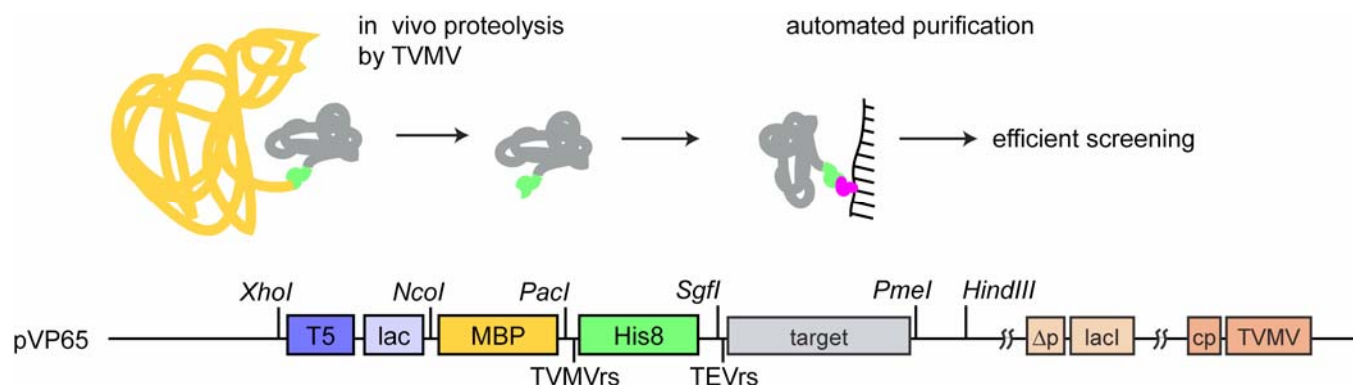


Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	017
Title	Customized Expression Vector Platform
Research Unit	Cloning
Authors	Blommel, P.G., Wrobel, R.L., and Fox, B.G.
Primary Contact	bgfox@biochem.wisc.edu



Summary

A series of expression vectors used for protein production and different research activities are available [1-6]. These vectors use Gateway, FlexiVector, or restriction digestion cloning methods. The vector backbones have been modularized for simple exchange of promoters, affinity tags and solubility tags. Over seventy versions are currently available and support expression in bacteria, wheat germ lysates, and insect lysates. The vector pVP65 is one example, and provides in vivo cleavage of MBP-target fusion proteins to liberate a His8-target that can be used with automated IMAC purification procedures. Sequence verified genes can be transferred between each of these different expression platforms by simple, highly efficient methods. These vectors are available by completion of standard biological materials transfer agreement, and will be deposited in the NIH PSI-Materials Repository.

Publication(s):

- [1] Blommel, P.G., Martin, P.A., Wrobel, R.L., Steffen, E., 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, 562-570.
- [2] Thao, S., Zhao, Q., Kimball, T., Steffen, E., Blommel, P. G., Ritters, M., Newman, C. S., Fox, B. G., and Wrobel, R.L. (2004) Results from high-throughput DNA cloning of *Arabidopsis thaliana* target genes using site-specific recombination. *J Struct Funct Genomics* 5, 267-276.
- [3] Blommel, P.G., Fox, B.G. (2005) Fluorescence anisotropy assay for proteolysis of specifically labeled fusion proteins. *Anal Biochem* 336, 75-86.
- [4] Blommel, P.G., Fox, B.G. (2007) A combined approach to improving large-scale production of tobacco etch virus protease. *Protein Expr Purif* 55, 53-68.
- [5] Blommel, P.G., Martin, P. A., Seder, K. D., Wrobel, R.L., and Fox, B.G. (2007) Flexi Vector Cloning. *Methods in Molecular Biology*, J.E. White, Editor. *Humana Press*, Totowa, NJ, in press.
- [6] 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.-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. *J Struct Funct Genomics*, in press.

Acquiring the Technology	Contact John Primm primm@nmrfam.wisc.edu .
Other Acknowledgements	Also supported by Promega Corporation, Madison WI (B. G. Fox, PI).
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 ; website: http://www.uwstructuralgenomics.org . This research funded by NIH / NIGMS Protein Structure Initiative grants U54 GM074901 and P50 GM064598.	