Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	007
Title	Wheat Germ Cell-Free Protein Production and Stable-Isotope Labeling Platform for NMR-Based Structural Proteomics
Research Unit	Cell-Free
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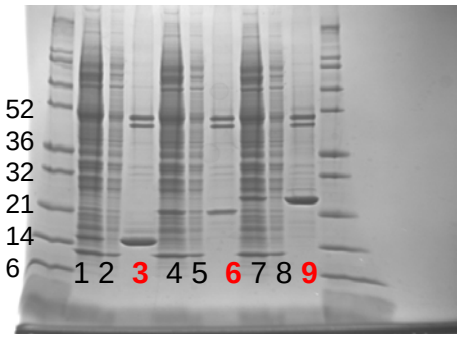
Protein Expression and Purification on DT-II



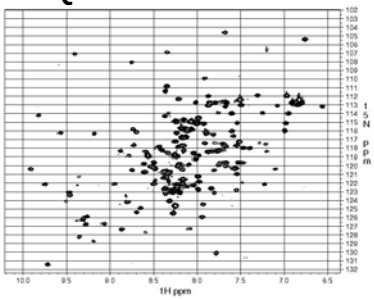
ORF80250: **lane 1 – synthesis**
lane 2 – flow through
lane 3 – purified soluble

ORF80230: **lane 4 – synthesis**
lane 5 – flow through
lane 6 – purified soluble

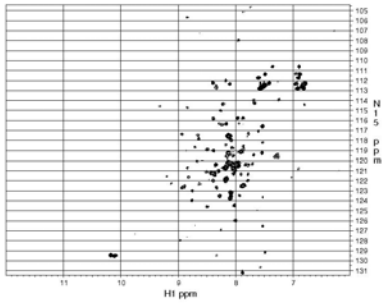
ORF80229: **lane 7 – synthesis**
lane 8 – flow through
lane 9 – purified soluble

NMR Analysis

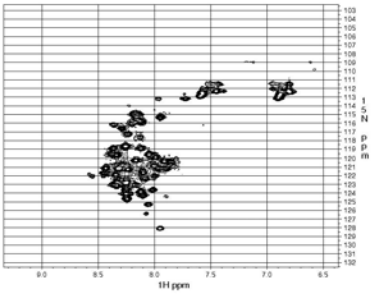
Orf 80229; 149 aa, w/His-tag
6 Pro; Peak No. 136/142; HSQC+



Orf 80230; 175 aa, w/His-tag
4 Pro; Peak No. 126/170; HSQC+/-



Orf 80250
HSQC-



Summary

In collaboration with Professor Yaeta Endo (Ehime University, Matsuyama, Japan) and CellFree Sciences (Yokohama, Japan), CESG has developed a platform that utilizes wheat germ cell-free technology to produce protein samples for NMR structure determinations [1-4]. In the first stage, cloned DNA molecules coding for proteins of interest are transcribed and translated on a small scale (25 microL) to determine levels of protein expression and solubility. The amount of protein produced (typically 2-10 micrograms) is sufficient to be visualized by polyacrylamide gel electrophoresis. The fraction of soluble protein is estimated by comparing gel scans of total protein and soluble protein. Targets that pass this first screen by exhibiting high protein production and solubility move to the second stage. In the second stage, the DNA is transcribed on a larger scale, and labeled proteins are produced by incorporation of [^{15}N]-labeled amino acids in a 4 mL translation reaction that typically produces 1-3 mg of protein. The [^{15}N]-labeled proteins are screened by ^{1}H-^{15}N HSQC NMR spectroscopy to determine whether the protein is a good candidate for solution structure determination. Targets that pass this second screen are then translated in a medium containing amino acids doubly labeled with ^{15}N and ^{13}C. These steps can be automated so that the labor costs involved are minimal. CESG uses an automated platform for wheat germ cell-free production of labeled proteins. Our current robotic systems (CellFree Sciences Co., Ltd., Japan) include the GeneDecoder1000™ (2-5 µg per well in 96-well format), the Protemist10^{TM} and the Protemist100^{TM} (1-2 mg per sample in eight samples format), and Protemist DT-II^{TM} (0.1-0.3 mg purified protein per well in 6-well format). The GeneDecoder1000^{TM} is used to produce samples to screen for expression, solubility, and, where appropriate, tag cleavage. The Protemist10^{TM} and the Protemist100^{TM}, coupled with ACTA PRIME purification systems, are used for expression and purification of sufficient quantities of labeled protein for NMR structural studies. Our cumulative experience with cell-free expression includes over 1000 different structural genomics targets from human, mouse, *Plasmodium*, and *Arabidopsis*. To date, CESG has deposited into the PDB 23

NMR structures of eukaryotic proteins produced by wheat germ cell-free methodology. The average yield of labeled purified proteins has been ~1.2 mg per ml of wheat germ lysate (OD260=200). We also report that the Protemist DT-II™ provides a cost-effective and rapid method for screening multiple constructs engineered to improve solubility or the folding state. Furthermore, CESG has begun structural investigations of membrane proteins using the automated translation and purification capabilities of the Protemist DT-II™. Several detergents have been identified to be compatible with wheat germ cell-free translation, and current efforts are aimed at developing efficient ways of protein concentration, detergent exchange, and preparation of structural samples (unpublished results).

Publication(s):

[1] Vinarov, D.A., Loushin Newman, C.L., Tyler, E.M., Markley, J.L. (2006) Protein Production using the Wheat Germ Cell-Free Expression System. *Current Protocols in Protein Science*, Wiley Interscience.

[2] Vinarov, D.A., Lytle, B.L., Peterson, F.C., Tyler, E.M., Volkman, B.F., Markley, J.L. (2004) Cell-free protein production and labeling protocol for NMR-based structural proteomics. *Nat Methods* 1(2):149-53.

[3] Vinarov, D.A., Markley, J.L. (2005) High-throughput automated platform for nuclear magnetic resonance-based structural proteomics. *Expert Rev Proteomics* 2(1):49-55.

[4] Vinarov, D.A., Newman, C.L., Markley, J.L. (2006) Wheat germ cell-free platform for eukaryotic protein production. *Febs J* 273(18):4160-9.

Acquiring the Technology	Wheat germ cell-free synthesis products and kits; CellFree Sciences, Inc. http://www.cfsciences.com/eg/index.html .
Other Acknowledgements	

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