

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 (μ 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 \(NMRFAM\)](#).

Workshop Schedule*		Wednesday, August 2, 2006	
Sunday, July 30, 2006		07:00-08:00 Continental breakfast and discussion plan for the day	
17:00-18:00	Arrival and registration	08:00-10:00	Large-scale expression and discussion plan for the day
18:00-18:30	Introduction to the workshop	10:00-10:30	Large-scale DNA prep
18:30-20:00	Workshop registration	10:30-11:00	Large-scale DNA prep
Monday, July 31, 2006		11:00-11:30	Large-scale DNA prep
08:00-09:00	Continental breakfast and discussion plan for the day	11:30-12:00	Large-scale DNA prep
09:00-11:00	Start small-scale transcription on bench	12:00-12:30	Large-scale DNA prep
11:00-11:30	Discussion: what was done today, plan for Tuesday	12:30-13:00	Large-scale DNA prep
11:30-11:45	Continental breakfast	13:00-13:30	Large-scale DNA prep
11:45-12:00	Start small-scale transcription manually	13:30-14:00	Large-scale DNA prep
12:00-12:30	Discussion: what was done today, plan for Tuesday	14:00-14:30	Large-scale DNA prep
12:30-13:00	Continental breakfast	14:30-15:00	Large-scale DNA prep
13:00-13:30	Start small-scale transcription manually	15:00-15:30	Large-scale DNA prep
13:30-14:00	Discussion: what was done today, plan for Tuesday	15:30-16:00	Large-scale DNA prep
14:00-14:30	Continental breakfast	16:00-16:30	Large-scale DNA prep
14:30-15:00	Start small-scale transcription manually	16:30-17:00	Large-scale DNA prep
15:00-15:30	Discussion: what was done today, plan for Tuesday	17:00-17:30	Large-scale DNA prep
15:30-16:00	Continental breakfast	17:30-18:00	Large-scale DNA prep
16:00-16:30	Start small-scale transcription manually	18:00-18:30	Large-scale DNA prep
16:30-17:00	Discussion: what was done today, plan for Tuesday	18:30-19:00	Large-scale DNA prep
17:00-17:30	Continental breakfast	19:00-19:30	Large-scale DNA prep
17:30-18:00	Start small-scale transcription manually	19:30-20:00	Large-scale DNA prep
18:00-18:30	Discussion: what was done today, plan for Tuesday		
18:30-19:00	Continental breakfast		
19:00-19:30	Start small-scale transcription manually		
19:30-20:00	Discussion: what was done today, plan for Tuesday		

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



Robotic System Currently in Operation at CESH

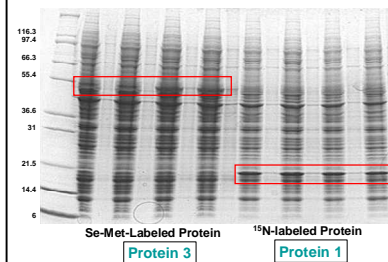
Workshop Small-Scale Expression Data Obtained on GeneDecoder1000

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	39.5	M	H
11.0	H	H	18.3	M	H	37.0	0	0
11.2	M	H	18.3	M	0	39.0	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	0	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	H	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

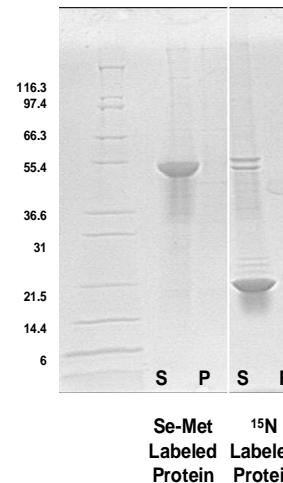
Summary of Workshop Small-Scale Expression Data Obtained on GeneDecoder1000

Expression	Over	<25	>25	High (H)	Solubility	Over	<25	>25	
(H)	all	all	all	(H)	(H)	all	all	all	
High (H)	0.5 - 1.0	33	20	13	>75%	28	22	6	
Medium (M)	0.1 - 0.5	26	21	5	50% - 75%	18	13	5	
Low (L)	<0.1	0	0	0	<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

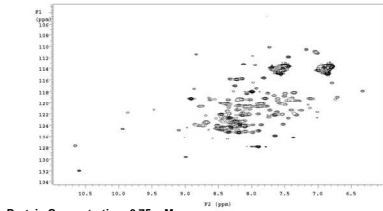


Large-Scale Protein Purification on ACTA Prime using HisTrap HP 1 ml Columns



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

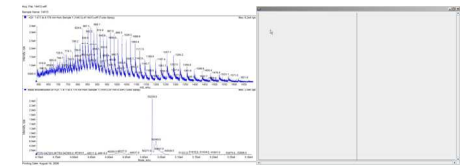


Protein Concentration: 0.75 mM
Buffer conditions: 10 mM NaPi, 50 mM NaCl, 1 mM DTT, 0.02% Na₃N, pH 6.0

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 μ 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 Scientists and Participants



For information about future workshops, visit: www.uwstructuralgenomics.org

