Journal of Biomolecular Techniques
13:179–186 © 2002 ABRF

ARTICLES

Proteomics in Mixtures: Study Results of ABRF-PRG02

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The trend in proteomics is to work with increasingly complex protein mixtures, limiting the protein separation steps prior to analysis. This is due in part to the difficulties encountered with detecting low abundance proteins, protein losses during SDS PAGE, and the limited separation capability of even 2D PAGE where a single protein spot may still contain multiple proteins. Hence, the ABRF-PRG02 sample was designed to study a simple protein mixture of five proteins at the ~2 pmol and ~200 fmol levels. The sample, after a tryptic digestion, was sent out by the Proteomics Research Group of the ABRF to interested member labs. A total of 41 labs participated in this study, with each participant using some type of mass spectrometric analysis. Laboratories that used μLC-NSI (microLC with nanospray ionization) with MS/MS analysis had a higher percent accuracy than labs using MALDI-MS (matrix assisted laser desorption ionization mass spectrometry).

KEY WORDS: mass spectrometry, proteomics, proteome, protein mixtures, MALDI-MS, NSI-MS

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The identification of multiple proteins in a single sample is a continuing challenge in studying proteomes. Typically, proteome analysis uses 1- and 2-dimensional gel electrophoresis (SDS PAGE) as the final protein purification step. This often leaves the protein of interest as a protein mixture and can be problematic for proteins that are highly acidic, basic, too hydrophobic, too large (to enter the gel), or too small (not retained in the gel).[1] In addition, SDS PAGE has a limited dynamic range, which causes difficulties with identification of the minor components in the proteome being studied. Other approaches such as immunoprecipitation, pull-down assays, protein complexes,[2] comparing mRNA and protein expression levels, and protein profiling experiments[3,4] all produce samples inherently composed of protein mixtures. Hence, there is a need to analyze ever increasingly complex protein samples.[5] For these reasons, the Proteomic Research Group (PRG) designed an ABRF study consisting of a limited protein mixture.

The last study performed by the ABRF Protein Identification Research Group (PIRG) in 1999 consisted of a mixture of two proteins at the 10- and 2-pmol level. These results showed that 97% of the responses at the 10-pmol level and only 23% of the identifications made at the 2-pmol level were correct. In fact, 77% of the identifications made at the 2-pmol

level were incorrect. Thus, the sample amount chosen for the ABRF-PRG02 (2 pmols for the major components) was based on this study, expecting that, with the new mass spectrometric techniques and advances in instrumentation, most laboratories should be able to identify the two major components. Identification of the three lower level components at 10-fold less material was expected to be more challenging. The proteins used were chosen to simulate a mixture that might be encountered in a real sample.

The goals of the study were (a) to learn if an improvement had been made in protein identification from the PIRG 1999 study; (b) to provide a mechanism for participants to evaluate their abilities with regard to protein identification; (c) to determine which approaches used were most successful; and (d) to help establish realistic expectations for proteomic analysis.

METHODS

ABRF-PRG02 Design

The ABRF-PRG02 sample contained a protein mixture of bovine protein disulfide isomerase (PDI, ~2 pmols), *Schistosoma japonicum* glutathione-*S*-transferase (GST, ~2 pmols), *Escherichia coli* GroEL (~200 fmols), bovine serum albumin (BSA, ~200 fmols), and bovine superoxide dismutase (SOD, ~200 fmols). This combination of proteins was chosen since it could mimic a possible recombinant protein mixture that a membership lab might receive to analyze. For example, the bovine PDI (53 kD) might be the recombinant protein of interest that is fused to GST (25 kD); the GroEL (57 kD) could be a copurifying contaminant from the *E. coli* host; and the BSA (66 kD) and SOD (24 kD) are possible sample "contaminants."

Proteins were purchased from Sigma and dissolved in 100% water at ~1 µg/µL. Amino acid analysis was performed on the stocks using a Beckman 6300 amino acid analyzer in order to accurately determine the protein concentration. Samples of each protein (400 pmols) were loaded in separate lanes on a 10% Tris/Tricine SDS PAGE and stained with Coomassie blue R-250 for 45 min followed by a 2-h destaining. Excised protein gel bands were reduced with 20 mM triscarboxyethylphosphine (TCEP)/25 mM ammonium bicarbonate (pH 8.0) and alkylated using 40 mM iodoacetamide in 25 mM ammonium bicarbonate. Each protein gel band was then digested separately in 40 mM ammonium bicarbonate using 0.02 µg/µL of trypsin (Promega, modified) for 18 h at 37°C. The protein digests were mixed in a 10:10:1:1:1 ratio so that each sample contained approximately 2 pmols of PDI and GST, and approximately 200 fmols of GroEL, BSA, and SOD.

Digested samples were speed-vacuumed dry and sent out to 123 participants (38 international and 85 USA). It was recommended that an acid/organic mixture such as 5% formic acid with 50% acetonitrile be used for resolublizing the protein. Participants were asked to analyze the sample using whatever technologies they had at their disposal. A survey was included to collect additional information on the sample preparation, type of analysis, instrumentation used (including age), database searched, and computer algorithms used for protein identification.

RESULTS AND DISCUSSION

The trypsin digestion step in this study was performed by the PRG. This was done to eliminate this variable from the study and so better determine the protein identification capabilities of the participating labs. A total of 41 laboratories participated in the study with 14 labs (34%) performing two types of mass spectrometric analysis for a total of 55 analyses. Proteins identified were scored as positive correct (PC), positive wrong (PW), tentative correct (TC), and tentative wrong (TW). The results analysis sheet returned from the participating laboratory asked them to classify their identification as positive or tentative. Thus, correct and wrong identifications were considered as either positive or tentative based on the laboratories' classification. The percent accuracy was calculated as the total correct (PC + TC) divided by the sum of the total correct plus total wrong (PW + TW). The percent identified was considered as the total correct divided by 5 (for the 5 expected proteins), and the percent confidence calculated as the positive correct (PC) divided by the total correct (PC + TC). Table 1 summarizes the individual analyses.

At the 2-pmol level, 96% (53/55) of the analyses correctly identified PDI as positive correct while 80% (44/55) identified the second ~2 pmol component GST as positive correct. This is a vast improvement over the 1999 PIRG study, in which 77% of the calls made at the 2-pmol level were incorrect. At the ~200-fmol level, 44% (24/55) identified GroEL; 27% (15/55) identified BSA; and 11% (6/55) identified SOD as positive correct. There were four laboratories that identified all five proteins as positive correct, with no tentative identifications made. There was at least one incorrect tentative or positive identification made in 18 (33%) of the analyses, and 37 analyses (67%) had

the PDI protein identified as positive correct with no wrong (PW or TW) identifications.

All participants used some type of mass spectrometry for analyzing ABRF-PRG02. MALDI-MS (matrix assisted laser desorption ionization mass spectrometry) and μLC-NSI (micro LC with nanospray ionization) with MS/MS were the most common types of mass spectrometry used with 49% (27/55) and 38% (21/55) of the analyses, respectively. Other types of MS were NSI (9%), LCLC-ESI (2%) and LC-ESI (2%). There were seven laboratories that identified all five proteins (PC + TC), with each analysis being performed using μLC-NSI with MS/MS. The average amino acid coverage of the known sequences was 20%. Of the 19 analyses that identified four or more proteins correctly, 17 used MS/MS for a 91% accuracy.

The highest number of MALDI-MS positive correct identifications with no positive or tentative wrong was four proteins. The percent coverage of the known sequences was 55% PDI, 54% GST, 28% GroEL, and 22% BSA. Three other MALDI-MS analyses had three proteins (PDI, GST, and GroEL/BSA) identified positively correct. The majority of the wrong calls were made from MALDI-MS analyses with three positive and 20 tentative wrong calls. This is not surprising since MALDI-MS analyses [except for the two analyses that did post-source decay (PSD)] contain no sequence information. Thus, it is necessary to use stringent search parameters when analyzing MALDI-MS spectra, with a high mass accuracy (better than 100 ppm) obtained on the instrumentation used. However, it should be noted that of the 23 wrong identifications, 87% were categorized as tentative. Overall, the percent accuracy for MALDI-MS was 74%. Table 2 summarizes the type of MS used along with the positive/tentative correct, and incorrect calls.

Figure 1 summarizes the database search programs used for each type of MS method. The two most commonly used programs for μLC-NSI with MS/MS were Mascot and Sequest; and for MALDI-MS data, ProFound, MS-FIT, and Mascot were used. There did not appear to be a trend for obtaining a better result or higher percent accuracy using one program over another.

Sample preparation for MS was done in a variety of ways, with all participants using formic, acetic, or trifluoroacetic acid in the solution used to dissolve the digest. The addition of an organic solvent to the solution had no appreciable difference in the results. Also, there was no clear advantage to desalting the sample prior to analysis. No laboratory that did desalt the sample identified SOD, which suggests desalting interfered with identifying this protein. One laboratory performed a 2D LC separation, and other laboratories that ran μLC-NSI with MS/MS analysis used a variety of reversed-phase columns ranging in ID from 50 μm to 1 mm.

There were no clear correlations between the proteins correctly identified and the type of mass spectrometer used for the analysis (make or model). There was also no correlation between the number of proteins correctly identified versus the age of the instrument, but the overall age was 2.4 years. Unfortunately, the survey did not include a question regarding the operators' years experience. Hence, no conclusions could be drawn on the impact that expertise may have had on this analysis.

There were 14 laboratories that ran two different types of MS analysis, as summarized in Table 3. Each used NSI with 13 responses also running MALDI-MS. Only one of the labs actually called all five proteins correctly, so the impact of running the sample using two different mass spectrometers was not clear.

CONCLUSIONS

The protein digestion step was performed by the PRG in order to eliminate this variable, thereby enabling a better determination of the participants' protein identification capabilities. A total of 41 laboratories participated in this study with 96% identifying one of the major components, PDI, at the 2-pmol level. This shows a marked improvement of the participants' ability to correctly identify proteins in a mixture over the PIRG study done in 1999, where only 23% of the participants identified the 2-pmol component in that two-protein mixture.

A variety of mass spectrometric techniques were used in this study, with MALDI-MS and μLC-NSI comprising the majority of the instrumentation. Identification of the three minor components at the ~200-fmol level proved to be most challenging, with only seven analyses correctly identifying all five proteins. Each of the fully correct analyses was done using μLC-NSI with MS/MS analysis. Overall, the percent accuracy was 91% for NSI with MS/MS and only 74% for MALDI-MS, demonstrating that MALDI-MS is more prone to false positive/tentative identifications.

Finally, the sample preparation (i.e., the solvent used to dissolve the sample), the age of the instrument, and the searching program used to process the data did not appear to impact the final identifications made. Overall, the results of this study have shown that it is realistic to routinely identify proteins at the 2-pmol level and that for several labs, even the 200-fmol level is reasonable.

TABLE 1

Summary of Data for ABRF-PRG02*

Label	Ioniz	MS	MS/MS	Alg	Instrument	% Acc	% ID	% Conf	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Wrong ID
45108	uLC-NSI		+	Mc	M-QT-2	100	100	100	P	P	P	P	P	
34479	uLC-NSI		+	Mc	S-QSP	100	100	100	P	P	P	P	P	
53427	uLC-NSI		+	Sq	F-LCQ-C	100	100	100	P	P	P	P	P	
38101	uLC-NSI		+	Mc,Mn	M-QT-2	100	100	100	P	P	P	P	P	
31255	uLC-NSI		+	Sq	F-LCQ	100	100	80	P	P	P	P	T	
01207	uLC-NSI		+	Mc,O	F-LCQ-D	100	100	80	P	T	P	T	P	
[illegible]	uLC-NSI		+	Mn	M-QT-2	100	100	20	P	T	T	T	T	
TB4446	MALDI	+		Mc,Pf	M-MALDI	100	80	100	P	P	P	P		
58152	uLC-NSI		+	Sq	F-LCQ-C	100	80	100	P	P	P	P		
37535	uLC-NSI		+	Mn	F-LCQ-D	100	80	100	P	P	P	P		
[illegible]	uLC-NSI		+	Mc,Sq	F-LCQ-X	100	80	100	P	P	P	P		
[illegible]	uLC-NSI		+	Sq,Mt	F-LCQ-D	100	80	100	P	P	P	P		
UB090	uLC-NSI		+	Mn	M-QT-1	100	80	100	P	P	P	P		
CC86L	uLC-NSI		+	Mn	F-LCQ-D	100	80	75	P	P	P	T		
34450	[illegible]	+	+	Mc,Mn	M-QT-2	100	80	73	P	P	P	T		
TB4449	NSI		+	Mc,Mn	M-QT-1	100	80	100	P	P	P			
9718	NSI	+		Pf	S-QS	100	80	100	P	P	P			
[illegible]	MALDI	+		Pf,O	M-TofSp-2E	100	80	100	P	P		P		
CC36L	MALDI	+		Mf	P-Voy-DS	100	80	100	P	P	P			
21255	MALDI	+		Pf,Mf	P-Voy-DS	100	80	100	P	P	P			
10061	uLC-NSI		+	Mn	M-QT-2	100	80	100	P	P	P			
10651	MALDI	+		Mf	P-Voy-DS	100	80	67	P	P	T			
[illegible]	MALDI	+		Pf,Mf	P-Voy-DP	100	80	67	P	P		T		
[illegible]	uLC-NSI		+	Sq	F-LCQ-D	100	80	67	P	P	T			
[illegible]	NSI		+	Mn,Pa,Mn	S-QS	100	80	67	P	P	T			
0718	LC-ESI		+	Mn	S-QS	100	40	100	P	P				
11128	MALDI	+		Mc,Pf	B-Biflex III	100	40	100	P	P				
[illegible]	MALDI	+		Mf	P-Voy-DP	100	40	100	P	P				
13039	MALDI	+		Pf	P-Voy-DP	100	40	100	P	P				
[illegible]	MALDI	+	Psd	Mf	K-Axima	100	40	100	P	P				
[illegible]	uLC-NSI		+	Mc	F-LCQ-D	100	40	100	P	P				
[illegible]	uLC-NSI		+	Sq	F-LCQ-D	100	40	100	P	P				
11120	NSI		+	Mn	S-API III+	100	40	100	P	P				
[illegible]	uLC-NSI		+	Mn	S-QS	100	40	100	P	P				
10251	[illegible]	+		Pf	B-Biflex	100	40	100	P	P				
UB830	[illegible]	+		Mf	P-Voy-DS	100	20	100	P					
[illegible]	[illegible]	+		Pf	P-Voy-DP	100	20	100	P					
[illegible]	uLC-NSI		+	Mc	M-QT-1	83	100	100	P	P	P	P	P	1T
34484	[illegible]	+		Mc,Pf,Mf	P-Voy-DP	80	80	78	P	P	P	T		1T
[illegible]	[illegible]	+		Mf	P-Voy-DS	75	80	100	P	P	P			1T
[illegible]	[illegible]	+		Mf	P-Voy-DP	75	80	100	P	P		P		1T
58770	[illegible]	+			P-Voy-DP	75	80	67	P	P		T		1T
[illegible]	uLC-NSI		+	Mn	S-API365	75	80	67	P	P	T			1T
[illegible]	MALDI	+		Mn	P-Voy-DS	75	80	67	P	P	T			1T
6247	MALDI	+		Pf	P-Voy-DP	75	80	33	P	T		T		1P
[illegible]	LCLC-ESI		+	Mc	F-LCQ-C	67	80	75	P	P	P	T		1P,1T
[illegible]	uLC-NSI		+	Mn	M-QT-2	67	80	78	P	T	P	P		2T
[illegible]	MALDI	+		Mf	P-Voy-DS	67	40	100	P	P				1T
12510	[illegible]	+		Pf	B-Reflex III	67	40	[illegible]	P			T		1T
10481	[illegible]	+	Psd	Mn	K-Axima-CFR	67	40	[illegible]	P	T				1T
[illegible]	[illegible]	+		Mn,Mf	M-TofSp-2E	50	50	67	P	P		T		2T
04121	[illegible]	+		Mc,Pf	P-Voy-DS	50	50	67	P	P		T		1P,2T
[illegible]	[illegible]	+		Pf	B-AutoFlex	20	20	100	P					1P,3T
[illegible]	[illegible]	+		Pp	M-MALDI	20	20			T				4T
03443	[illegible]	+		Pf	M-Tof Spec II									1T

Ioniz Legend
- MALDI matrix assisted laser desorption ionization
- uLC-NSI microLC with nanospray ionization
- LCLC-ESI 2 dimensional LC with electrospray ionization
- NSI nanospray ionization
- LC-ESI LC with electrospray ionization

MS Search Algorithm Legend
- Mn: Manual
- Pf: Profound
- Mc: Mascot
- Pp: [illegible]
- Mf: MS-Fit
- Sq: Sequest
- Mt: MS-Tag
- O: Other
- [illegible]

*These data have been sorted according to % Accuracy > % Identified > % Confidence > Average % Coverage. Analyses with all correct assignments are in the top portion of the table.

% Coverage										Additional Information			
[illegible]		[illegible]		[illegible]		[illegible]		[illegible]		[illegible]	Vol	%	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	µl	[illegible]	
30	35	62	23	28	12	12	8	10	2	0.1% FA	8	75	
31	16	21	7	24	10	12	7	23	3	0.25% Ac	30	33	
30	10	38	8	16	8	8	5	17	2	0.1% FA	30	10	
22	14	11	7	17	7	7	8	6	1	5%CN/1%Ac	10	50	
28	15	28	10	18	8	12	8	23	3	0.1%TFA	10	20	
	10		8		8		1		2	5%CN/0.1%TFA	60		
32	13	28	8	12	6	1	1	1	1	10%FA	8	40	
65	32	64	17	28	12	22	13			50%CN/0.1%TFA	8	10	[illegible]
40	16	34	11	28	11	30	11			5%Ac	20	5	
38	19	23	7	22	10	12	8			3%TFA	8	25	CapTrap
38	25	30	8	12	8	7	4			2%CN/1%FA	8	40	
24	12	32	10		3		8			50%CN/1%FA	10	50	
18	7		4		6		2			0.1%FA	20	25	CapTrap
27	15	30	6	14	2		1			50%CN/0.3%TFA	8	45	
	9		4		2	20	12			50%CN/5%FA	10	80	C18 Zip
68	32	54	17	28	12					50%CN/0.1%TFA	6	10	[illegible]
63	30	62	21	24	15					0.1%FA	4	80	
47	21	47	12			23	11			50%CN/0.2%TFA	23	25	C18 Zip
27	15	30	6	14	8					60%CN/0.3%TFA	6	45	
28	17	22	15	18	12					0.1%TFA	10	20	
22	5		3		2					5%FA	10	30	C18 Zip
41	19	38	9	16	6					50%CN/5%FA	19	10	
34	19	28	6			12	8			50%CN/0.05%TF/	10	20	Zip
34	19	22	6							50%CN/0.05%TF/	16	25	
18	10	11	3	7	6					0.5%FA	28	60	[illegible]
63	30	58	21							0.1%FA	4	50	
33	15	32	8							80%CN/0.1%TFA	3	78	C18 Zip
27	14	37	14							0.1%TFA	50	60	C18 Zip
27		36								60%CN/0.1%TFA	6	29	
24	12	32	10							40%CN/1%FA	10	60	
24	12	14	7							60%CN/0.1%TFA		8	
21	11	17	4							0.1%TFA	50	26	
	4		3							50%CN/0.1%TFA		25	C18 Zip
	7		4							40%CN/0.1%TFA	5	40	C18 Zip
	8		3		2					5%FA	10	28	
18	7									0.1%FA	20	26	CapTrap
										2%CN/1%FA	8	40	
24	13	27	9	22	6	15	6	18	2	5%CN/0.1%FA	50	33	
31	18	37	18	14	8	14	7			60%CN/0.1%TFA	5	10	
41	22	40	14	23	9					1%TFA	10	10	
24	12	14	7			29	14			60%CN/0.1%TFA		5	
34	16	37	13			19	14			0.1%TFA	20	50	C18 zip
	7		5		3					2%CN/0.05%TFA		3.5	other
	7		5		3					2%CN/0.05%TFA		3.5	other
63	26	43	9			19	12			5%FA	20	5	
32	32	22	21	27	12	11	9			20%CN/0.1%FA	20	100	CapTrap
29	12	17	6	13	6	4	3			0.1%FA	10	40	
28	14	28	11							0.1%TFA	10	80	C18 Zip
28	17					15	9			0.1%TFA	10	30	C18 Zip
24	11		1							50%CN/0.1%TFA	5	5	
27	18	30	11			13	8			10%FA	20	80	C18 Zip
25	8	23	4			8	3			50%CN/0.1%TFA	20	50	
24	12									60%CN/1%FA	10	3	[illegible]
		30	9							50%CN/0.1%TFA	5	100	zip
										50%CN/0.1%TFA	10	10	C18 Zip

Instrument Legend

B	[illegible]	
F	[illegible]	C:Classic D:Deca XP
K	[illegible]	
M	[illegible]	[illegible]
P	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

% legend

% Accuracy	Total Correct / (Total Correct+Total Wrong)
% Identified	Total Correct / (5 expected proteins)
% Confidence	Positive Count / Total Count
% Coverage	Average of protein coverage

TABLE 2

Summary of Proteins Identified Using the Indicated Mass Spectrometric Approach

		Major Proteins		Minor Proteins			
Type of MS	No. samples	PDI (no. correct)	GST (no. correct)	GroEL (no. correct)	BSA (no. correct)	SOD (no. correct)	No. wrong calls
μLC-NSI MSMS	21	21 P	18 P 3 T	15 P 3 T	12 P 3 T	6 P 2 T	0 P 4 T
MALDI-MS	25	23 P	18 P 2 T	5 P 2 T	3 P 7 T		3 P 19 T
MALDI-MS with PSD	2	2 P	1 P 1 T				1 T
Nano ESI MSMS	4	4 P	4 P	2 P 1 T	1 T		
Nano ESI	1	1 P	1 P	1 P			
LC-ESI	1	1 P	1 P				
LCLC-ESI	1	1 P	1 P	1 P	1 T		1 P 1 T

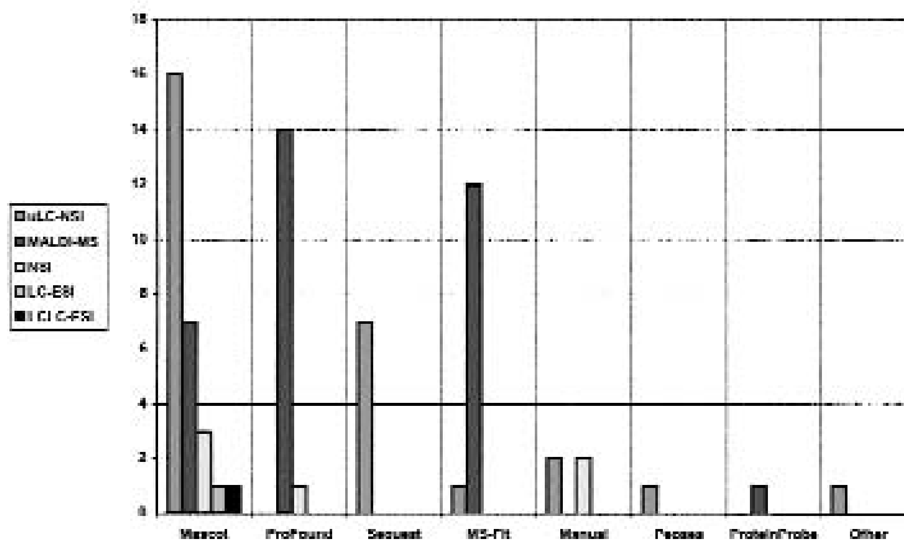

FIGURE 1

Summary of search programs used in analysis. The type of search program used depended on the type of analysis performed. For μLC-NSI, Mascot was used most often, while for MALDI-MS, ProFound and MS-FIT were used most often.

T A B L E 3

Results for Laboratories That Ran two Types of MS Analyses

% Accuracy	Total Correct / (Total Correct + Total Wrong)
% Identified	[illegible]
% Confidence	Positive Correct / Total Correct

Total/Positive/Tentative Correct Calls; [illegible]; Positive or Tentative Call

Lab#	Inlet	MS	MS2	Alg	Instrument	Age	% Acc	% ID	% Conf	Correct Tot	Correct PC	Correct TC	Wrong Tot	Wrong PW	Wrong TW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Wrong ID
[illegible]	nLC-NSI		+	[illegible]	[illegible]	[illegible]	100	80	100	4	4					P	P	P	P		
U8036	MALDI	+		Mf	P-Voy-DS	4	100	20	100	1	1					P					
[illegible]	NSI		+	[illegible]	[illegible]	[illegible]	100	60	100	3	3					P	P	P			
184M8	MALDI	+		Mc,Pf	M-MALDI	0.5	100	80	100	4	4					P	P	P	P		
[illegible]	nLC-NSI		+	[illegible]	[illegible]	[illegible]	100	60	[illegible]	[illegible]	3	1				P	P	P	T		
CC88L	MALDI	+		Mf	P-Voy-DS	2.5	100	60	100	3	3					P	P	P			
[illegible]	nLC-NSI		+	[illegible]	[illegible]	[illegible]	100	80	[illegible]	[illegible]	[illegible]	1				P	P	T			
87000	MALDI	+		Pf,Mf	P-Voy-DP	2	100	60	67	3	2	1				P	P		T		
[illegible]	nLC-NSI		+	[illegible]	[illegible]	3	100	80	100	4	4					P	P	P	P		
63798	MALDI	+	[illegible]	Mf	K-Axima	1	100	40	100	2	2					P	P				
[illegible]	nLC-NSI		+	[illegible]	[illegible]	2	100	40	100	2	2					P	P				
5823	MALDI	+		Mf	P-Voy-DP	3	75	60	100	3	3		1		1	P	P		P		1T
[illegible]	nLC-NSI		+	[illegible]	[illegible]	4	75	60	67	3	2	1	1		1	P	P	[illegible]			[illegible]
56388	MALDI	+		Mc	P-Voy-DS	5	75	60	67	3	2	1	1		1	P	P	T			1T
[illegible]	nLC-NSI		+	[illegible]	[illegible]	0.5	100	80	100	4	4					P	P	P	P		
48583	MALDI	+		Pf	P-Voy-DP	1.5	100	20	100	1	1					P					
[illegible]	nLC-NSI		+	[illegible]	[illegible]	1	100	40	100	2	2					P	P				
29836	MALDI	+		Mf	P-Voy-DP	3	100	40	100	2	2					P	P				
[illegible]	nLC-NSI		+	[illegible]	[illegible]	3	100	100	80	[illegible]	4	1				P	P	P	P	1	
21288	MALDI	+		Pf,Mf	P-Voy-DS	1	100	60	100	3	3					P	P	P			
18001	nLC-NSI		+	[illegible]	[illegible]	1	100	60	100	3	3					P	P	P			
15381	MALDI	+		Pf	B-Biflex	3	100	40	100	2	2					P	P				
[illegible]	nLC-NSI		+	[illegible]	[illegible]	1	100	40	100	2	2					P	P				
13038	MALDI	+		Pf	P-Voy-DP	2	100	40	100	2	2					P	P				
[illegible]	NSI		+	[illegible]	[illegible]	[illegible]	100	40	100	2	2					P	P				
11128	MALDI	+		Mc,Pf	B-Biflex III	2	100	40	100	2	2					P	P				
0715	NSI	+		Pf	[illegible]	[illegible]	100	60	100	3	3					P	P	P			
0715	LC-ESI		+	Mc	S-DS	2	100	40	100	2	2					P	P				

Lab#	Inlet	[illegible] %COV	PDI %COV	PDI [illegible]	[illegible] %COV	[illegible]	[illegible] %COV	[illegible]	BSA %COV	BSA [illegible]	SOD %COV	SOD [illegible]	Buffer used to dissolve	Vol µl	% organic	Desalt method
[illegible]	nLC-NSI	4	18	7		4		5		2			0.1%TFA	20	25	Cap trap
U8036	MALDI	4	18	7									0.1%TFA	20	25	
[illegible]	NSI	27	55	32	54	17	20	12					50%CN/0.1%TFA	5	10	Poros C18/Oligo R3/Graphite
184M8	MALDI	32	55	32	54	17	28	12	22	13			50%CN/0.1%TFA	5	10	
[illegible]	nLC-NSI	14	77	15	38	6	14	7		1			50%CN/0.1%TFA	5	45	
CC88L	MALDI	14	27	18	30	9	14	6					50%CN/0.2%TFA	5	45	
[illegible]	nLC-NSI	11	34	19	22	6							[illegible]	10	20	
87000	MALDI	14	34	10	22	6			12	8			50%CN/0.05%TFA	10	20	Zip
[illegible]	nLC-NSI	11	24	10	32	10		3		3			[illegible]	10	50	
63798	MALDI	11	24	12	32	10							50%CN/1%TFA	10	50	
[illegible]	nLC-NSI	8	24	10	14	7							[illegible]		5	
5823	MALDI	13	24	12	14	T			28	14			50%CN/0.1%TFA		5	
[illegible]	nLC-NSI			7		6		3					[illegible]		3.5	other
56388	MALDI			T		5		3					2%CN/0.05%TFA		3.5	
[illegible]	nLC-NSI	16	38	26	29	8	10	6	7	4			[illegible]	6	40	
48583	MALDI												2%CN/1%TFA	5	40	
[illegible]	nLC-NSI	8	21	11	17	4							0.1%TFA	60	25	
29836	MALDI	13	27	14	27	14							0.1%TFA	50	50	C18 Zip
[illegible]	nLC-NSI	[illegible]	29	18	26	10	10	8	12	8	28	8	0.1%TFA	10	20	
21288	MALDI	14	29	17	22	18	19	12					0.1%TFA	10	20	
18001	nLC-NSI	4	22	6		3		2					5%FA	10	20	C18 Zip
15381	MALDI			8		3		2					5%FA	10	20	
[illegible]	nLC-NSI			7		4							50%CN/0.1%TFA	5	40	C18 Zip
13038	MALDI	13	27		26								50%CN/0.1%TFA	5	20	
[illegible]	NSI			4		3							50%CN/0.1%TFA		25	C18 Zip
11128	MALDI	13	33	13	32	8							50%CN/0.1%TFA	2	35	
0715	NSI	28	58	33	52	21	24	15					0.1%FA	4	50	
0715	LC-ESI	21	53	39	52	21							0.1%TFA	4	50	

Search Algorithm Key: Mn: Manual; Mc: Mascot; Mf: MS-Fit; Mt: MS-Tag; Pf: Profound; Pp: Pepsea; Sn: Sequest; O: Other

MS Instrument Key: K: Kratos; P: Perseptive; [illegible]

	# Labs
NSI & MALDI same # IDs	4
NSI more Correct IDs than MALDI	6
MALDI more correct IDs than NSI	2
NSI & MALDI differ in the proteins ID'd	1
NSI more Correct IDs than LC-ESI	1

There were 14 laboratories that ran two types of MS on the sample. Each of these laboratories ran NSI, with 13 responses also running MALDI-MS. Only one of these laboratories called all five proteins correctly (4PC, 1TC); four laboratories identified four proteins. Overall, it is difficult to determine the impact that two different types of MS analysis had on the interpretation of the data.

REFERENCES

1. Gygi SP, Corthals GL, Zhang Y, Rochon Y, Aebersold R. Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. *Proc Natl Acad Sci* 2000;97:9390–9395.
2. Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, Morris DR, Garvik BM, Yates, JR. Direct analysis of protein complexes using mass spectrometry. *Nat Biotechnol* 1999;17:676–682.
3. Gygi SP, Rochon Y, Franza BP, Aebersold R. Correlation between protein and mRNA abundance in yeast. *Mol.Cell Biol* 1999;19:1720–1730.
4. Lian Z, Yamaga S, Bonds W, Beazer-Barclay Y, Kluger Y, Gerstein M, Newburger PE, Berliner N, Weissman SM. Genomic and proteomic analysis of the myeloid differentiation program. *Blood* 2001;98:513–524.
5. Peng J, Gygi SP. Proteomics: the move to mixtures. *J Mass Spectrom* 2001;36:1083–1091.