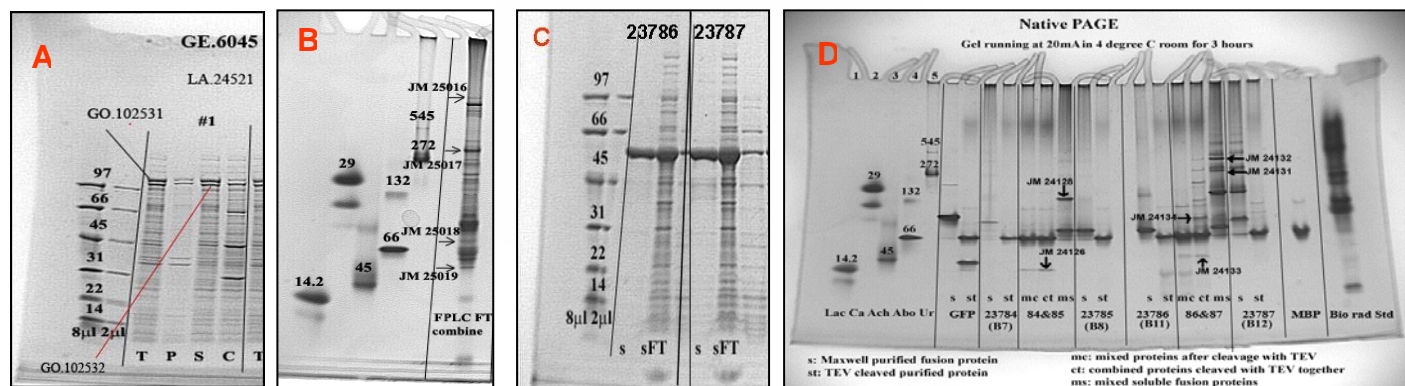


# Center for Eukaryotic Structural Genomics

## Technology Dissemination Report

<b>CESG Tech Report No.</b>	029
<b>Title</b>	Identification of Protein-Protein Complex Formation and Co-Expression in Cell-Based Platform
<b>Research Unit</b>	Protein Production
<b>Authors</b>	Gromek, K.A., Li H., Frederick, R.O., Bergeman L.F., Nichols, K.W., Beebe, E.T., Hwang, S., Aceti, D.J., Vojtik, F.C., Primm, J.G., Markley, J.L., Phillips, G.N., and Fox, B.G.
<b>Primary Contact</b>	<a href="mailto:bgfox@biochem.wisc.edu">bgfox@biochem.wisc.edu</a>

**A.** Co-expression of MBP fusion 1JEQ in *E.coli*. **B.** Native (non-denaturing) PAGE of co-expressed 1jeq (subunits A and B). The band labeled JM.25017 was identified as complex. **C.** Automated Maxwell 16 purification of 2O0J (23786–subunit A and 23787 – subunit B). s-eluted MBP fusion protein, sFT- flow-through. **D.** Native PAGE of 2PQA (LA.24125 and LA.24127) and 2O0J (LA.24129 and LA.24130). Bands labeled as JM.24126 and JM.24133 were identified as complex 2PQA and 2O0j, respectively.



### Summary

Protein complexes play many essential roles in cellular functional pathways. As a pilot project we have tested and developed cell based techniques for the formation of these complexes using proteins obtained through small-scale expression and purification. Proteins known to form complexes were expressed in *Escherichia coli* B834-pRARE2 cells with auto-induction system for efficient protein labeling with seleno-methionine. All expression plasmids included MBP (Maltose Binding Protein) partner as solubility enhancer [1]. We successfully tested co-expression of two partner proteins cloned into vectors with different antibiotic resistance (pVP68K and pVP80) in one host cell line in presence of 3 different antibiotics to assure stability of plasmids [2].

The research procedures included small-scale purification of expressed protein on Maxwell 16 automated purification system (Promega, USA), amylose plate purification and FPLC-IMAC (1 mL resin volume) purification for amount of wet cell paste exceeding 1g [3]. The partner proteins were either purified separately and then mixed to allow the complex formation or purified together starting with cell breakage by sonication. The purification on Maxwell 16 automated purification system included several modifications for the amount of resin used, the volume of elution buffer and number of purification cycles [4]. The fusion partner MBP was removed by cleavage with TEV or 3C protease (depending on results of preliminary screens) and followed by batch purification on Ni-NTA resin (Qiagen, USA). Protein yields were estimated by densitometry (BioRad Stain Free Imager) and BCA assay. Purified proteins were concentrated on Amicon Ultra 0.5mL Centrifugal Filters (Millipore, USA) with buffer exchange to provide the required pH and salt concentration for given complex. To detect complex formation we applied separation by means of discontinuous native (non-denaturing conditions) PAGE [5] followed by visualization by Coomassie Blue or silver staining. For each separation ~ 10-20 µg of protein was used to improve detection. The bands that were identified as potential complexes (as compared to several controls and standard) were submitted for final confirmation by mass spectrometry [6].

[1] Fox, J.D. and Waugh, D.S. (2003) Maltose-binding protein as a solubility enhancer. *Methods Mol Biol.* 205:99-117.

[2] Yang, W., Zhang, L., Lu, Z., Tao, W. and Zhai, Z. (2001) A new method for protein coexpression in *Escherichia coli* using two incompatible plasmids. *Protein Expr Purif* 22:472-8.

[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.G., 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.

[4] 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.* 19 (3):570-8.

[5] Gallagher, S.R. (2001) One-dimensional electrophoresis using nondenaturing conditions. *Curr Protoc Protein Sci*, Chapter 10: p. Unit 10 3.

[6] Mackun, K. and Downard, K.M. (2003) Strategy for identifying protein-protein interactions of gel-separated proteins and complexes by mass spectrometry. *Anal Biochem* 318(1): p. 60-70.

<b>Acquiring the Technology</b>	Contact John Primm <a href="mailto:primm@nmrfam.wisc.edu">primm@nmrfam.wisc.edu</a> .
---------------------------------	---

<b>Other Acknowledgements</b>	
-------------------------------	--

Center for Eukaryotic Structural Genomics (CESG), University of Wisconsin-Madison Biochemistry Department, 433 Babcock Drive, Madison, WI 53706-1549; phone: 608.263.2183; fax: 608.890.1942; email: [cesginfo@biochem.wisc.edu](mailto:cesginfo@biochem.wisc.edu); website: <http://www.uwstructuralgenomics.org>. This research funded by NIH / NIGMS Protein Structure Initiative grant U54 GM074901