
PERG

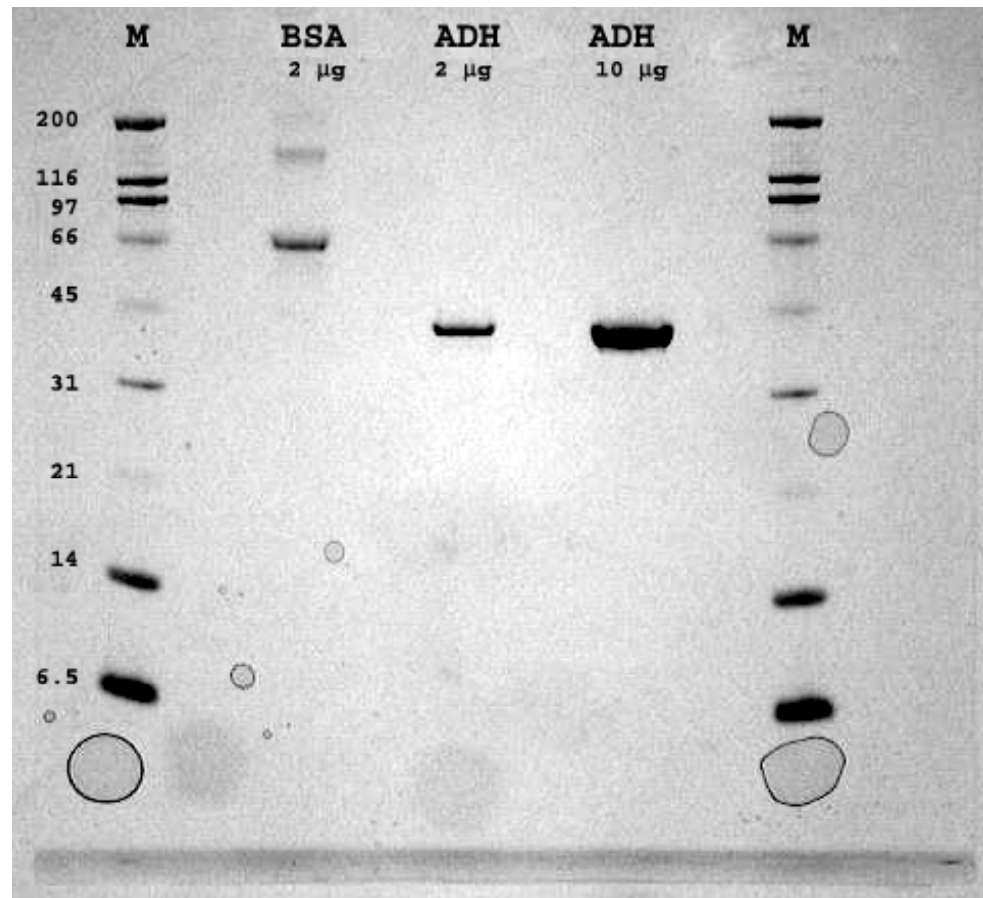
Recombinant Protein Expression and Purification Study

Each participant was sent

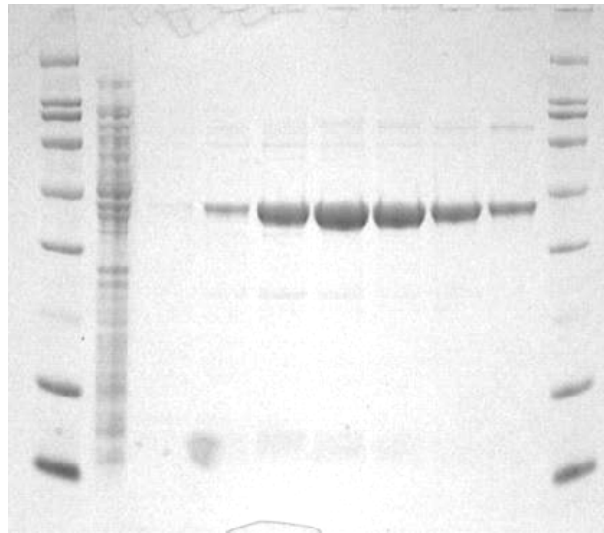
- Plasmid
- Instructions
- Link to Survey Monkey for data reporting
- Over 50 plasmid samples sent out...

	Lab 1a	Lab 1b	Lab 2a	Lab 2b	Lab 3	Lab 4
Strain	BL21(DE3)	Rosetta 2 (DE3)	BL21-T1R	BL21-T1R	BL21-CodonPlus	BL21
Media	LB	LB	TB	TB	LB	TY
Volume	1 L	1 L	250 mL	250 mL	5 L	3L
OD at Induction	0.6	0.8	0.5	0.5	0.6	0.5
[IPTG]	0.5 mM	1 mM	1 mM	1 mM	0.5 mM	0.5 mM
Temp	30°C	37°C	37°C	37°C	23°C	26°C
Time	6 h	3 h				
Lysis	Microfluidizer	Microfluidizer	CellLytic Express (Sigma)	CellLytic B (Sigma)	French Press	Sonication
Matrix	5 ml Ni-NTA	5 mL GE HisTrap	3 mL His-Select	3 mL His Select	40 mL Ni NTA (Qiagen)	10 mL Ni-NTA (Qiagen)
Yield	15 mg/L	18 mg/L	22 mg/L	16 mg/L	27 mg/L	9 mg/L
Method	Bradford (γ -globulin)	Bradford (γ -globulin)	BCA	BCA	Amino acid analysis	Bradford (BSA)
Sp Act (nmol/min/mg)	300	1650	83	23	22	70

Sample Purification Gel

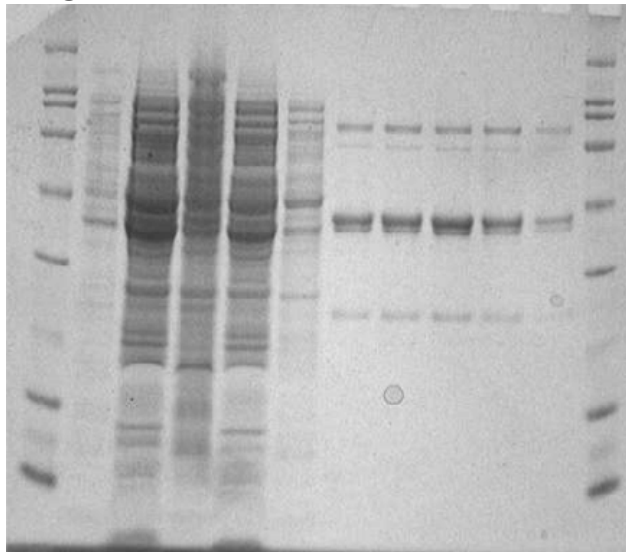


ADH Purification from the Workshop

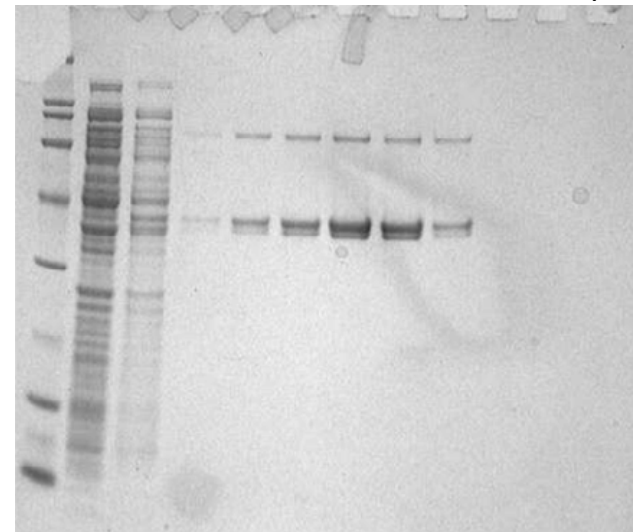


AKTA purification
His-Trap Column (GE)

Benchtop
Ni-NTA (Qiagen)



Benchtop
Talon (Clontech)



9. What was the composition of the lysis buffer used?

- Lab 1a
 - 20 mM Tris 7.9 500 mM NaCl 10% glycerol
- Lab 1b
 - 20 mM Tris.Cl, pH 8.0; 500 mM NaCl; 0.1% Triton X-100
- Lab 2a and b
 - Proprietary, however buffers contained Benzonase and Lysozyme
- Lab 3
 - 25 mM Tris-HCl, pH = 8.0, 0.3 M NaCl, 0.2% TritonX100, 10 mM imidazole
- Lab 4
 - 25 mM Tris, pH 8.0, 0.5M NaCl, 0.2% Tween 20, 10 mM imidazole, 5 mM DTT

11. What was the composition of the wash buffers used prior to elution? What was the volume of each wash buffer?

- Lab 1a
 - 20 mM Tris 7.9 500 mM NaCl 40 mM Immidazole
- Lab 1b
 - Used AKTA*Explorer*
 - Wash out unbound with 2 CV (10ml) of lysis buffer.
 - Wash with 6 CV (30ml) of 40 mM imidazole in lysis buffer
 - Elute protein with a 40 to 500 mM gradient of imidazole over 10 CV.
- Lab 2a and b
 - His-Select Wash Buffer- 50 mM NaPO₄, 300 mM NaCl, 10 mM Imidazole pH 8.0
 - The column was washed with 5 column volumes (15 mL) using a vacuum apparatus
- Lab 3
 - Same as the lysis buffer
- Lab 4
 - Same as the lysis buffer but without detergent.
 - A series of washes with 10 mM, 20 mM, 40 mM, and 60 mM imidazole in succession, using 30 ml buffer for each. This was time consuming, but is our standard procedure for many proteins with Qiagen Ni-NTA.

15. Please add any information regarding this study, your procedures, your results, or questions and concerns you may have regarding this study.

- Lab 1a
 - Specific activity may be low because it sat over the weekend at 4 degrees.
- Lab 1b
 - Protein correctly ID'd by mass spec finger printing. Noted some ADH ran as a dimer in NuPage gels if not enough reducing agent present in loading buffer.
- Lab 2
 - It appears as if an integrated lysis method not only yields the most protein, but also more pure protein. This method was used in the screening of clones as well as in a purification method. (Lab 2)
- Lab 2
 - My main concern is the fact that only one of each experiment was performed. I wish I had more time to perform the experiment in order to get some %CVs. (lab 2)

Conclusions

- Too few samples and too many variables to make any statistical conclusions about differences in protocols
- Even though a protocol was sent, each investigator made their own changes
 - No one standard protocol for expression
- All methods gave pure, active proteins
 - For a protein that expresses well in *E. coli*, the specifics don't matter

Past Projects

- 2007 Survey about Protein Expression in Core labs
- 2007 Satellite Meeting at ABRF 2007 (Tampa)
- 2007 Expression Study using a simple *E. coli* expression vector.
- 2009 Protein Expression Laboratory (St. Jude)

Potential Future Projects

- Development of an On-line Resource for Protein Expression
 - a web site with problems and solutions and shared experiences
- Difficult Expression Study
 - insoluble, or unstable, or low expression
 - The PERG would identify a good candidate protein
- Joint Study with the PRG
 - Development of an “artificial proteome” recombinant protein sample set as a test sample for proteomic applications
 - PERG study could have participants either make the whole set in high throughput mode, OR have each participant make different sub-sets of proteins to make up the test set for PRG study
- Joint Study with the MIRG
 - PERG participants make protein-protein interaction partners for use in MIRG biophysical measurement study
 - PERG study would collect data on variations of protein production, and provide protein samples for MIRG study
- Labeled Standards for 2D gels
 - PERG participants could make protein set of standards with varied pIs and sizes to allow easy standardization of 2D gels
- Test Protein for Edman vs Mass Spec Sequencing
 - Engineer a protein lacking trypsin cleavage sites to test protein sequencing methods
- To be continued... **1 pm Ballroom D**