
ABRF 2009
Protein Expression Research Group
Recombinant Protein Laboratory

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St Jude Children's Research Hospital

Screening for Solubility

Solubility is important for almost every downstream application of recombinant proteins.

Many applications require a high concentration of protein.

Storage conditions are important for long-term stability.

Screening for Solubility

- Protein Solubility can be screened after purification. This is different from “soluble expression” or “insoluble expression”.
- Insoluble expression results from misfolding and inclusion body formation.
- Soluble proteins don't always remain soluble after purification, unless proper storage conditions are determined.

Screening for Solubility

- Solubility is dependant on sequence, so every protein is different.
- Solubility can be enhanced by changes in pH, ionic strength, detergents, polyols, ligands.

Screening for Solubility

- Example: A protein kinase in my laboratory was highly expressed and easily purified, yielding 100mg/L culture.
- This protein is very hydrophobic. Within one hour after purification the protein started precipitating. After freezing it was mostly precipitated.
- Removal of salts and addition of 50% glycerol resulted in long lasting solubility at high concentrations

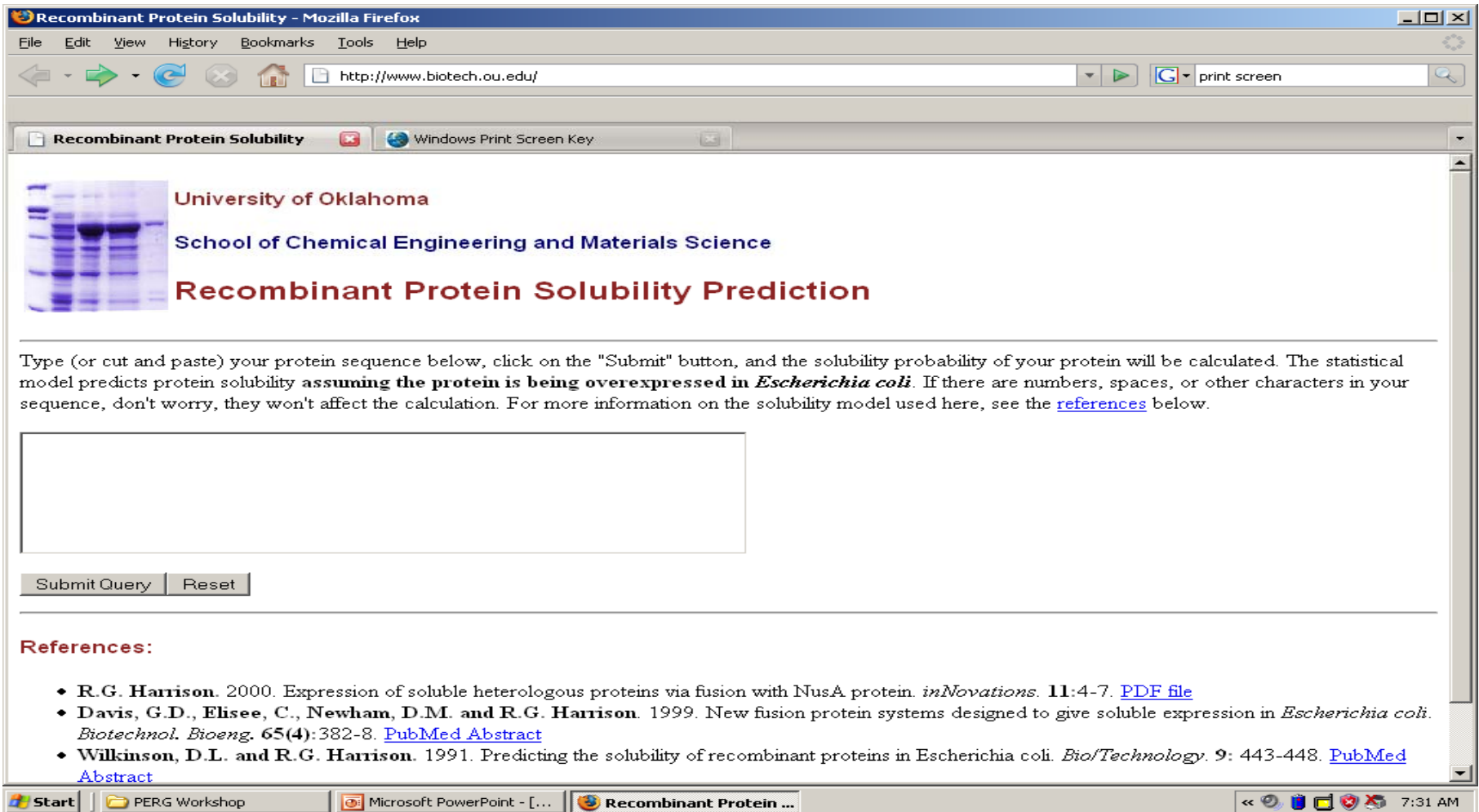
Screening for Solubility

- There are published methods for prediction of solubility (Wilkinson, D.L., and Harrison, R.G. (1991). Predicting the solubility of recombinant proteins in Escherichia coli. Biotechnology (N Y) 9, 443-448.)

Screening for Solubility

- There are on-line solubility predictors.
- One example: <http://www.biotech.ou.edu/>
- These are quick and easy, but just “predictions”.

Screening for Solubility

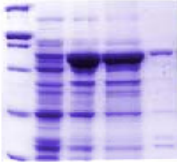


Recombinant Protein Solubility - Mozilla Firefox

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http://www.biotech.ou.edu/ print screen

Recombinant Protein Solubility Windows Print Screen Key

 **University of Oklahoma**
School of Chemical Engineering and Materials Science
Recombinant Protein Solubility Prediction

Type (or cut and paste) your protein sequence below, click on the "Submit" button, and the solubility probability of your protein will be calculated. The statistical model predicts protein solubility **assuming the protein is being overexpressed in *Escherichia coli***. If there are numbers, spaces, or other characters in your sequence, don't worry, they won't affect the calculation. For more information on the solubility model used here, see the [references](#) below.

Submit Query Reset

References:

- ♦ **R.G. Harrison.** 2000. Expression of soluble heterologous proteins via fusion with NusA protein. *inNovations*. **11**:4-7. [PDF file](#)
- ♦ **Davis, G.D., Elisee, C., Newham, D.M. and R.G. Harrison.** 1999. New fusion protein systems designed to give soluble expression in *Escherichia coli*. *Biotechnol. Bioeng.* **65(4)**:382-8. [PubMed Abstract](#)
- ♦ **Wilkinson, D.L. and R.G. Harrison.** 1991. Predicting the solubility of recombinant proteins in *Escherichia coli*. *Bio/Technology*. **9**: 443-448. [PubMed Abstract](#)

Start | PERG Workshop | Microsoft PowerPoint - [...] | **Recombinant Protein ...** | 7:31 AM

Screening for Solubility

- There are published methods for screening solubility conditions.
- Screens range from 24 or 96 wells to microarrays and even scanning electron microscopy.

Screening for Solubility

- The easiest method is a sparse matrix using 24 well plates.



Screening for Solubility

- A Sparse Matrix is a grid that covers a range of conditions to be tested (pH, salts, additives)
- Protein is mixed with each reagent and then screened visually or by various analytical methods.
- A dilution screen can also be produced where the protein starts in high salt and is serially diluted.

Screening for Solubility

Detergent Screen 1 Formulation - Hampton Research Corp.
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Reagent #	Detergent	MW	CMC (mM)	[Actual] (mM)	Type
1	C12E9	avg. ~ 583	0.05	0.5	N
2	C12E8	538.8	0.11	1.1	N
3	n-Dodecyl- β -D-maltoside	510.6	0.17	1.7	N
4	Sucrose monolaurate	524.6	0.30	3.0	N
5	CYMAL®-6	508.5	0.56	5.6	N
6	TRITON® X-100	650.0	0.90	9.0	N
7	CTAB	364.46	1.00	10.0	I
8	Big CHAP, Deoxy	862.1	1.40	14.0	N
9	n-Decyl- β -D-maltoside	482.6	1.80	18.0	N
10	LDAO	229.41	2.00	20.0	N
11	CYMAL®-5	494.5	5.00	50.0	N
12	ZWITTERGENT® 3-12	335.6	4.00	40.0	Z
13	n-Nonyl- β -D-glucoside	306.4	6.50	65.0	N
14	n-Octyl- β -D-thioglucoside	308.4	9.00	90.0	N
15	DDAO	201.35	10.40	104.0	N
16	HECAMEG®	335.4	19.50	195.0	N
17	n-Octanoylsucrose	468.5	24.40	244.0	N
18	n-Heptyl- β -D-thioglucopyranoside	294.4	30.00	300.0	N
19	n-Octyl- β -D-glucoside	292.4	20.00	200.0	N
20	CYMAL®-3	466.5	34.50	345.0	N
21	C-HEGA®-10	377.5	35.00	350.0	N
22	ZWITTERGENT® 3-10	307.6	40.00	400.0	Z
23	MEGA®-8	321.4	79.00	790.0	N
24	n-Hexyl- β -D-glucopyranoside	264.3	250.00	2500.0	N

Screening for Solubility

Grid Screen Sodium Chloride Hampton Research Corp.

Reagent n	[Precipitant]	[Precipitant] units	Precipitant	[Buffer]	[Buffer] units	Buffer	pH
1		1 M	Sodium Chloride		0.1 M	citric acid	4.0
2		1 M	Sodium Chloride		0.1 M	citric acid	5.0
3		1 M	Sodium Chloride		0.1 M	MES	6.0
4		1 M	Sodium Chloride		0.1 M	HEPES	7.0
5		1 M	Sodium Chloride		0.1 M	Tris	8.0
6		1 M	Sodium Chloride		0.1 M	bicine	9.0
7		2 M	Sodium Chloride		0.1 M	citric acid	4.0
8		2 M	Sodium Chloride		0.1 M	citric acid	5.0
9		2 M	Sodium Chloride		0.1 M	MES	6.0
10		2 M	Sodium Chloride		0.1 M	HEPES	7.0
11		2 M	Sodium Chloride		0.1 M	Tris	8.0
12		2 M	Sodium Chloride		0.1 M	bicine	9.0
13		3 M	Sodium Chloride		0.1 M	citric acid	4.0
14		3 M	Sodium Chloride		0.1 M	citric acid	5.0
15		3 M	Sodium Chloride		0.1 M	MES	6.0
16		3 M	Sodium Chloride		0.1 M	HEPES	7.0
17		3 M	Sodium Chloride		0.1 M	Tris	8.0
18		3 M	Sodium Chloride		0.1 M	bicine	9.0
19		4 M	Sodium Chloride		0.1 M	citric acid	4.0
20		4 M	Sodium Chloride		0.1 M	citric acid	5.0
21		4 M	Sodium Chloride		0.1 M	MES	6.0
22		4 M	Sodium Chloride		0.1 M	HEPES	7.0
23		4 M	Sodium Chloride		0.1 M	Tris	8.0
24		4 M	Sodium Chloride		0.1 M	bicine	9.0

Screening for Solubility

SaltRx Formulation - Hampton Research Corp.
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Reagent #	[Precipitant] units	[Precipitant] Precipitant	pH	[Buffer] units	[Buffer] Buffer	pH
1	1.8 M	Sodium acetate trihydrate	7.0	0.1 M	BIS-TRIS propane	7.0
2	2.8 M	Sodium acetate trihydrate	7.0	0.1 M	BIS-TRIS propane	7.0
3	1.5 M	Ammonium chloride		0.1 M	Sodium acetate trihydrate	4.6
4	1.5 M	Ammonium chloride		0.1 M	BIS-TRIS propane	7.0
5	1.5 M	Ammonium chloride		0.1 M	Tris	8.5
6	3.5 M	Ammonium chloride		0.1 M	Sodium acetate trihydrate	4.6
7	3.5 M	Ammonium chloride		0.1 M	BIS-TRIS propane	7.0
8	3.5 M	Ammonium chloride		0.1 M	Tris	8.5
9	2.2 M	Sodium chloride		0.1 M	Sodium acetate trihydrate	4.6
10	2.2 M	Sodium chloride		0.1 M	BIS-TRIS propane	7.0
11	2.2 M	Sodium chloride		0.1 M	Tris	8.5
12	3.2 M	Sodium chloride		0.1 M	Sodium acetate trihydrate	4.6
13	3.2 M	Sodium chloride		0.1 M	BIS-TRIS propane	7.0
14	3.2 M	Sodium chloride		0.1 M	Tris	8.5
15	1.0 M	Ammonium citrate dibasic		0.1 M	Sodium acetate trihydrate	4.6
16	1.8 M	Ammonium citrate dibasic		0.1 M	Sodium acetate trihydrate	4.6
17	1.0 M	Ammonium citrate tribasic	7.0	0.1 M	BIS-TRIS propane	7.0
18	2.0 M	Ammonium citrate tribasic	7.0	0.1 M	BIS-TRIS propane	7.0
19	0.7 M	Sodium citrate tribasic dihydrate		0.1 M	BIS-TRIS propane	7.0
20	0.7 M	Sodium citrate tribasic dihydrate		0.1 M	Tris	8.5
21	1.2 M	Sodium citrate tribasic dihydrate		0.1 M	BIS-TRIS propane	7.0
22	1.2 M	Sodium citrate tribasic dihydrate		0.1 M	Tris	8.5
23	0.4 M	Magnesium formate dihydrate		0.1 M	Sodium acetate trihydrate	4.6
24	0.4 M	Magnesium formate dihydrate		0.1 M	BIS-TRIS propane	7.0
25	0.4 M	Magnesium formate dihydrate		0.1 M	Tris	8.5
26	0.7 M	Magnesium formate dihydrate		0.1 M	BIS-TRIS propane	7.0
27	2.0 M	Sodium formate		0.1 M	Sodium acetate trihydrate	4.6
28	2.0 M	Sodium formate		0.1 M	BIS-TRIS propane	7.0
29	2.0 M	Sodium formate		0.1 M	Tris	8.5
30	3.5 M	Sodium formate		0.1 M	Sodium acetate trihydrate	4.6
31	3.5 M	Sodium formate		0.1 M	BIS-TRIS propane	7.0
32	3.5 M	Sodium formate		0.1 M	Tris	8.5
Reagent #	[Precipitant] units	[Precipitant] Precipitant	pH	[Buffer] units	[Buffer] Buffer	pH
33	1.2 M	DL-Malic acid	7.0	0.1 M	BIS-TRIS propane	7.0
34	2.2 M	DL-Malic acid	7.0	0.1 M	BIS-TRIS propane	7.0

Screening for Solubility

For this workshop we will use the Hampton Research “Grid Screen” to set up a sparse matrix and examine solubility.

This is a matrix designed for crystallization trials, not solubility screening, but will provide an easily visualized range of solubilities.

Screening for Solubility

Procedure:

1. Add 2-5 microliters of protein to the post on each well of a 24-well plate.
2. Add an equal volume of purified protein and mix by pipetting.
3. Examine the drops under a microscope.
(drops could be examined over time or at different temperatures. Activity could be monitored over time.)

Screening for Solubility

What you can expect to see:

1. Clear drop (soluble)
2. Light precipitation
3. Heavy aggregation
4. Phase separation (looks like oil droplets)
5. Small crystals (this can be valuable as a purification step)
6. Color can be informative