was subjected to crystallization screening, and yielded crystals under several conditions. Single crystals were subsequently produced and optimized by the hanging drop method. The structure was solved by molecular replacement at a resolution of 1.7 Å. This approach provides an efficient way to carry out several key target screening steps that are essential for successful operation of proteomics pipelines with eukaryotic proteins: examination of total expression, determination of proteolysis of fusion tags, quantification of the yield of purified protein, and suitability for structure determination.

### Publication(s):

- [1] 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.
- [2] 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.
- [3] Frederick, R.O., Bergeman, L., Blommel, P.G., Bailey, L.J., Song, J., Meske, L., Bingman, C.A., Ritters, 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.

<b>Acquiring the Technology</b>	Maxwell: <a href="http://www.promega.com/default.asp">http://www.promega.com/default.asp</a> Caliper LC90: <a href="http://www.caliperls.com/">http://www.caliperls.com/</a>
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