

*Proteomics Standards
Research Group*

sPRG2006: A Proteomics Standard

www.abrf.org/sprg



Proteomics Standards Research Group (sPRG) Members

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Phil Andrews - University of Michigan

David Arnott - Genentech, Inc.

Mary Ann Gawinowicz - Columbia University

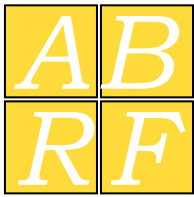
Jeffrey A. Kowalak - National Institutes of Health (Chair)

William S. Lane - Harvard University (EB Liaison)

Kathryn S. Lilley - University of Cambridge

Larry Martin - East West University

Stephen E. Stein - NIST



Mission Statement

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- Promote and support the development and use of standards in Proteomics.
- Identify and implement technical standards that reflect the ABRF's commitment to accuracy, clarity, and consistency.
- Standards include, but are not limited to, reference materials, data sets, conditions and procedures that give researchers independent criteria to evaluate their abilities to produce predictable, consistent results.
- Strongly support efforts for standardization of the recording and reporting of proteomics experiments.



sPRG Website

www.abrf.org/sprg

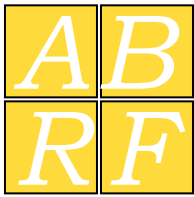
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The screenshot shows the ABRF website interface. At the top, the ABRF logo and the text "The Association of Biomolecular Resource Facilities" are displayed. Navigation links include "Home", "ABRF Sponsors", "Contact Us", and "Help". Below this, there are links for "White Pages", "Yellow Pages", "Log In", and "Join ABRF".

On the left side, there is a search bar with a "GO" button and a link to "Advanced Search". Below the search bar is a vertical navigation menu with the following items: "About ABRF", "Forms and Documents", "News and Announcements", "Communications", "Research Groups" (circled in red), "Open Research Studies", "Committees", "Reference", "Sponsorship", "Flyout Menu", and "Featured Sponsor: Thermo ELECTRON CORPORATION".

A dropdown menu is open from "Research Groups", listing various research areas: "Amino Acid Analysis", "Computational Biology", "DNA Sequencing", "Edman Sequencing", "Fragment Analysis", "Microarray research group (MARG)", "Molecular Interactions", "Nucleic Acids", "Proteomics", "Proteomics Standards Research Group" (circled in red), "Survey", and "Former Research Groups".

The main content area features a news article titled "Roger Tsien to receive the 2006 ABRF Award". The article text includes: "Scientist cited for his pioneering work in the of in vivo fluorescence methods for the molecular processes." and "eting ranked in the top 10! 2006 and see why Genome Technology did it as one of the best!". There are "Read more..." links for both paragraphs. Below the article, there is a link for "Atlantic Macromolecular phy Meeting" and the address "Wake Forest University, Winston-Salem, North Carolina, United States".



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Proteomics Standards in the Literature

Keller, A., Purvine, S., Nesvizhskii, A.I., Stolyar, S., Goodlett, D.R. and E. Kolker, E. (2002) “Experimental Protein Mixture for Validating Tandem Mass Spectral Analysis” *OMICS* **6**: 207-212

Authors described a data set of peptide tandem mass spectra generated from a control mixture of 18 purified proteins that can be employed to evaluate strategies for tandem mass spectral analysis.

Purvine, S., Picone, A.F., and E. Kolker, E. (2004) “Standard Mixtures for Proteome Studies” *OMICS* **8**: 79-92

Authors introduced a mixture of 23 peptides and 12 protein digests to serve as possible standard for proteome analyses.



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Materials

- Human proteins, purified from their biological source or recombinantly expressed.
- Subjected to multiple analytical methodologies, e.g., 1D PAGE, IEF, and RP-HPLC, in order to assess required levels of purity ($\geq 95\%$).
- Protein concentration was determined by amino acid analysis.
- Five picomole aliquots of each protein were combined and lyophilized in a 1 mL polypropylene tube.



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Standard Validation

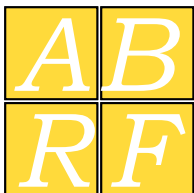
- Prior to distribution to participating labs, the prototype protein standard was distributed to the sPRG committee members and analyzed by 5 different member laboratories for validation.
- In three RG laboratories, the standard was analyzed using a shotgun approach by digestion with trypsin of the entire protein mixture followed by on-line 1D LC-MS/MS, on-line 2D LC-MS/MS, off-line capillary RP-HPLC, TOF/TOF
- In two RG laboratories, the protein mixture was partially separated by 1D SDS-PAGE, followed by in-gel tryptic digestion and on-line 1D LC-MS/MS.



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Results Submission

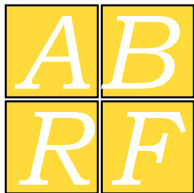
- Results were submitted anonymously through an on-line survey and included experimental details, instrument(s) used, and a complete list of all proteins identified with accession numbers.
- A web site (www.proteomecommons.org/dev/abrf/) was provided for the voluntary submission of raw mass spectral data to be made available for future studies.



60.5% Data Return

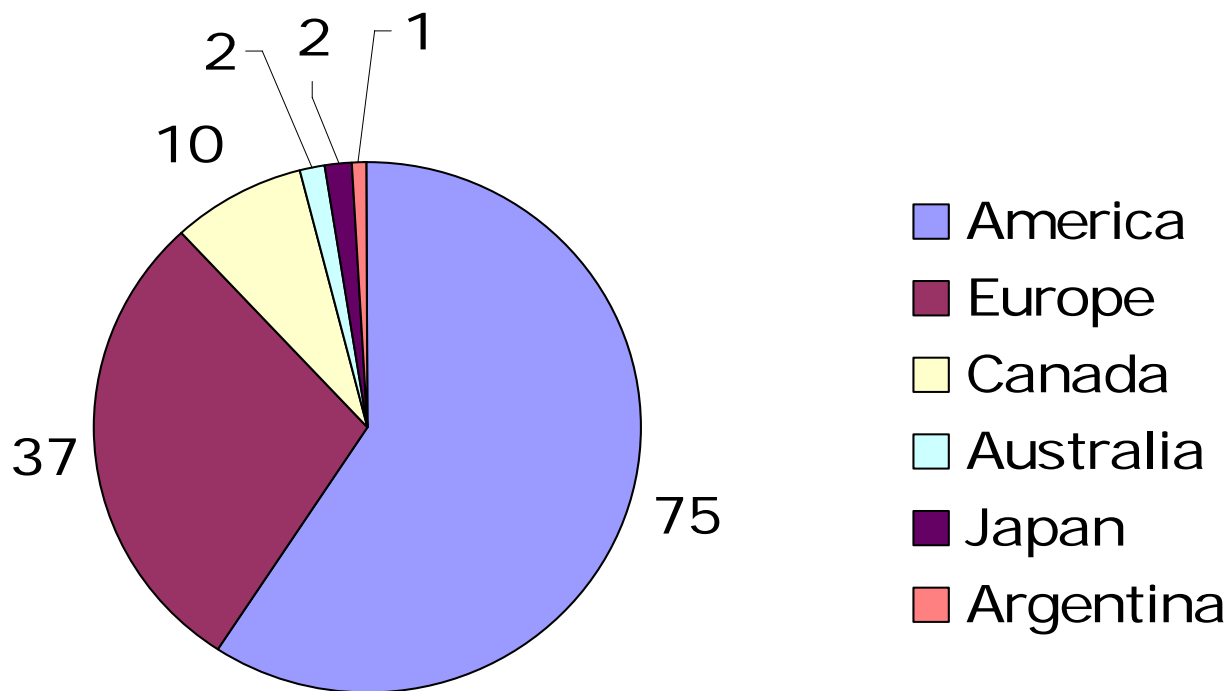
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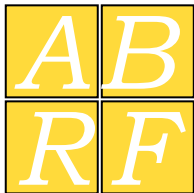




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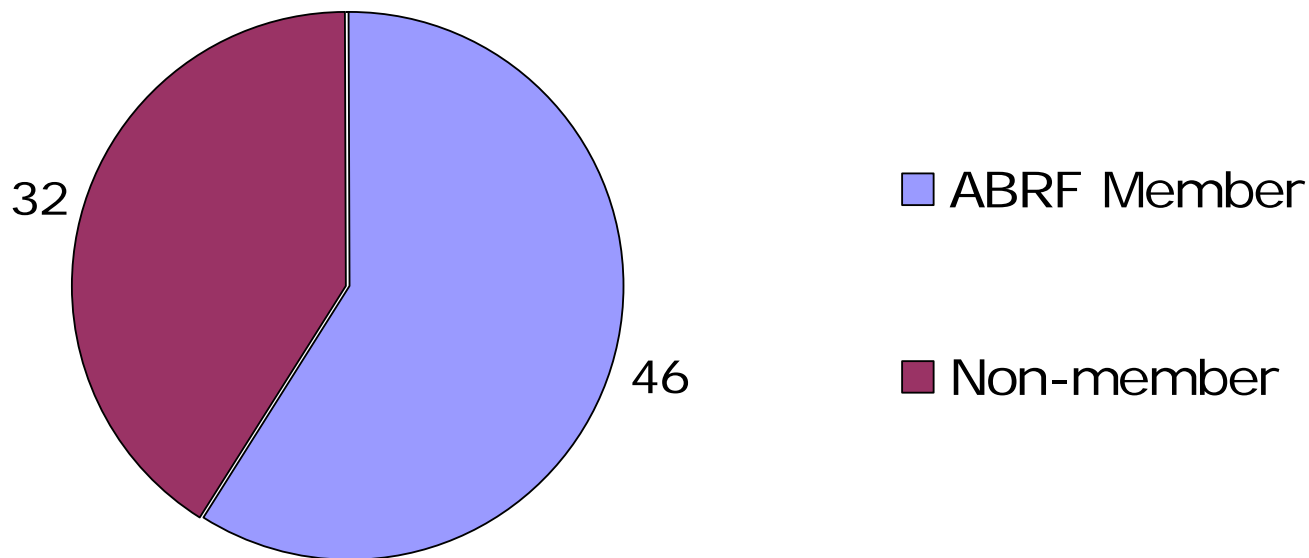
sPRG2006 Requester Demographics





sPRG2006 ABRF Membership

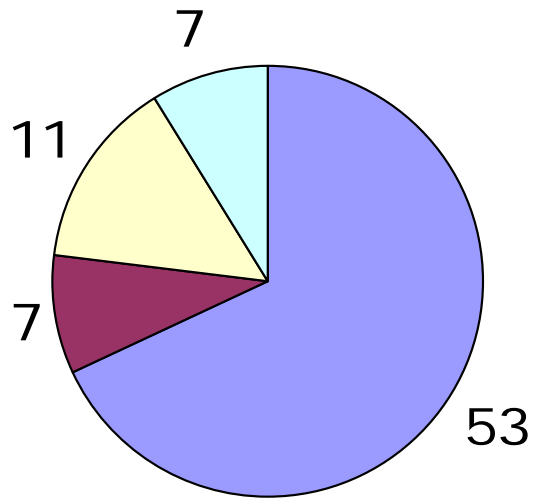
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sPRG2006 Laboratories

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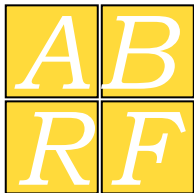


Academic

Biotech/Pharma/Industrial

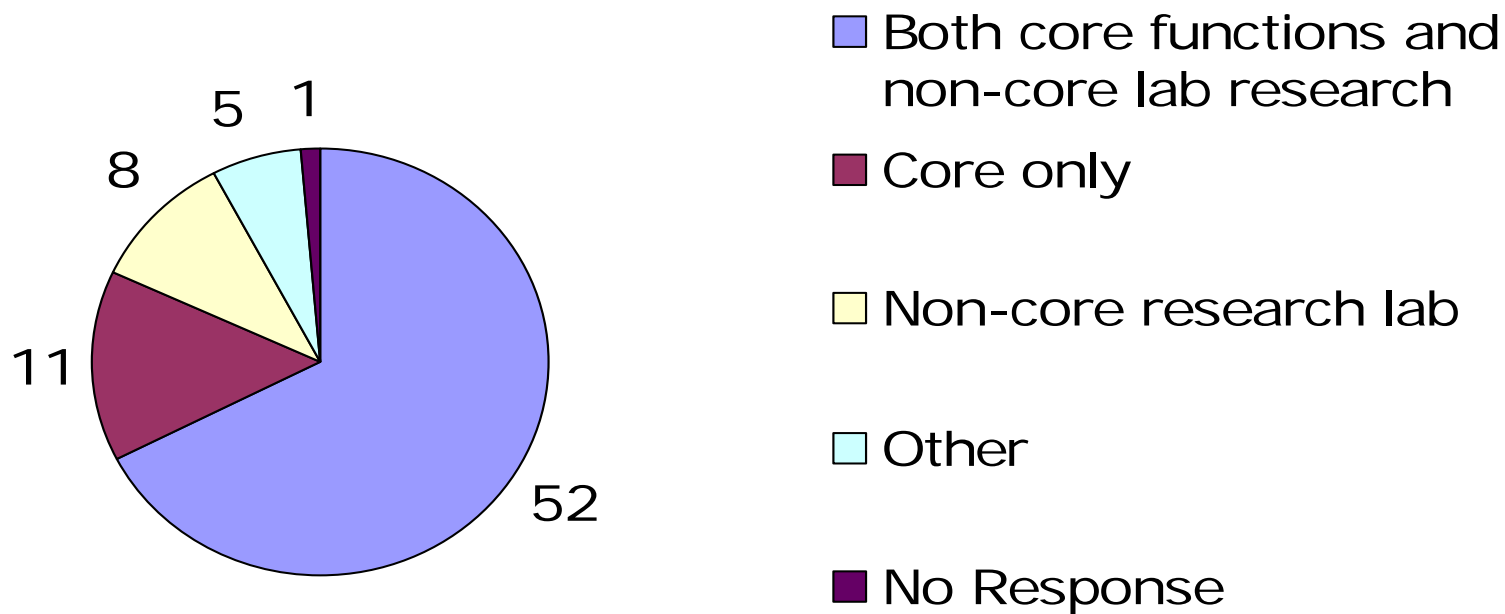
Government

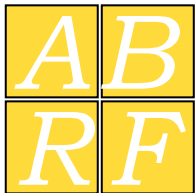
Manufacturer/Vendor



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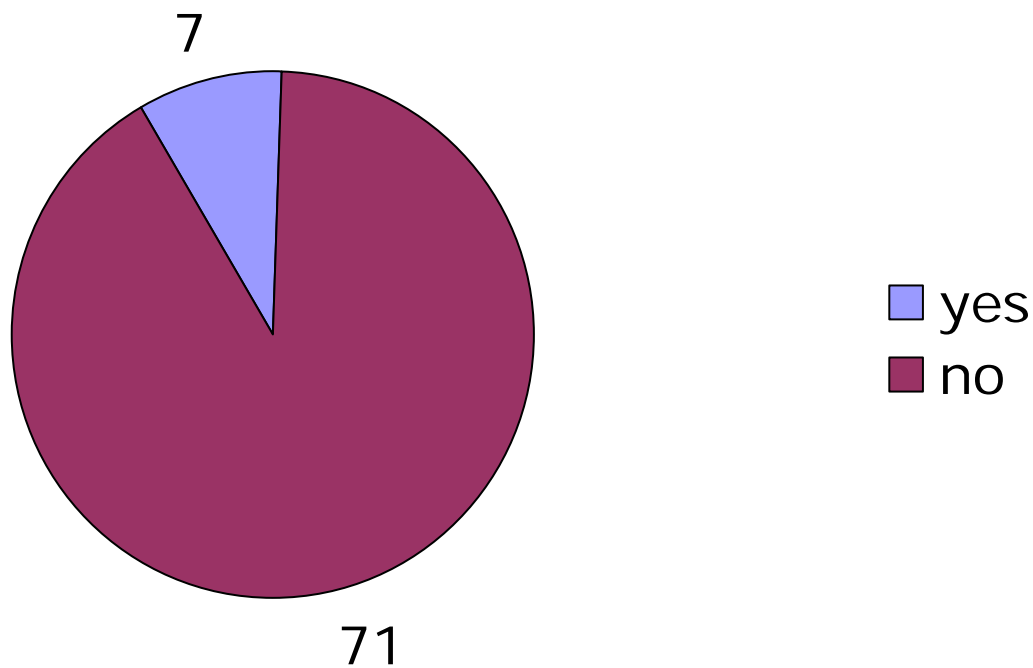
sPRG2006 Facility Type

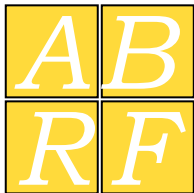




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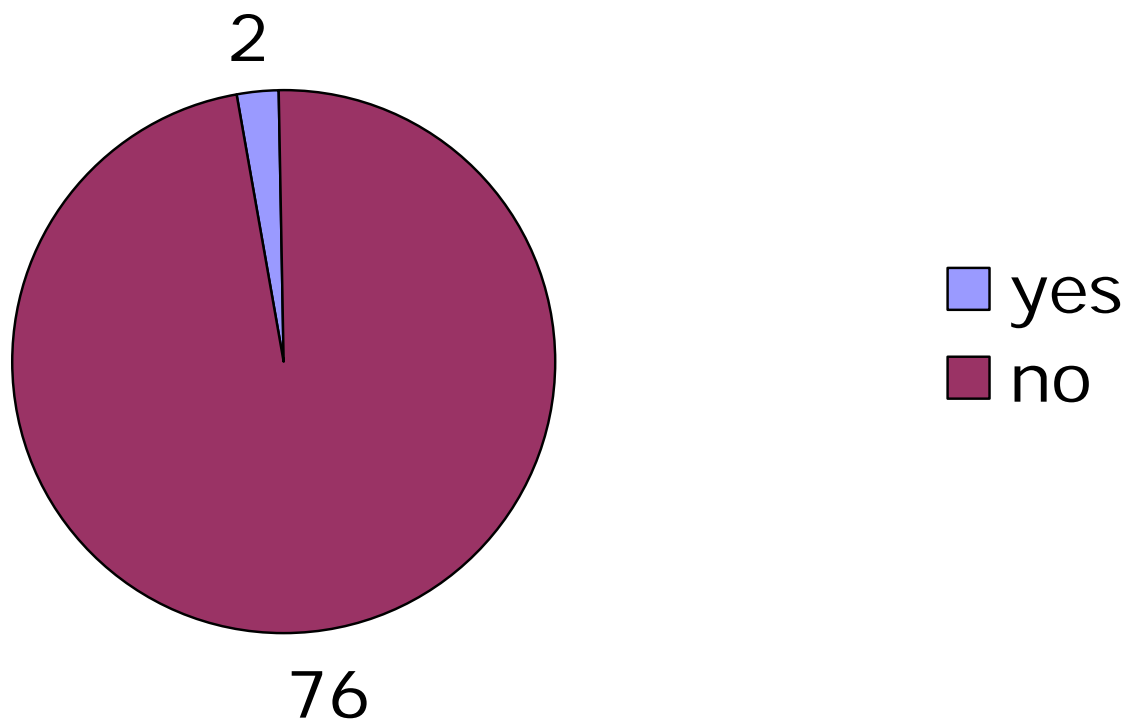
Did you receive more than 1 sample?





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Did you have prior knowledge
of another lab's results?



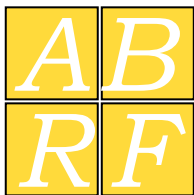


sPRG2006 Proteomics Standard

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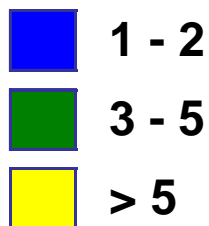
Accsn	Entry	Protein	Accsn	Entry	Protein
P01008	ANT3_HUMAN	Antithrombin-III precursor	P01343	IGF1A_HUMAN	Insulin-like growth factor IA
P02768	ALBU_HUMAN	Serum albumin	P12081	SYH_HUMAN	Histidyl-tRNA synthetase
P08758	ANXA5_HUMAN	Annexin A5	P02788	TRFL_HUMAN	Lactotransferrin
P55957	BID_HUMAN	BH3 interacting domain death agonist	P41159	LEP_HUMAN	Leptin
P00167	CYB5_HUMAN	Cytochrome b5	P61626	LYSC_HUMAN	Lysozyme C
P99999	CYC_HUMAN	Cytochrome c	P00709	LALBA_HUMAN	Alpha-lactalbumin
P04040	CATA_HUMAN	Catalase	P61769	B2MG_HUMAN	Beta-2-microglobulin
P62937	PPIA_HUMAN	Peptidyl-prolyl cis-trans isomerase A	P02144	MYG_HUMAN	Myoglobin
P02741	CRP_HUMAN	C-reactive protein	Q15843	NEDD8_HUMAN	Neddylin
P00915	CAH1_HUMAN	Carbonic anhydrase 1	P01127	PDGFB_HUMAN	Platelet-derived growth factor B-chn
P08311	CATG_HUMAN	Cathepsin G	Q06830	PRDX1_HUMAN	Peroxiredoxin 1
P01031	CO5_HUMAN	Complement C5	P16083	NQO2_HUMAN	NRH dehydrogenase [quinone] 2
P00918	CAH2_HUMAN	Carbonic anhydrase 2	P02753	RETBP_HUMAN	Retinol-binding protein
P07339	CATD_HUMAN	Cathepsin D	P01112	RASH_HUMAN	GTPase HRas
P06732	KCRM_HUMAN	Creatine kinase M-type	P63165	SUMO1_HUMAN	Small ubiquitin-related modifier 1
P15559	NQO1_HUMAN	Quinone reductase 1	P00441	SODC_HUMAN	Superoxide dismutase [Cu-Zn]
P01133	EGF_HUMAN	Pro-epidermal growth factor	P02787	TRFE_HUMAN	Serotransferrin
P05413	FABPH_HUMAN	Fatty acid-binding protein	P01375	TNFA_HUMAN	Tumor necrosis factor
P06396	GELS_HUMAN	Gelsolin	P10599	THIO_HUMAN	Thioredoxin
P08263	GSTA1_HUMAN	Glutathione S-transferase A1	P10636	TAU_HUMAN	Microtubule-associated protein tau
P09211	GSTP1_HUMAN	Glutathione S-transferase P	P62988	UBIQ_HUMAN	Ubiquitin
P69905	HBA_HUMAN	Hemoglobin alpha chain	P51965	UB2E1_HUMAN	Ubiquitin-conjugating enz E2 E1
P68871	HBB_HUMAN	Hemoglobin beta chain	P63279	UBE2L_HUMAN	Ubiquitin carrier protein 9
P10145	IL8_HUMAN	Interleukin-8	O00762	UBE2C_HUMAN	Ubiquitin-conjugating enzyme E2 C
P01344	IGF2_HUMAN	Insulin-like growth factor II			



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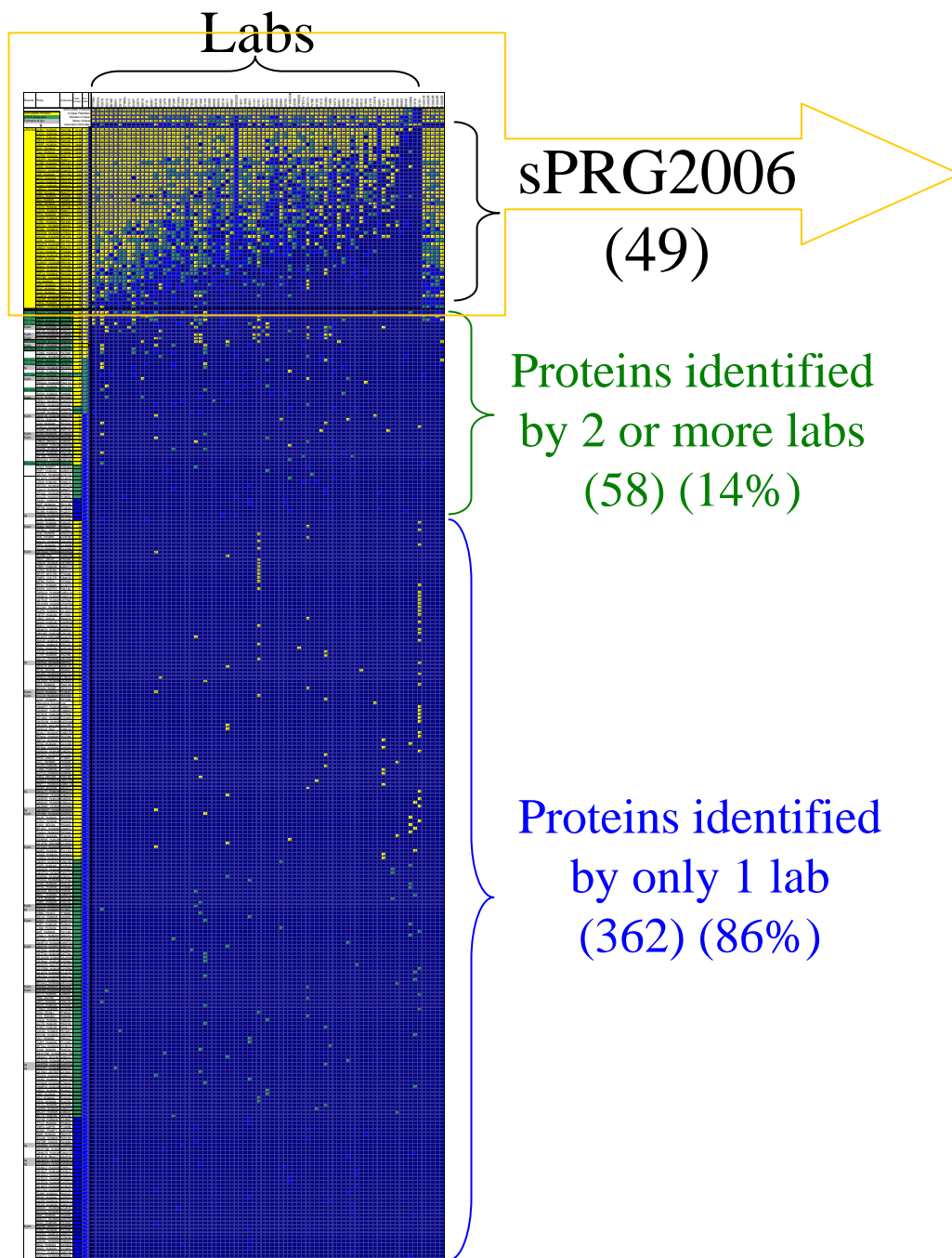
sPRG2006 Protein Identification

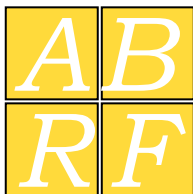
Legend



www.abrf.org/sprg

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sPRG2006 Protein Identification

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Legend:



1 - 2



3 - 5



> 5

Source	Entry	Accession	Total Unique	# of Labs
sPRG2006 Proteins:				
Unique Peptides:				
Median Unique:				
Mean Unique:				
Incorrect (Only Lab):				
sPRG50	ALBU HUMAN	P02768	2102	76
	CATA HUMAN	P04040	1153	74
	ANT3 HUMAN	P01008	1100	74
	GELS HUMAN	P06396	1190	73
	TRFE HUMAN	P02787	1898	71
	SYH HUMAN	P12081	1066	70
	TAU HUMAN	P10636	1059	70
	KCRM HUMAN	P06732	1224	68
	GSTP1 HUMAN	P09211	426	68
	CYB5 HUMAN	P00167	322	68
	TRFL HUMAN	P02788	1252	67
	PRDX1 HUMAN	Q06830	549	67
	LEP HUMAN	P41159	353	66
	MYG HUMAN	P02144	439	65
	CAH2 HUMAN	P00918	712	64
	CAH1 HUMAN	P00915	554	64
	UBE2C HUMAN	O00762	428	64
	HBB HUMAN	P68871	425	64
	UBIQ HUMAN	P62988	493	63
	BID HUMAN	P55957	383	63
	HBA HUMAN	P69905	288	63
	CATD HUMAN	P07339	521	62
	CATG HUMAN	P08311	452	62
	PPIA HUMAN	P62937	378	61
	RASH HUMAN	P01112	422	60
	LYSC HUMAN	P61626	255	58
	GSTA1 HUMAN	P08263	368	57
	SODC HUMAN	P00441	247	57
	NEDD8 HUMAN	Q15843	181	57
	ANXA5 HUMAN	P08758	438	55
	COS HUMAN	P01031	159	52
	PDGFB HUMAN	P01127	259	50
	UBE2I HUMAN	P63279	241	49
	THIO HUMAN	P10599	169	49
	NQO2 HUMAN	P16083	208	47
	CRP HUMAN	P02741	147	42
	SUMO1 HUMAN	P63165	133	42
	NQO1 HUMAN	P15559	184	39
	B2MG HUMAN	P61769	113	39
	IGF1A HUMAN	P01343	106	35
	ITLB HUMAN	P02753	122	34
	IGF2 HUMAN	P01344	85	33
	EGF HUMAN	P01133	97	30
	CYC HUMAN	P99999	79	26
	FABPH HUMAN	P05413	107	24
	LALBA HUMAN	P00709	40	19
	TNFA HUMAN	P01375	33	15
	UBZ1 HUMAN	P51965	40	14

Full sPRG2006 HeatMap available at www.abrf.org/sprg



Isoform Issue

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IGF1A_HUMAN

MGKISSLPTQLFKCCFCDFLKVKMHTMSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCG
DRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSARSVRAQRHTDMPKTQK
EVHLKNASRGSAGNKNYRM

IGF1B_HUMAN

MGKISSLPTQLFKCCFCDFLKVKMHTMSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCG
DRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSARSVRAQRHTDMPKTQK
YQPPSTNKNTKSQRRKGWPKTHPGGEQKEGTEASLQIRGKKKEQRREIGSRNAECRGKKKGK

IGF1A_HUMAN

GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA

IGF1B_HUMAN

GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA



Molecular and Cellular Proteomics Workshop

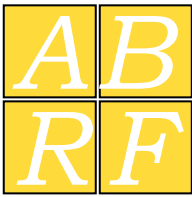
Paris - May 2005

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Revised Guideline 7.

- Identical peptide sequences can occur as elements of multiple unique protein sequences due to biological variation.
- Shotgun proteomics (proteolytic digestion of protein mixtures) introduces the complication of loss of connectivity between peptides and their protein precursors.
- Assignment of peptide sequences results in two outcomes; *distinct* peptides that map to only one protein sequence or *shared* peptides that map to more than one protein sequence.
- Detection of shared peptides introduces an uncertainty bioinformatics redundancy versus physical redundancy.
- The apparent ambiguity in peptide assignment requires reporting of a protein group.

Sean Seymour - de novo sequencing/DB searching tutorial session - Tuesday 11 am

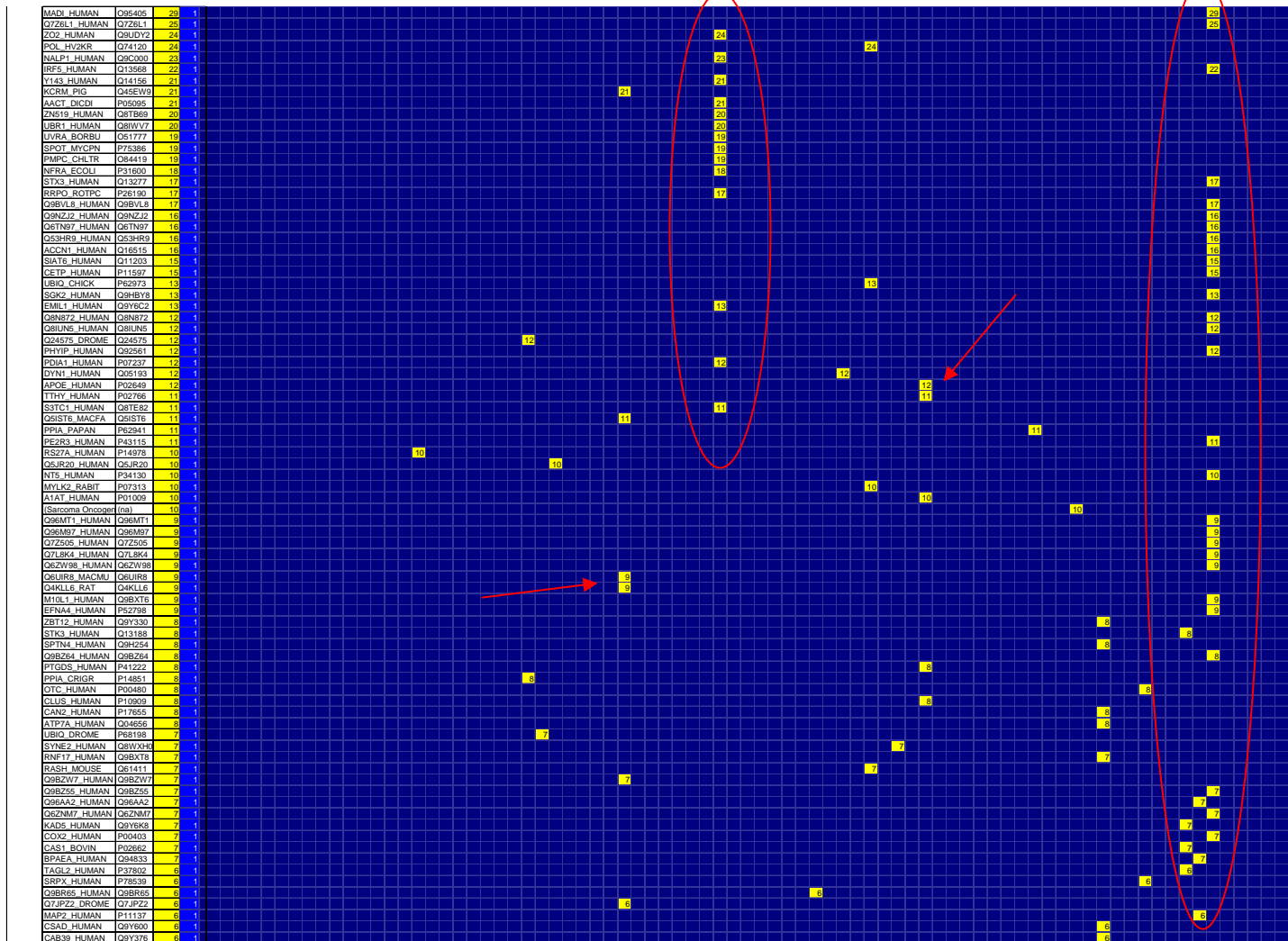


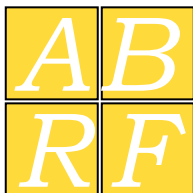
Incorrect IDs: High Peptide Count

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Legend: ■ 1 - 2 ■ 3 - 5 ■ > 5

INCORRECT PROTEINS (Non-Epithelial, Non-IgG, Proteins detected by only one lab)





Protein Identification Accuracy

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sPRG						
R0001R	49		381	6	8	
R0002R	47		408	6	9	
R0003R	49		317	4	6	
R0004R	48		390	6	8	
R0005R	48		645	8	13	

Total Correct / (Correct + Incorrect)

% Identified x Accuracy

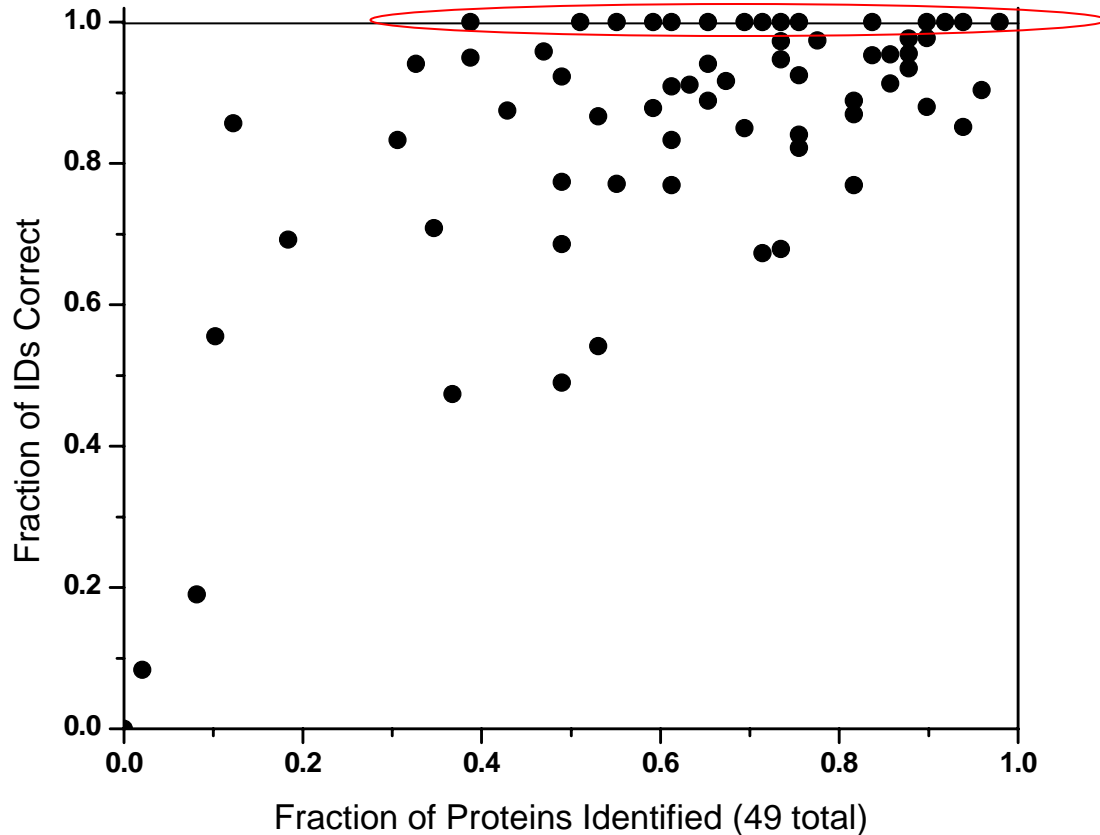
Lab	sPRG50 Identified		Incorrect	Accuracy	Overall	Unique Peptides		
	Proteins	%				Peptides	Median	Mean
77708	48	98%	0	100%	98%	808	11.5	16.8
29504	48	98%	0	100%	98%	497	6.0	10.4
72079	47	96%	5	90%	87%	883	13.0	18.8
65215	46	94%	0	100%	94%	646	9.0	14.0
98166	46	94%	0	100%	94%	576	9.0	12.5
58941	46	94%	0	100%	94%	568	9.5	12.3
29115	46	94%	8	85%	80%	562	8.5	12.2
27960	45	92%	0	100%	92%	712	11.0	15.8
17017	45	92%	0	100%	92%	702	12.0	15.6
42457	45	92%	0	100%	92%	547	10.0	12.2
12874	45	92%	0	100%	92%	442	7.0	9.8
46013	44	90%	0	100%	90%	520	8.5	11.8
0715	44	90%	0	100%	90%	477	8.0	10.8
26081	44	90%	1	98%	88%	349	5.5	7.9
92616	44	90%	6	88%	79%	419	5.5	9.5
22455	43	88%	1	98%	86%	631	10.0	14.7
21079	43	88%	2	96%	84%	347	5.0	8.1
10085	43	88%	2	96%	84%	222	4.0	5.2
37266	43	88%	3	93%	82%	306	5.0	7.1
97330	42	86%	2	95%	82%	36	5.0	6.2
53908	42	86%	4	91%	78%	340	5.5	8.1
77526	41	84%	0	100%	84%	185	3.0	4.5
74643	41	84%	2	95%	80%	381	6.0	9.3
62562	40	82%	5	89%	73%	462	8.0	11.6
27406	40	82%	6	87%	71%	219	4.5	5.5
00144	40	82%	12	77%	63%	570	7.5	14.2
22069	38	78%	1	97%	76%	195	4.0	5.1
98506	37	76%	0	100%	76%	160	3.0	4.3
40003	37	76%	3	93%	70%	118	2.0	3.2
25519	37	76%	7	84%	63%	177	4.0	4.8

06511	37	76%	8	82%	62%	338	7.0	9.1
14997	36	73%	0	100%	73%	190	4.0	5.3
1062000	36	73%	0	100%	73%	36	1.0	1.0
11641	36	73%	1	97%	71%	221	4.0	6.1
51958	36	73%	2	95%	70%	198	4.0	5.5
54601	36	73%	17	68%	50%	290	5.0	8.1
91741	35	71%	0	100%	71%	328	8.0	9.4
72791	35	71%	17	67%	48%	410	8.0	11.7
96751	34	69%	0	100%	69%	224	4.5	6.6
05013	34	69%	6	85%	59%	235	4.5	6.9
69089	33	67%	3	92%	62%	109	2.0	3.3
26402	32	65%	0	100%	65%	80	1.0	2.5
18984	32	65%	2	94%	61%	253	7.0	7.9
00700	32	65%	4	89%	58%	100	2.5	3.1
8165883487	31	63%	3	91%	58%	337	6.0	10.9
23258	30	61%	0	100%	61%	156	4.0	5.2
4788620	30	61%	3	91%	56%	158	3.5	5.3
47631	30	61%	6	83%	51%	122	3.5	4.1
53017	30	61%	9	77%	47%	371	7.0	12.4
70788	29	59%	0	100%	59%	111	3.0	3.8
29105	29	59%	4	88%	52%	213	6.0	7.3
21013	27	55%	0	100%	55%	222	7.0	8.2
10266	27	55%	8	77%	43%	383	8.0	14.2
71489	26	53%	4	87%	46%	128	4.0	4.9
14474	26	53%	22	54%	29%	95	2.0	3.7
38501	25	51%	0	100%	51%	283	8.0	11.3
58696	24	49%	2	92%	45%	317	5.0	13.2
F4755	24	49%	7	77%	38%	191	6.0	8.0
01903	24	49%	11	69%	34%	70	2.0	2.9
28629	24	49%	25	49%	24%	162	3.5	6.7
10812	23	47%	1	96%	45%	311	12.0	13.5
31960	21	43%	3	88%	38%	77	3.0	3.7
53178	19	39%	0	100%	39%	137	6.0	7.2
213479	19	39%	1	95%	37%	179	9.0	9.4
43691	18	37%	20	47%	17%	18	1.0	1.0
74187	17	35%	7	71%	25%	156	9.0	9.2
20014	16	33%	1	94%	31%	193	11.0	12.1
13895	15	31%	3	83%	26%	117	7.0	7.8
32344	9	18%	4	69%	13%	101	12.0	11.2
39563	6	12%	1	86%	10%	13	1.5	2.2
39440	5	10%	4	56%	6%	5	1.0	1.0
150866	4	8%	17	19%	1.6%	23	5.5	5.8
91919	1	2%	11	8%	0.2%	1	1.0	1.0
32181	0	0%	32	0%	0%	0	0.0	0.0

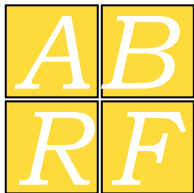


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Protein Identification Performance for 74 Labs

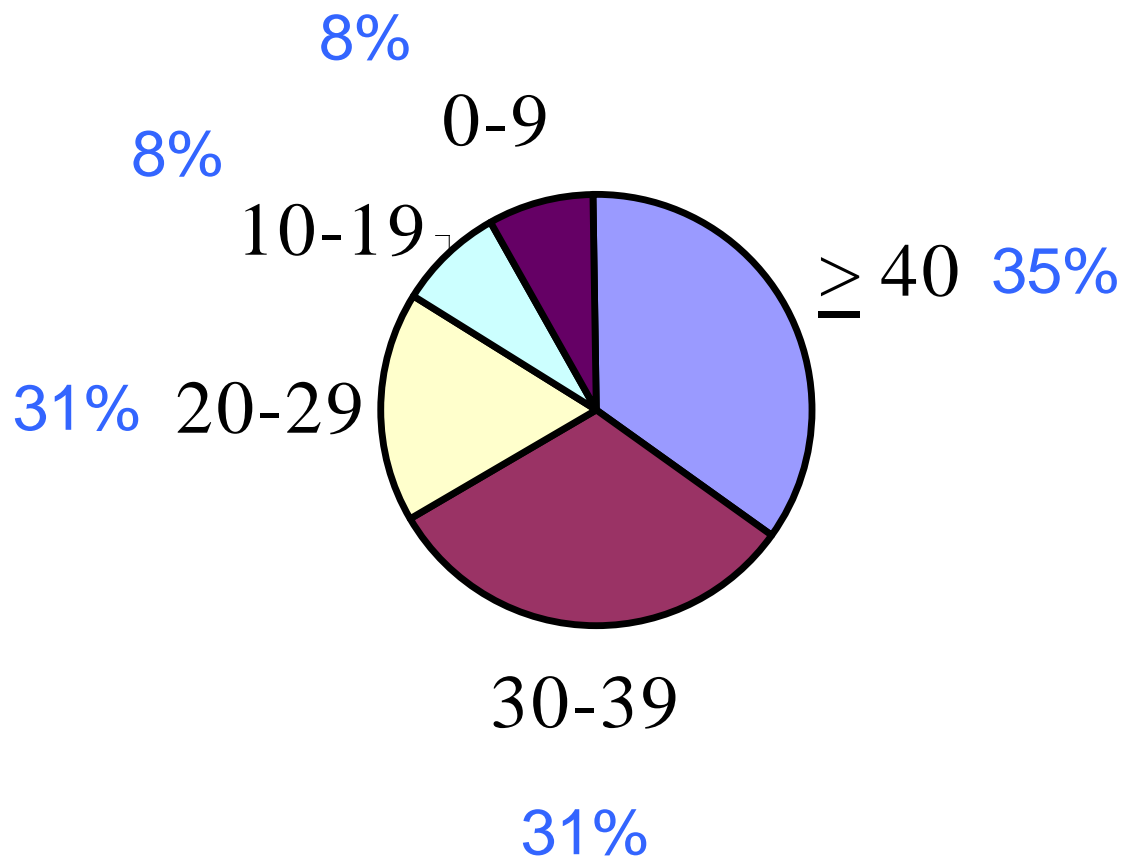


The performance of each lab is represented by a point defined by the fraction of all known proteins identified and the fraction of all reported identifications that were correct. Those labs with no false identifications fall along the upper axis.



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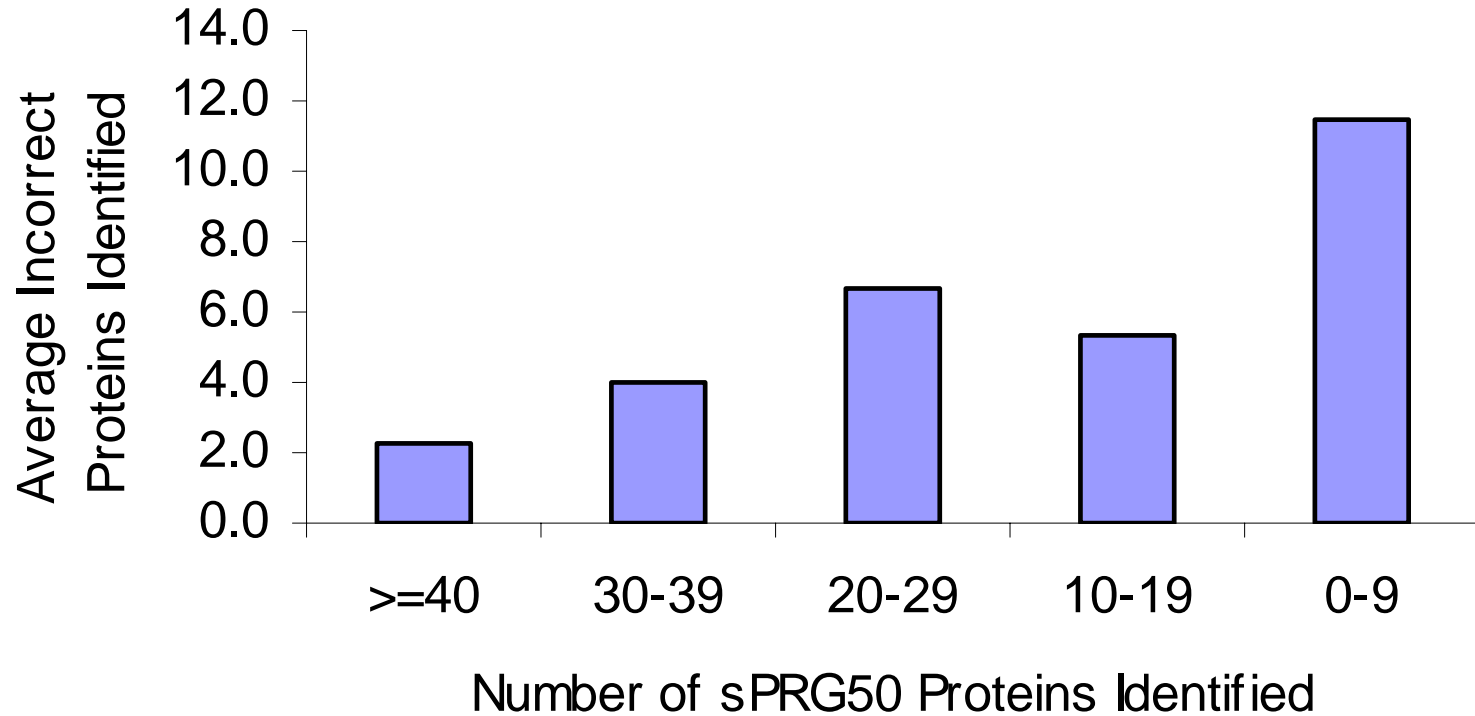
Number of Proteins Identified by Respondents





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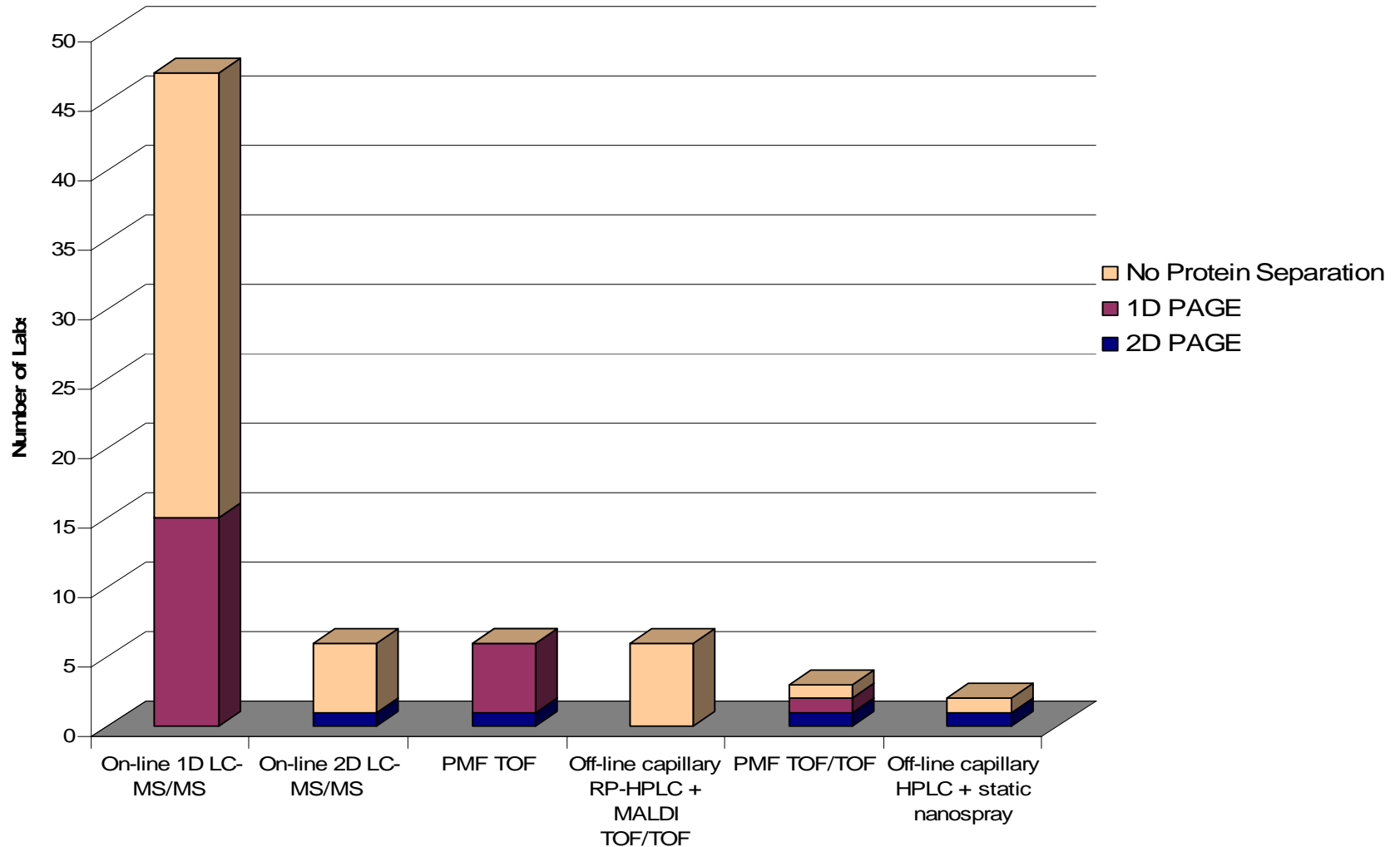
Labs detecting fewer correct proteins tended to identify more incorrect proteins

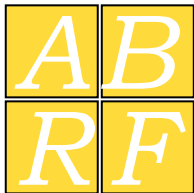




Primary Method of Analysis

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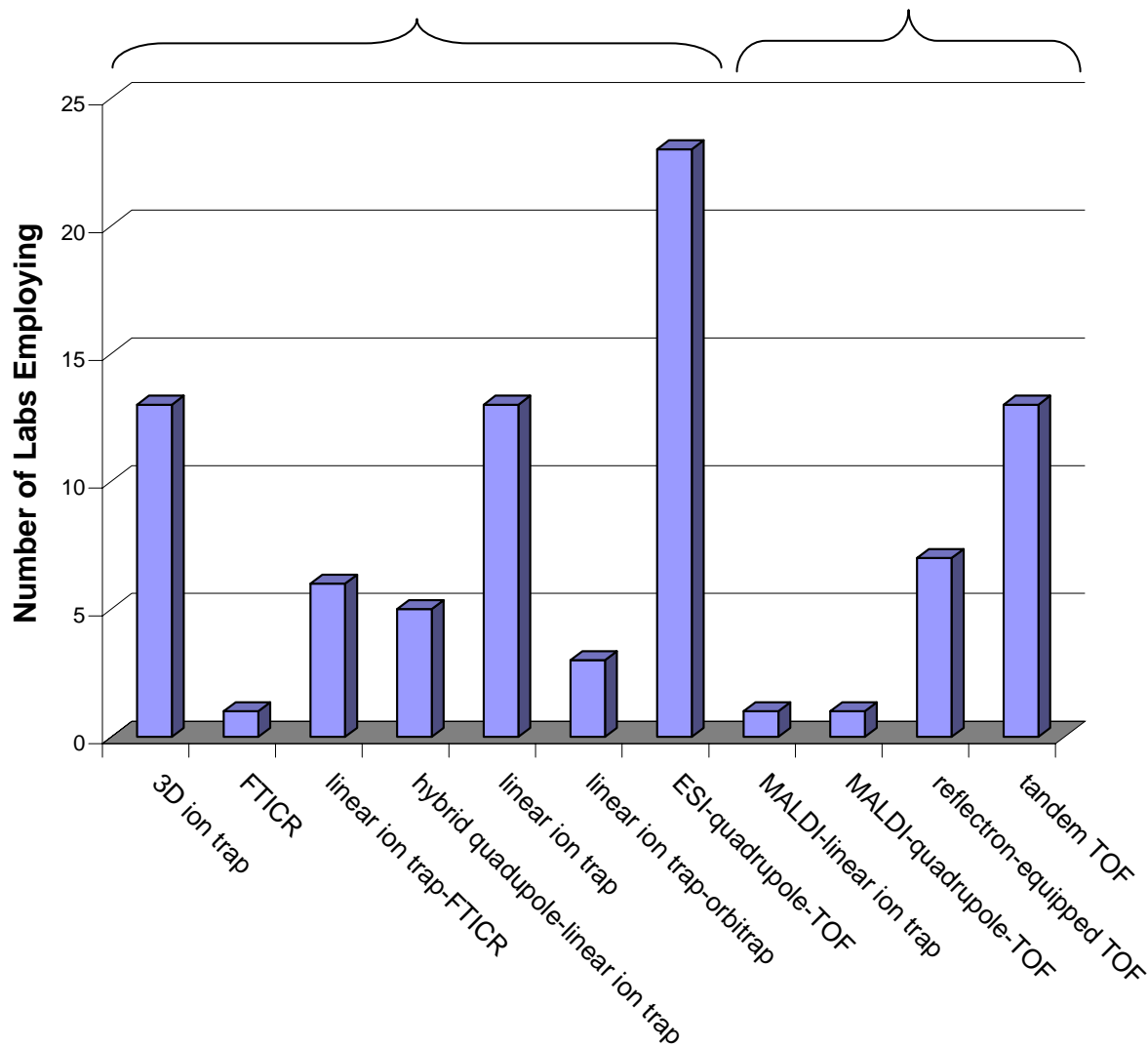


Mass Spectrometers Used

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Electrospray

MALDI



Identification Accuracy Using Different Experimental Approaches

Each data point represents the instrument(s) used and the accuracy achieved, where

Accuracy = total correct identifications / (correct identifications + incorrect identifications)

Brackets enclose range of scores for each method; slash indicates multiple instruments

	1	20	40	60	80	100
<u>Separation performed at the protein level</u>						
1D PAGE/LC-ESI-MS/MS			[g e d	a	dd d g a g e]	
1D PAGE/MALDI-MS	[i h	h				h]
1D PAGE/ESI and MALDI			[(a/h)	x	(a/i)]	
2D PAGE/MALDI			[(i/c)	i]		
1D Protein HPLC/ESI-MS/MS			[d		e]	

"Shotgun proteomic" approaches

LC-ESI-MS/MS			[dg	c	d df	gcg g dgdgc e gga]
LC-MALDI-TOF/TOF				[i	i	i i]
LC-MALDI and ESI			[(i/a)			(i/g)]
2D LC-ESI-MS/MS		[a	g	d	a g	b]

Split sample and did both protein separation and "shotgun" techniques

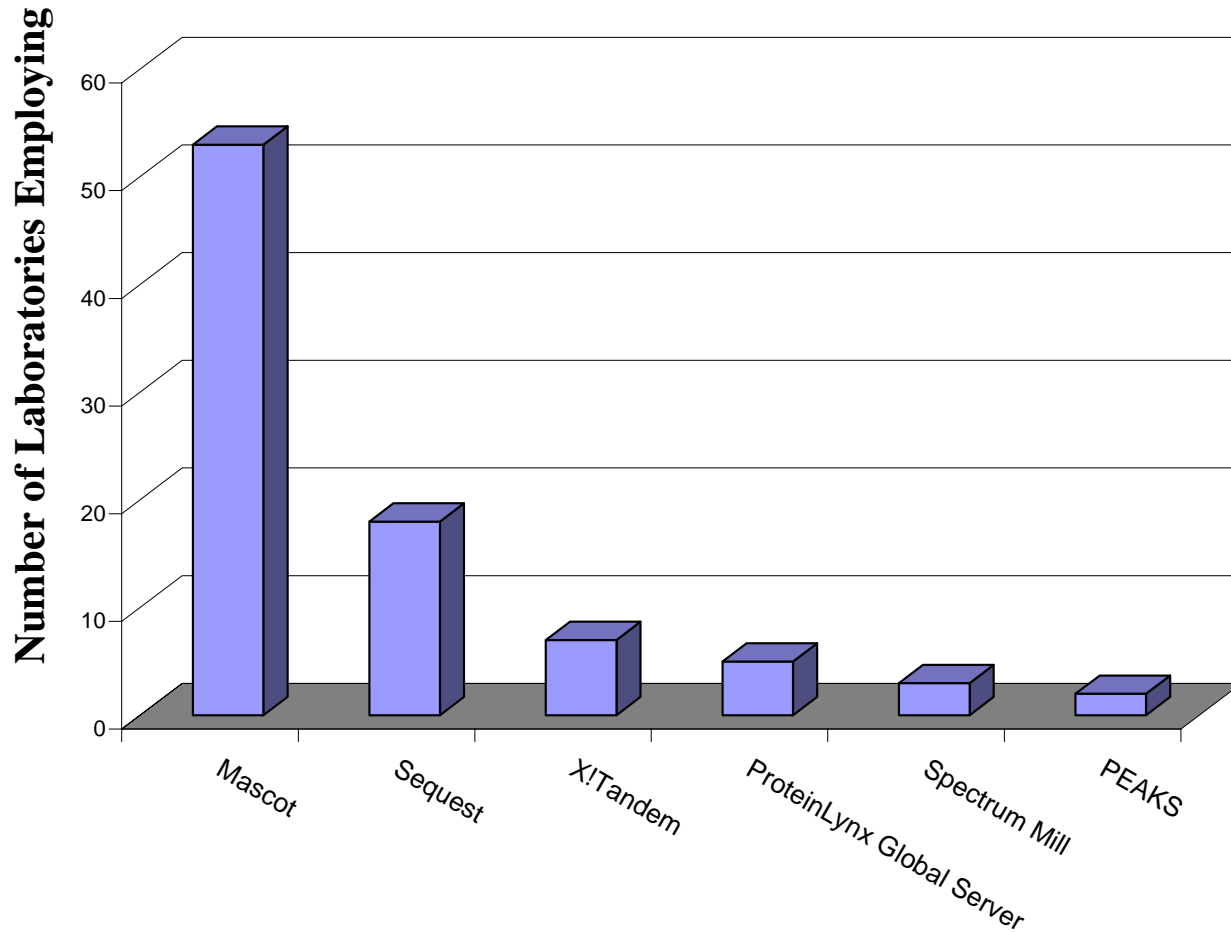
Shotgun + 1D PAGE				[g	g g	a x	i]
Combination w/ 2D PAGE					[(g/i)		g]
Truly miscellaneous	[(g/h)	a	x	a]			

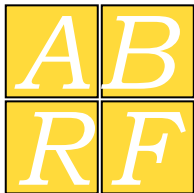
a=3D ion trap, b=FTICR, c=hybrid quadrupole-linear ion trap, d=linear ion trap
 e=linear ion trap-FTICR, f=linear ion trap-orbitrap, g=quadrupole-TOF
 h=reflectron-equipped TOF, i=tandem TOF, x=other combination



Search Engines Used for Protein Identification from Tandem Mass Spectra

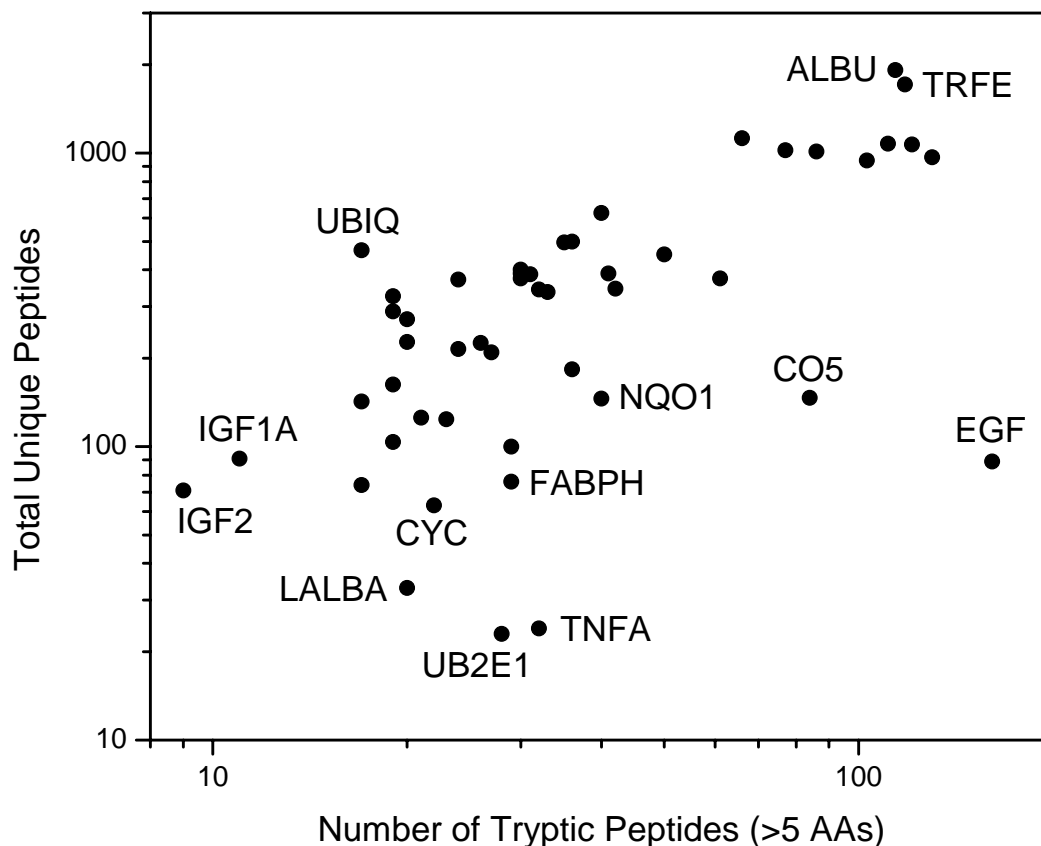
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Comparison of Observed vs Expected Unique Tryptic Peptides

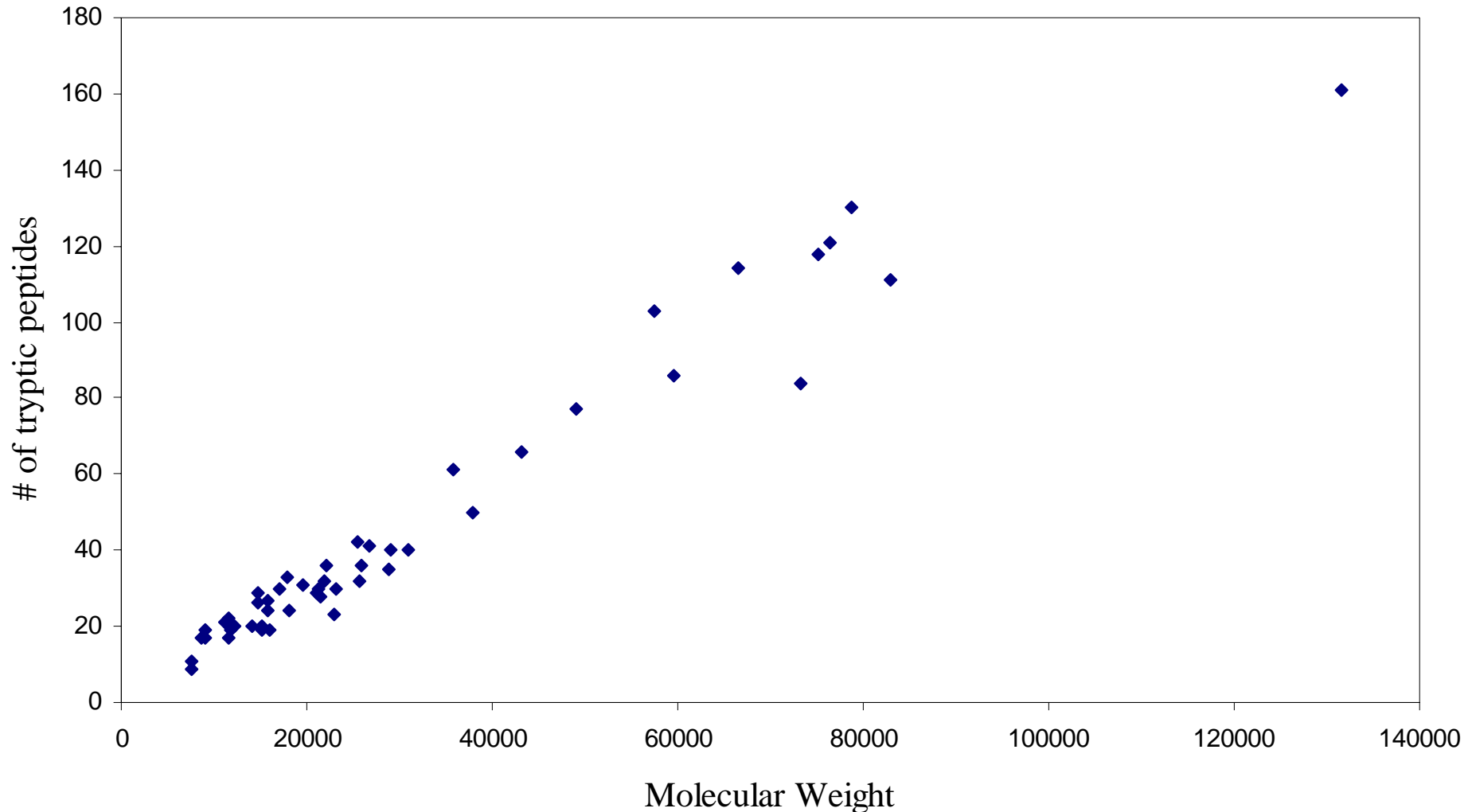


For each protein in the test mixture, the total number of reported unique peptides is compared to the maximum expected number of different simple tryptic peptides. Since an effort was made to prepare the proteins in eqimolar amounts (5 pmol), if all peptides were formed and detected with equal efficiency, a straight line would result. The origin of large dispersion is not clear, but outliers at the lower right are glycoproteins (CO5_HUMAN and EFG_HUMAN) while the highest yields are from secreted proteins (ALBU_HUMAN and TRFE_HUMAN). Other protein at the extremities are also labeled.



Theoretical number of tryptic peptides vs molecular weight

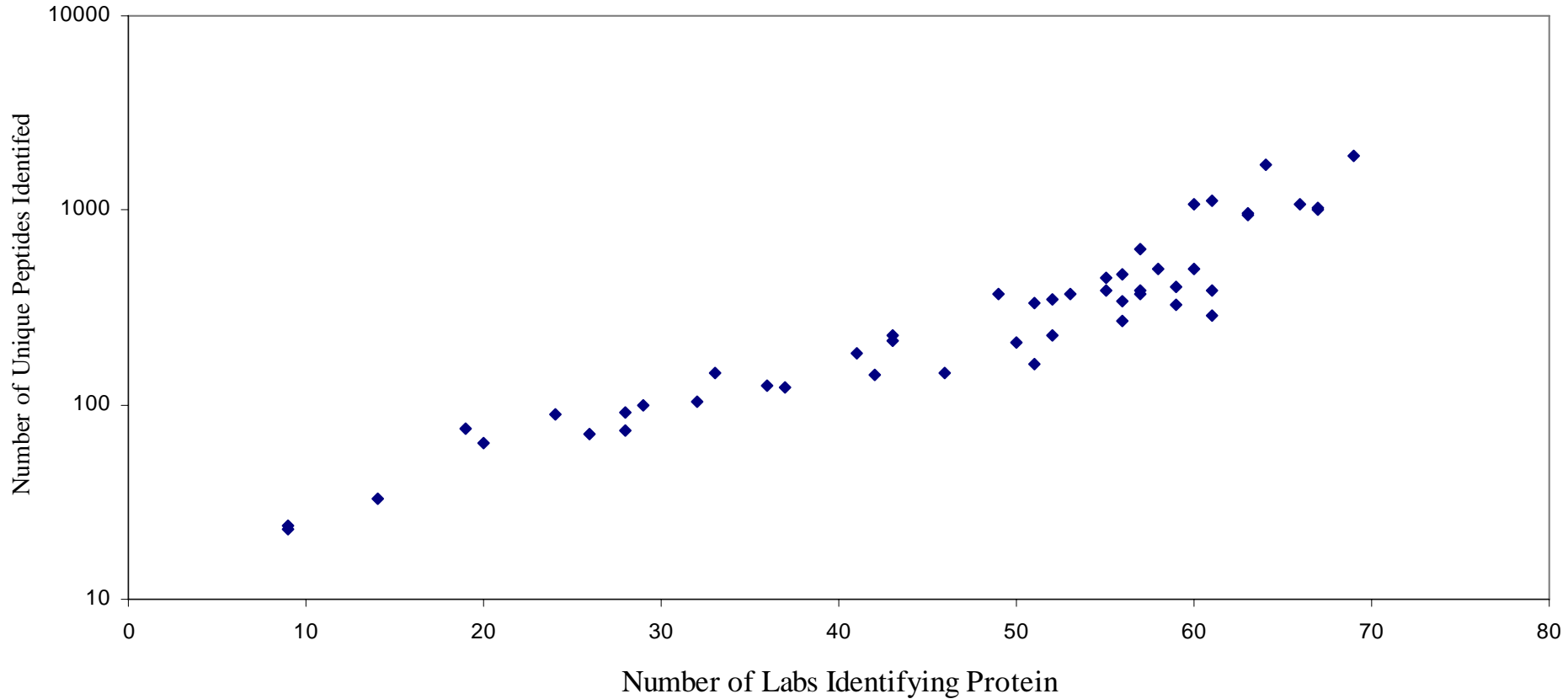
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Unique Peptides vs Successful IDs

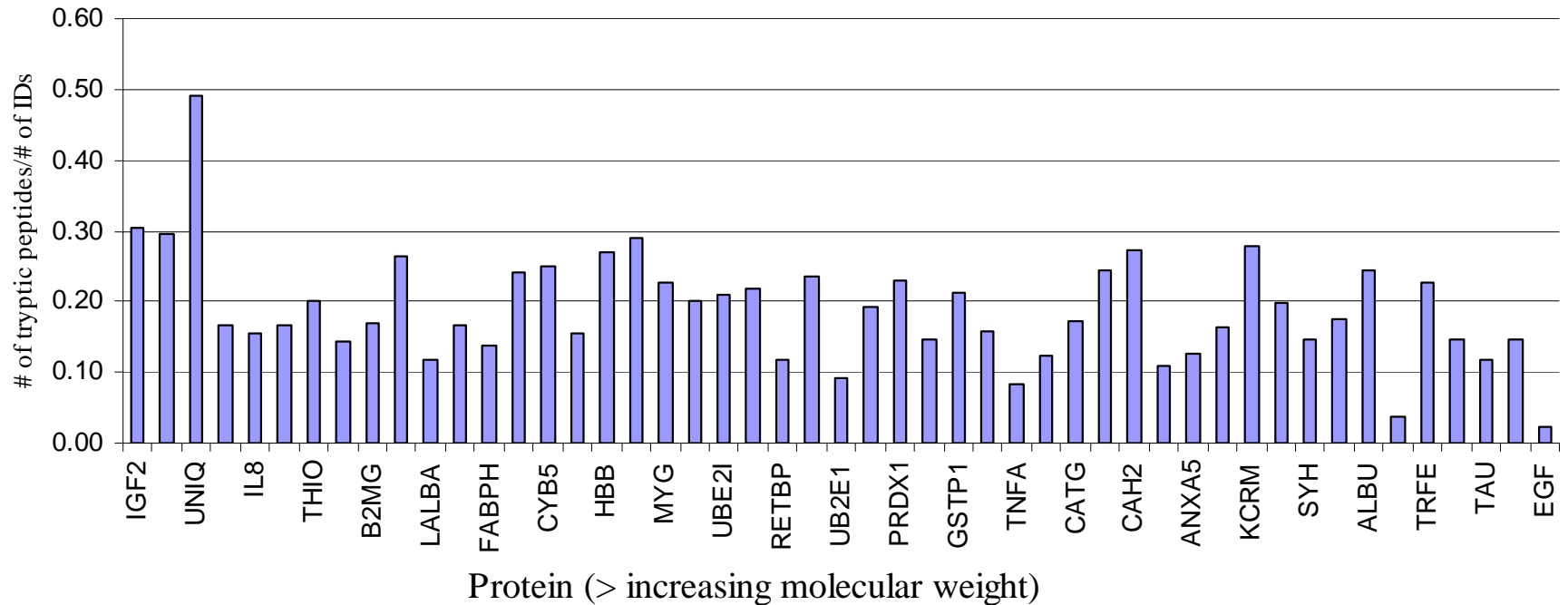
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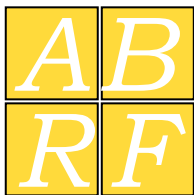




Protein Sequence Coverage: Molecular Weight

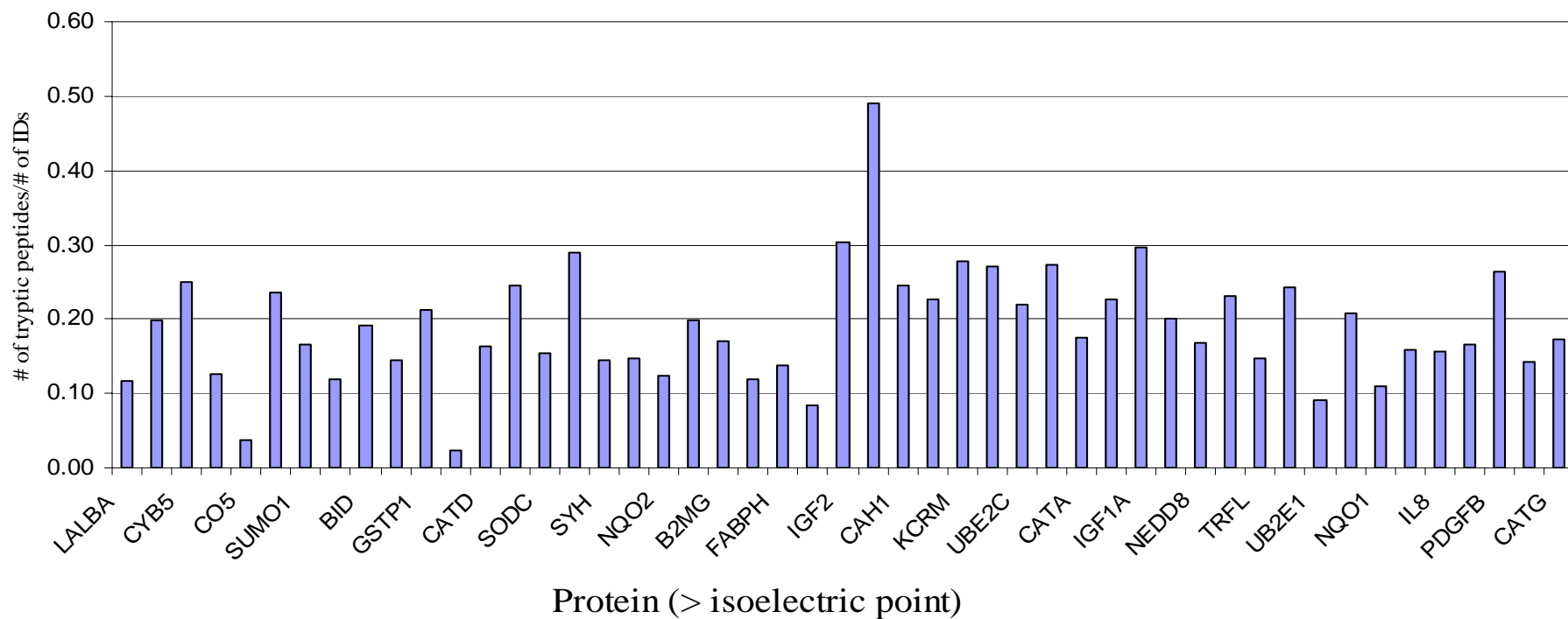
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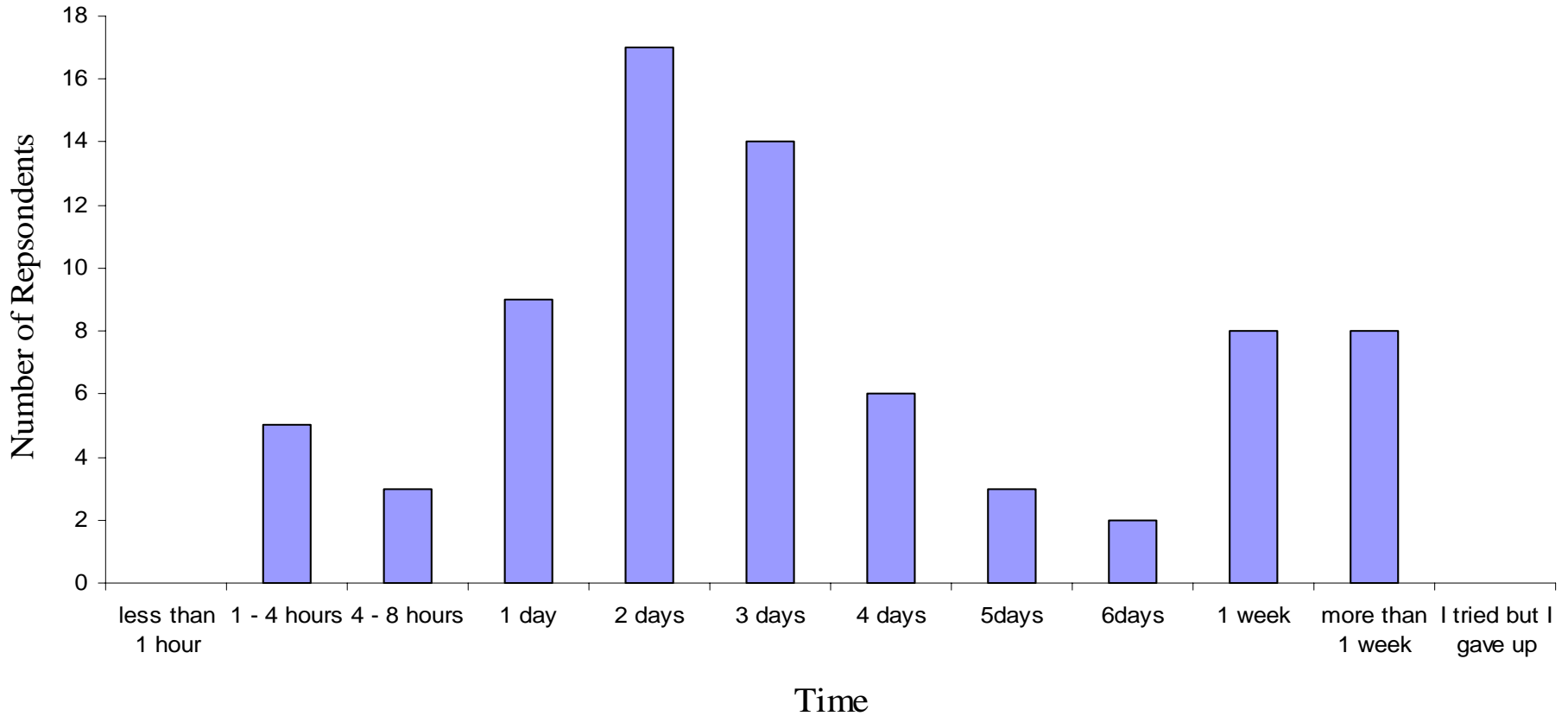
Protein Sequence Coverage: Isoelectric Point





Time to Prepare and Analyze

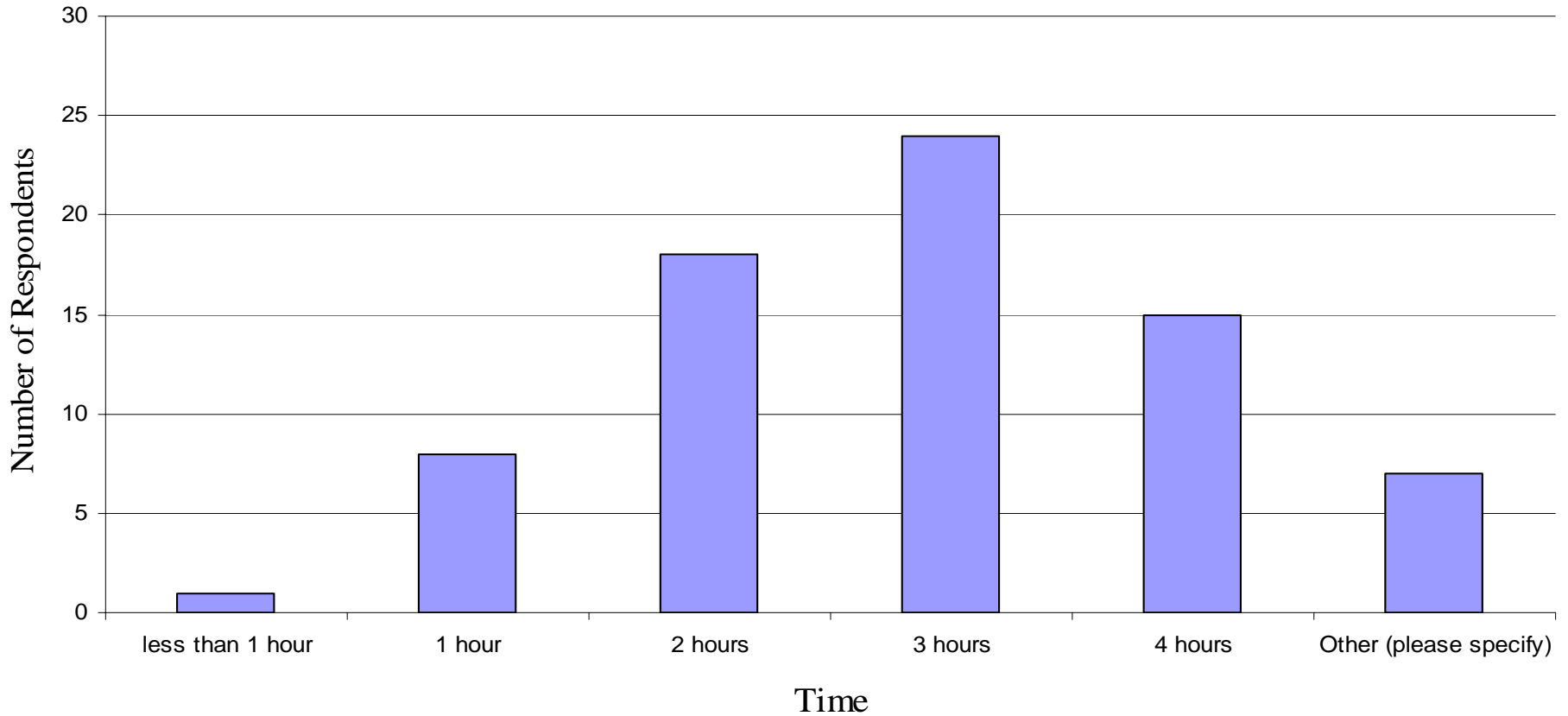
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Time to Complete Survey

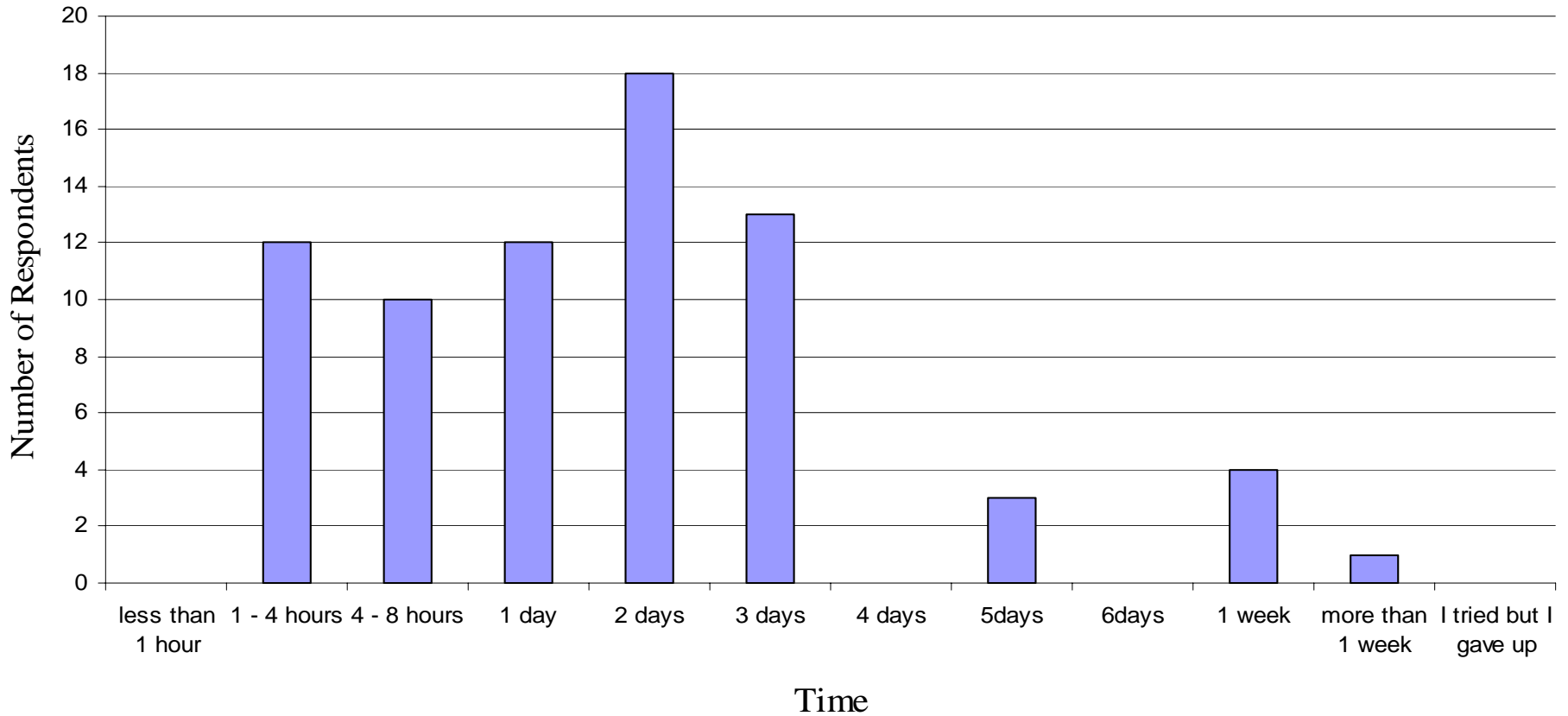
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Time for Data Analysis

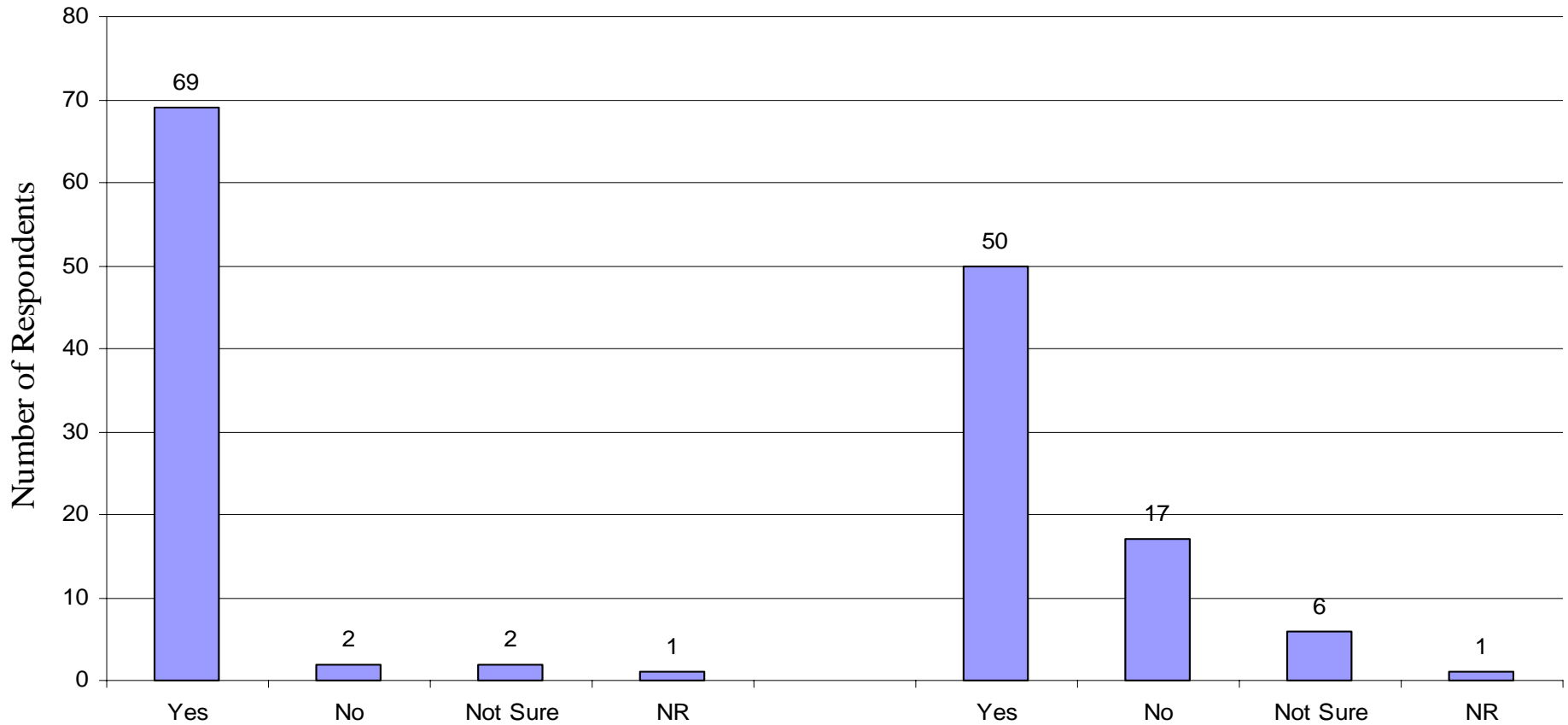
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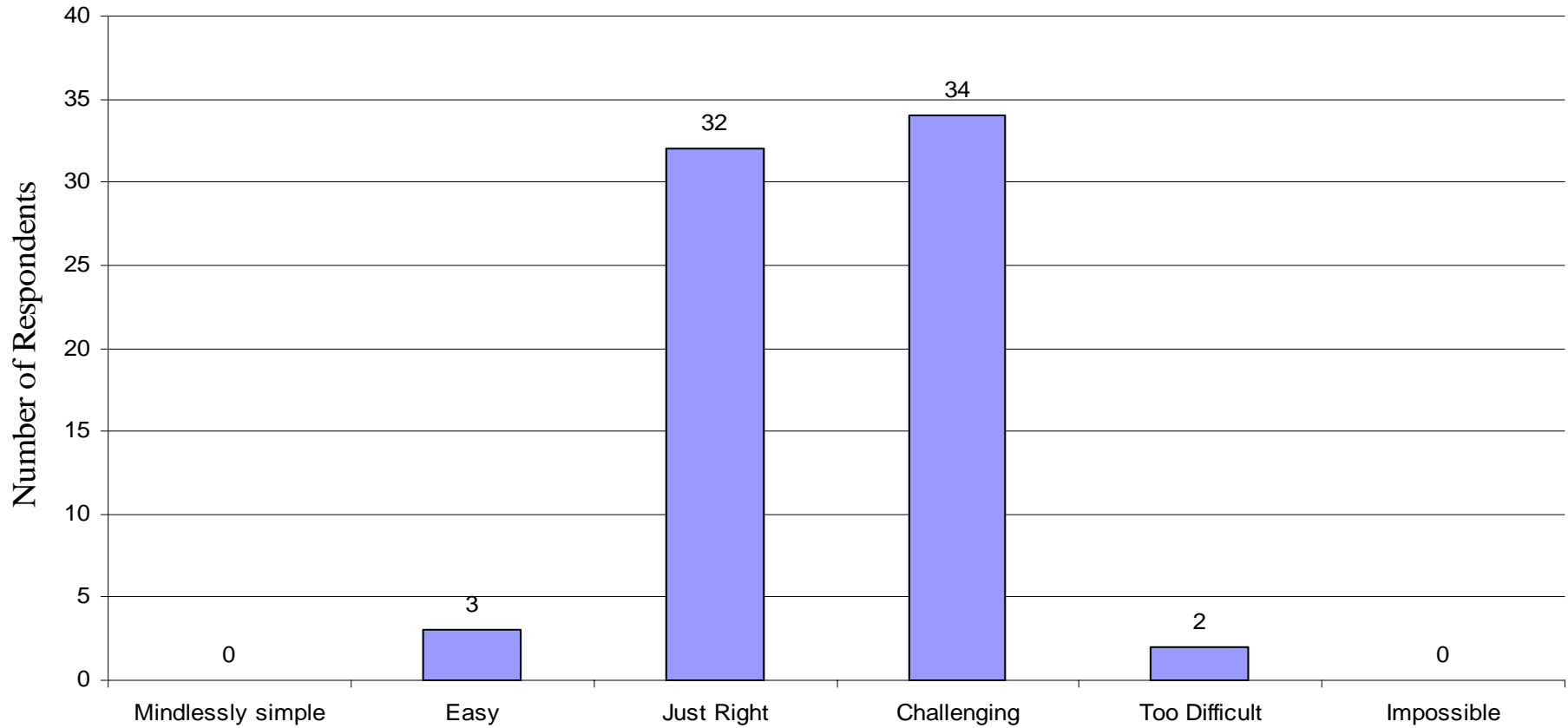
Utility





Level of Difficulty

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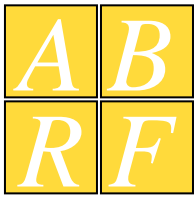




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Conclusions

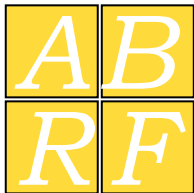
- A standard protein mixture has been developed that has broad usefulness for a variety of proteomics strategies.
- The fact that no single approach performed better than others may suggest that success is not so much method-dependent as possibly experience-, or technique-dependent (technique of the individual performing the analysis).
- Good results are achievable by a lab which does not necessarily have the latest instruments but optimizes variables within its control.



Conclusions (cont.)

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- Many labs can reliably identify a large fraction of proteins at these concentrations with few false positive results.
- The nature of variability expected in a semi-complex protein mixture analysis will have to be dealt with in future standards design and analysis.
- We demonstrated in a tangible way, problems resulting from the myriad representations of proteins and lack of data representation standards.
- The study has led to a publicly available set of raw data files for analysis of a known protein mixture by a number of independent labs.



Acknowledgements

*Proteomics Standards
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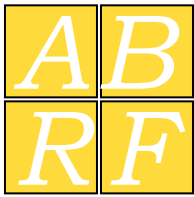
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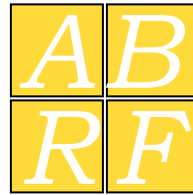
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Kathryn S. Lilley - University of Cambridge

Larry Martin - East West University

Stephen E. Stein - NIST



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THANK YOU

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