

Madison,  
Wisconsin  
USA



### 2006 CESH / NMRFAM Wheat Germ Cell-Free Protein Production Workshop

Biochemistry  
UNIVERSITY OF WISCONSIN MADISON

W A R F  
Wisconsin Alumni Research Foundation



PSI National Institute for General Medical Sciences grant number 1 U54 GM074901-01



Professor  
Endo's  
Seminar

The first CESH/NMRFAM Wheat Germ Cell-Free Protein Synthesis Workshop was held at the Center for Eukaryotic Structural Genomics in Madison, WI from July 31, 2006 to August 4, 2006. The workshop gave participants a unique opportunity to obtain hands-on experience with cell-free protein production utilizing the wheat germ cell-free technology. The workshop covered various aspects of small-scale expression screening ( $\mu$ g quantities of attendee-nominated target protein) and large-scale production (mg quantities of target proteins selected from attendee efforts) of labeled proteins for NMR spectroscopy ( $^{15}$ N) or crystallization screening (Se-Met). Both manual and automated (supported by robotic systems installed at the CESH) procedures were demonstrated. The workshop schedule also included seminars and company presentations on topics related to cell-free protein production and proteomics technologies in general.

The workshop was sponsored by [CellFree Sciences Co., Ltd.](#), [Cambridge Isotope Laboratories](#), [Promega](#), [Wisconsin Alumni Research Foundation](#), and the [Nuclear Magnetic Resonance Facility at Madison \(NMRFAM\)](#).



Robotic System Currently  
in Operation at CESH

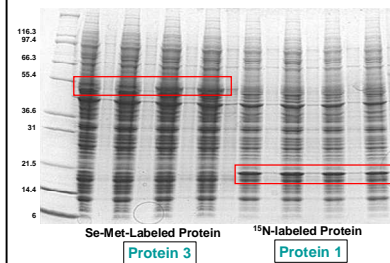
#### Workshop Small-Scale Expression Data Obtained on GeneDecoder1000

MW	E	S	MW	E	S	MW	E	S
6.7	H	M	17.9	M	M	39.9	H	L
8.9	H	M	18.0	M	H	39.8	H	0
8.6	M	H	18.0	M	H	39.2	H	H
9.0	H	M	18.2	H	0	35.5	M	H
11.0	H	H	18.3	M	H	37.0	0	0
11.2	M	H	18.3	M	0	40.0	0	0
11.3	M	H	18.5	M	M	41.4	0	M
12.4	H	M	18.6	H	0	42.0	H	M
12.5	M	H	18.5	M	H	45.6	H	0
13.1	M	0	18.9	M	H	46.0	0	0
13.5	0	0	18.9	0	H	47.6	M	H
13.7	M	M	19.0	H	H	48.7	H	H
14.0	M	M	19.0	M	H	51.4	H	M
14.1	M	M	19.3	H	0	55.0	H	0
14.5	0	M	19.2	M	H	57.0	0	0
14.6	M	M	19.3	0	0	59.6	0	0
16.0	H	M	19.4	H	L	63.0	H	L
16.6	M	M	19.6	0	0	65.6	H	0
16.8	H	M	19.7	H	H	68.0	M	H
16.8	H	0	20.0	H	H	71.0	M	0
16.8	0	0	20.0	0	H	71.5	0	0
17.0	M	M	20.0	H	H	78.0	M	0
17.2	0	0	20.0	0	0	83.1	H	H
17.5	H	H	21.3	M	H	86.5	0	0
17.7	H	H	21.4	0	0	105.0	H	M
17.8	H	H	27.5	M	M	117.0	H	0

#### Summary of Workshop Small-Scale Expression Data Obtained on GeneDecoder1000

Expression	Over	all	<25	>25	High (H)	Solubility	Over	<25	>25
High (H)	0.5 - 1.0	33	20	13	High (H)	>75%	28	22	6
Medium (M)	0.1 - 0.5	26	21	5	Medium (M)	50% - 75%	18	13	5
Low (L)	<0.1	0	0	0	Low (L)	<50%	3	1	2
0	no expression	19	9	10	0	insoluble	10	5	5
Total		78	50	28			59	41	18

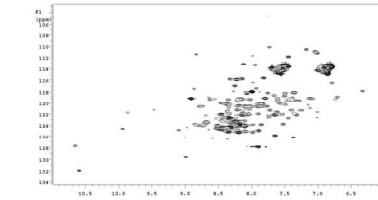
#### Large-Scale Protein Purification on Protomist10 and Protomist100: Total Synthesis



Se-Met-Labeled Protein  $^{15}$ N-Labeled Protein

#### HSQC Analysis of Workshop Protein "1"

MW (with His tag) 18934 Da; Protein was expressed on  $^{15}$ N-labeled AA on large-scale on the Protomist10

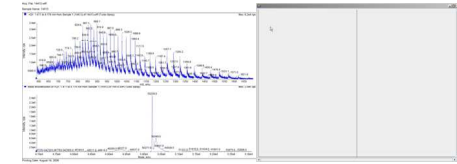


Protein Concentration: 0.75 mM  
Buffer conditions: 10 mM NaPi, 50 mM NaCl, 1 mM DTT, 0.02% Na<sub>3</sub>N, pH 6.0

#### Mass Spec Analysis of Workshop Protein "3"

MW (with His tag) 50374 Da; Protein was expressed on Se-Met-labeled AA on large scale on the Protomist100

ESI/MS analysis  
100  $\mu$ g of ~10 mg/ml protein used

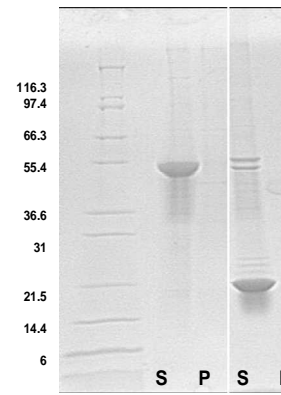


Se-Met labeling is essentially 100%.  
Shown below, peak distribution in the case of 100% Se-Met labeling

#### Workshop Seminars

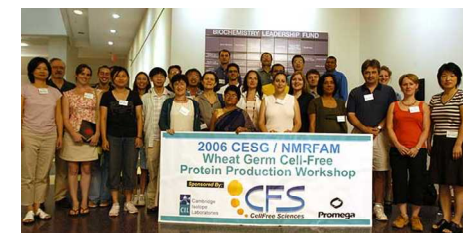
- [Professor Yaeta Endo, Ehime University](#)  
HT- Materialization of Genetic Information Using the Wheat Germ Cell-Free System  
[CellFree Sciences Co., Ltd.](#)  
ENDEXT® Technology: Wheat Germ Cell-Free Protein Synthesis System
- [Professor George Phillips, CESH](#)  
Crystallography at CESH
- [Professor John Markley, CESH](#)  
NMR at CESH: The National Magnetic Resonance Facility at Madison (NMRFAM)
- [Dr. Michael R. Slater, Promega Corporation](#)  
Flexi® Vector System: Promega's flexible system for cloning and expressing protein coding regions  
[Caliper Life Sciences](#)  
LabChip 90 Overview and Applications

#### Large-Scale Protein Purification on ACTA Prime using HisTrap HP 1 ml Columns



Se-Met Labeled Protein  $^{15}$ N Labeled Protein

#### Workshop Scientists and Participants



For information about future workshops, visit: [www.uwstructuralgenomics.org](http://www.uwstructuralgenomics.org)

