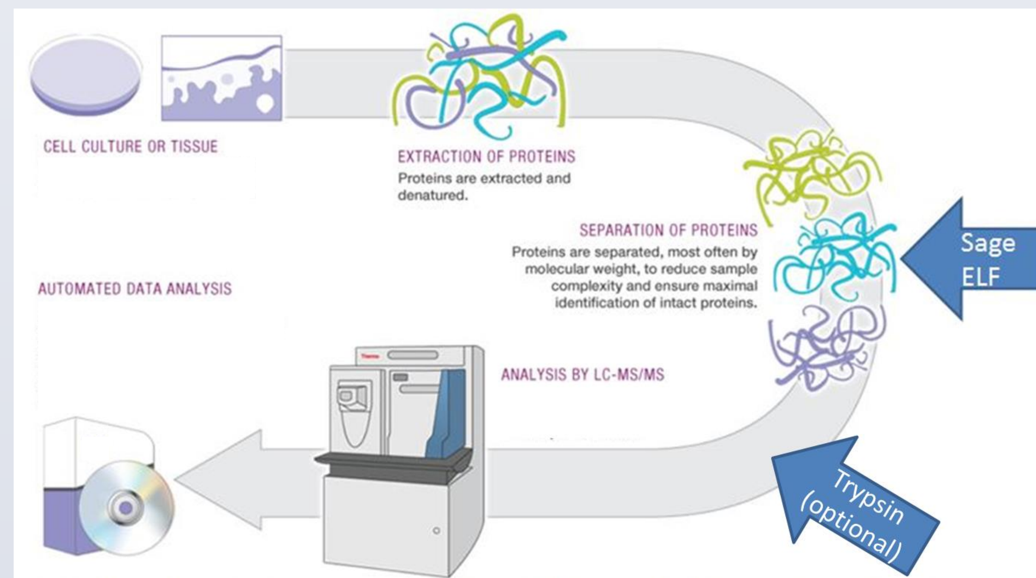
The ELF preparative electrophoresis system for size-based proteome fractionation

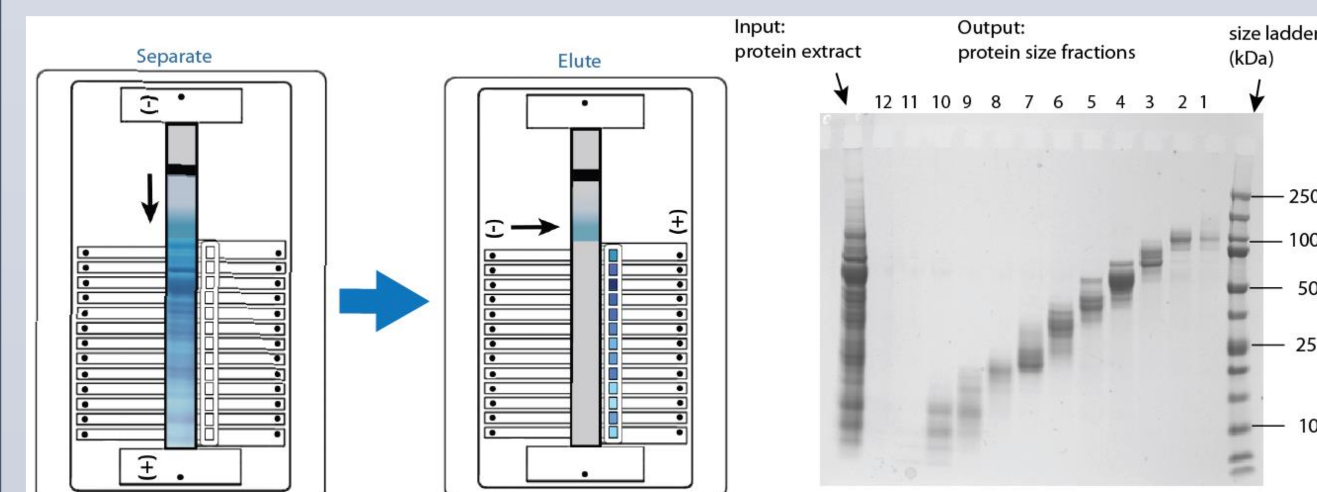
David Cook, Sadaf Hoda, Danny Yun, Bryan Spencer, Ezra S. Abrams, T. Christian Boles

Sage Science, Inc., Beverly, MA 01915

Introduction

Although the resolution of LS-MS systems is improving every year, size fractionation of complex samples prior to MS analysis is still very useful for many proteomic studies. Despite the promise of automated 2D LC systems, SDS PAGE remains a popular, but laborious, pre-fractionation method for MS. We have developed an automated preparative electrophoresis system (the ELF system), to fractionate complex protein samples into 12 contiguous size fractions. The system uses a two-dimensional process: first, proteins are electrophoresed through an SDS agarose gel column to separate the proteins by size, and second, the separated proteins are electrophoresed sideways out of the agarose column into a linear array of membrane-bounded elution modules that are positioned alongside the separation gel column. The fractionated samples are recovered in SDS buffer, and can be processed for LC-MS using commercial detergent removal spin columns and standard trypsin digestion methods.

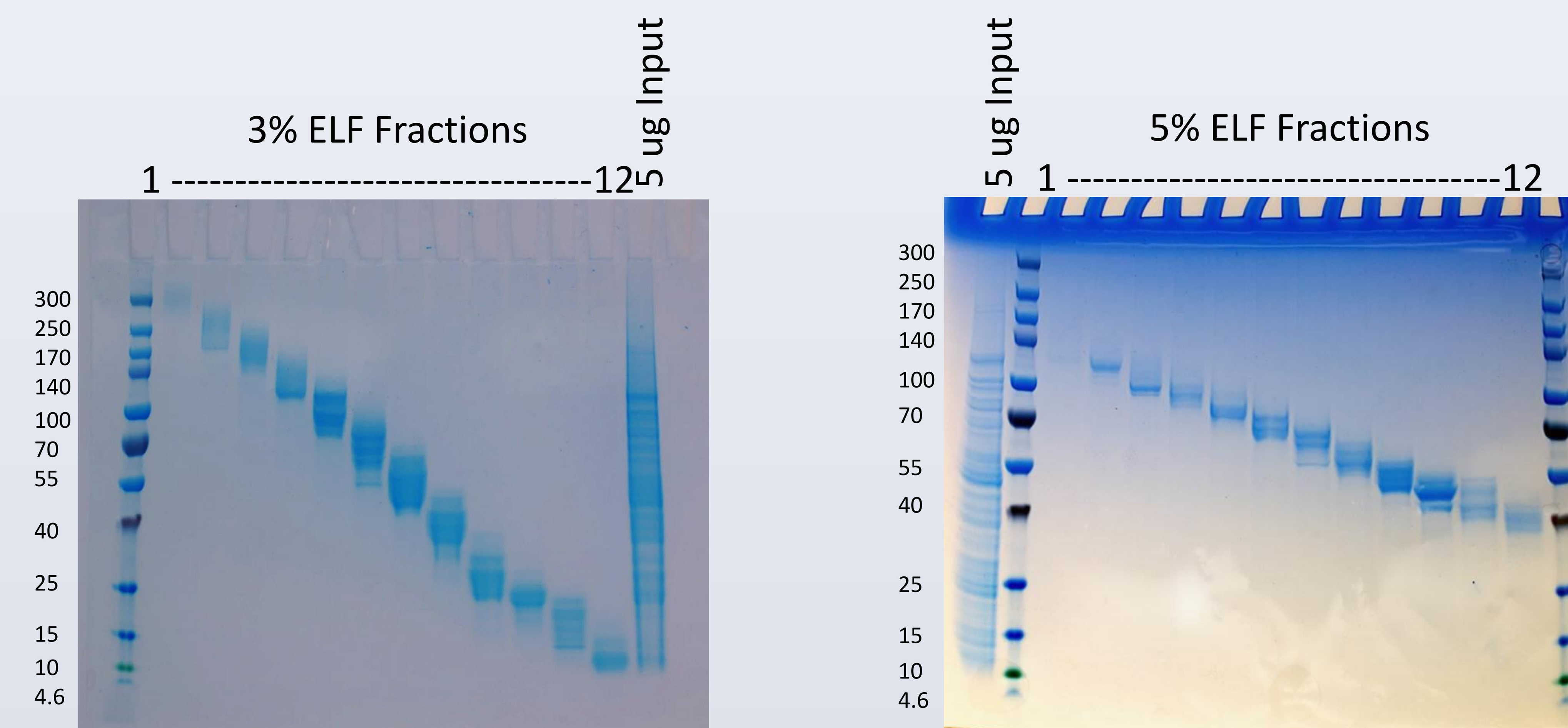




Experimental Design

To evaluate the performance of the system, a commercial E. coli protein extract (E. coli DH 5a lysate, MCLAB, San Francisco, CA) was fractionated using 3% and 5% SDS agarose cassettes. 200 micrograms of total E. coli extract were loaded per lane. Samples were reduced with TCEP and denatured by heating in SDS loading solution immediately prior to loading. Fractionated samples were recovered from the ELF elution modules in 28 microliters of electrophoresis buffer, and 15 microliters aliquots were analyzed on SDS PAGE gels. The gels were stained with Coomassie Blue and photographed. The minimum and maximum protein sizes from each fraction were determined with reference to marker ladders using TotalLab software.

Results: Gel images of typical 3% and 5% ELF fractionation products.



Results: Estimates of range, bandwidth, resolution of fractionation

Example of 3% ELF Analysis (kDa) (not from run pictured above)

Elution lane	low	high	average	range	CV	Difference in average size, n vs n+1
1	165	273	219	108	12.4%	13.1%
2	134	247	190	113	14.8%	30.2%
3	91	175	133	84	15.9%	20.4%
4	75	137	106	62	14.6%	19.1%
5	61	111	86	50	14.6%	25.0%
6	49	79	64	30	11.6%	22.4%
7	39	60	50	21	10.5%	16.8%
8	28	55	41	27	16.1%	21.5%
9	23	42	33	19	14.3%	24.3%
10	16	33	25	18	17.8%	19.0%
11	13	26	20	13	16.3%	19.0%
12	10	22	16	12	19.1%	--

Example of 5% ELF Analysis (kDa) (from run pictured above)

Elution lane	low	high	average	range	CV	Difference in average size, n vs n+1
1	112	135	123.5	23	4.7%	4.9%
2	105	130	117.5	25	5.3%	11.1%
3	85	124	104.5	39	9.3%	10.0%
4	76	112	94	36	9.6%	10.1%
5	68	101	84.5	33	9.8%	10.1%
6	59	93	76	34	11.2%	13.8%
7	56	75	65.5	19	7.3%	10.7%
8	52	65	58.5	13	5.6%	10.3%
9	45	60	52.5	15	7.1%	7.6%
10	39	58	48.5	19	9.8%	4.1%
11	37	56	46.5	19	10.2%	9.7%
12	34	50	42	16	9.5%	--

CV's were estimated as the (fraction bandwidth divided by 4) divided by the average fraction size, and expressed as a percentage.

Results (cont.)

For 3% agarose cassettes, the useful fractionation range was from 220 (fraction 1) to 16 kDa (fraction 12). The bandwidth, expressed as CV, varied from between 11 and 19%, and the average size difference between adjacent fractions varied between 18 and 30%.

For the 5% agarose cassettes, the useful fractionation range was from 120 to 10 kDa. Bandwidths varied between 6 and 11% CV, or roughly half the bandwidth obtained with the 3% ELF cassette. Average size difference between adjacent fractions was 5-11%.

In both cases the variation in size difference between adjacent fractions was mainly due to the influence of uneven distribution of protein sizes throughout the E. coli extract (which strongly biases calculation of the average size of the collected fraction).

Fractionation runs could be completed in less than 2.5 hrs.

Conclusions

The Sage ELF is a fully automated preparative electrophoresis platform that is well suited for protein fractionation by size prior to LC-MS analyses. The 3% cassettes can fractionate a complex sample into 12 "slices" extending from 10kDa out to nearly 300kDa in a single run. The 5% cassettes offer higher resolution (narrow size slices) with a more limited overall range.

In either case, proteins are electroeluted without user intervention into electrophoresis buffer, ready for buffer exchange (i.e., Pierce HiPPR detergent removal columns) and trypsin digestion.

Early customers are using the ELF to increase the number of protein IDs in discovery proteomics, and for increasing LC-MS sensitivity for detection of minor protein species in samples that have one (or a few) extremely abundant proteins (i.e., serum and plasma samples, or samples of biotherapeutic proteins).

Contact

Poster inquiries: David Cook, Ph.D., dave@sagescience.com
Sales inquiries: sales@sagescience.com
Technical inquiries: support@sagescience.com
General inquiries: info@sagescience.com

www.sagescience.com
http://www.sagescience.com/products/sageelf/

RESEARCH POSTER PRESENTATION DESIGN © 2012
www.PosterPresentations.com