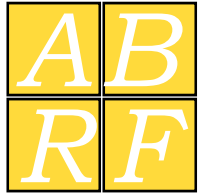


*Proteomics Standards
Research Group*

sPRG2007:
Development and Evaluation of a
Phosphoprotein Standard

www.abrf.org/sprg



*Proteomics Standards
Research Group*

Proteomics Standards Research Group (sPRG) Members

Phil Andrews - University of Michigan

David Arnott - Genentech, Inc.

Mary Ann Gawinowicz - Columbia University (Chair)

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

William S. Lane - Harvard University (EB Liaison)

Kathryn S. Lilley - University of Cambridge

Rachel Loo – University of California Los Angeles (Chair Elect)

Larry Martin - East West University

Stephen E. Stein - NIST



Proteomics Standards
Research Group

sPRG Website

www.abrf.org/sprg

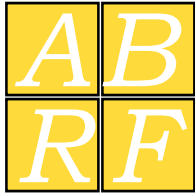
The screenshot shows the ABRF website interface. At the top, the ABRF logo and the text "The Association of Biomolecular Resource Facilities" are displayed. Navigation links include "Home", "ABRF Sponsors", "Contact Us", and "Help". Below this, there are links for "White Pages", "Yellow Pages", "Log 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 "ABRF News" with two articles. The first article is titled "Roger Tsien to receive the 2006 ABRF Award" and describes his work in in vivo fluorescence methods. The second article is titled "Ranking ranked in the top 10!" and mentions the 2006 Genome Technology ranking. Both articles have "Read more..." links.

At the bottom, there is a link for "Atlantic Macromolecular Chemistry Meeting" at Wake Forest University, Winston-Salem, North Carolina, United States.



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Materials

Catalase - 10 pmol

Troponin T - 25 pmol

Osteopontin - 50 pmol

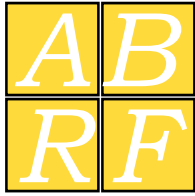
Ovalbumin - 50 pmol

Cystatin - 100 pmol

α -S1-casein - 250 pmol

α -S2-casein - 250 pmol

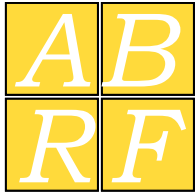
Proteins were determined to be phosphorylated based on positive staining with two phosphoprotein-specific stains and a phosphate-specific chemical reaction



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Results Submission

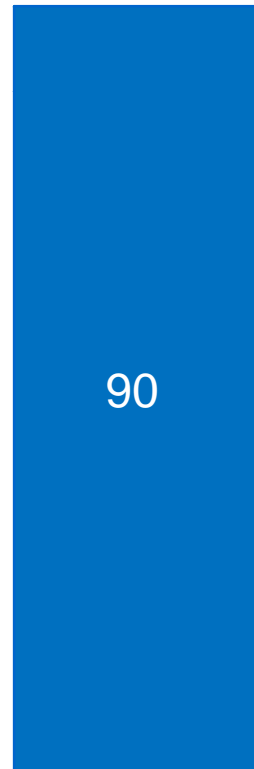
- Results were submitted anonymously through an on-line survey and included experimental details, instrument(s) used, a list of identified proteins and a list of identified phosphopeptides including site(s) of phosphorylation.
- A web site (www.proteomecommons.org/dev/abrf/) was provided for the required submission of annotated mass spectra of phosphopeptides.



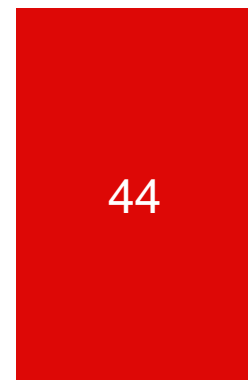
***Proteomics Standards
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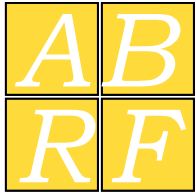
48.9% Data Return

Sample Requests



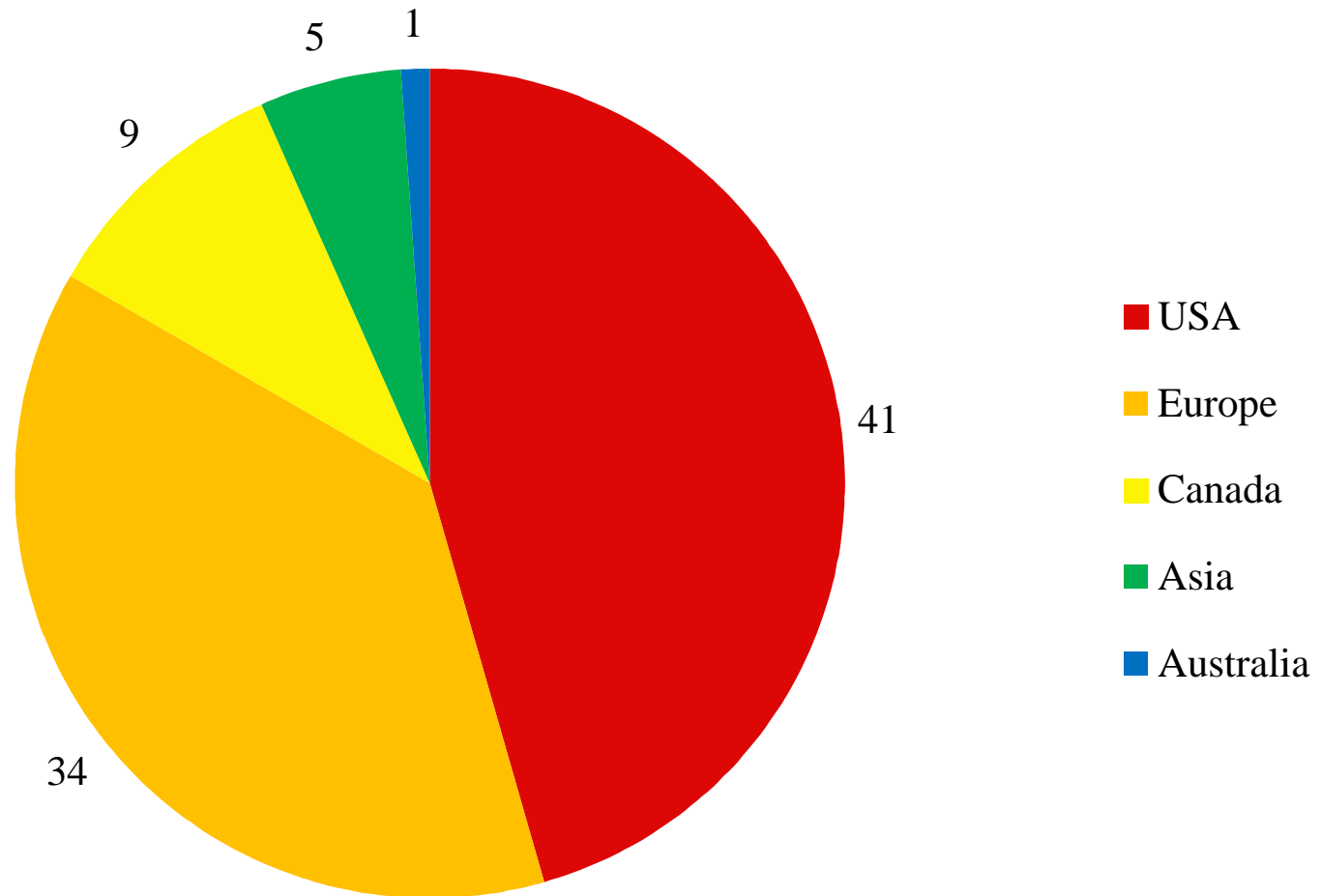
Datasets





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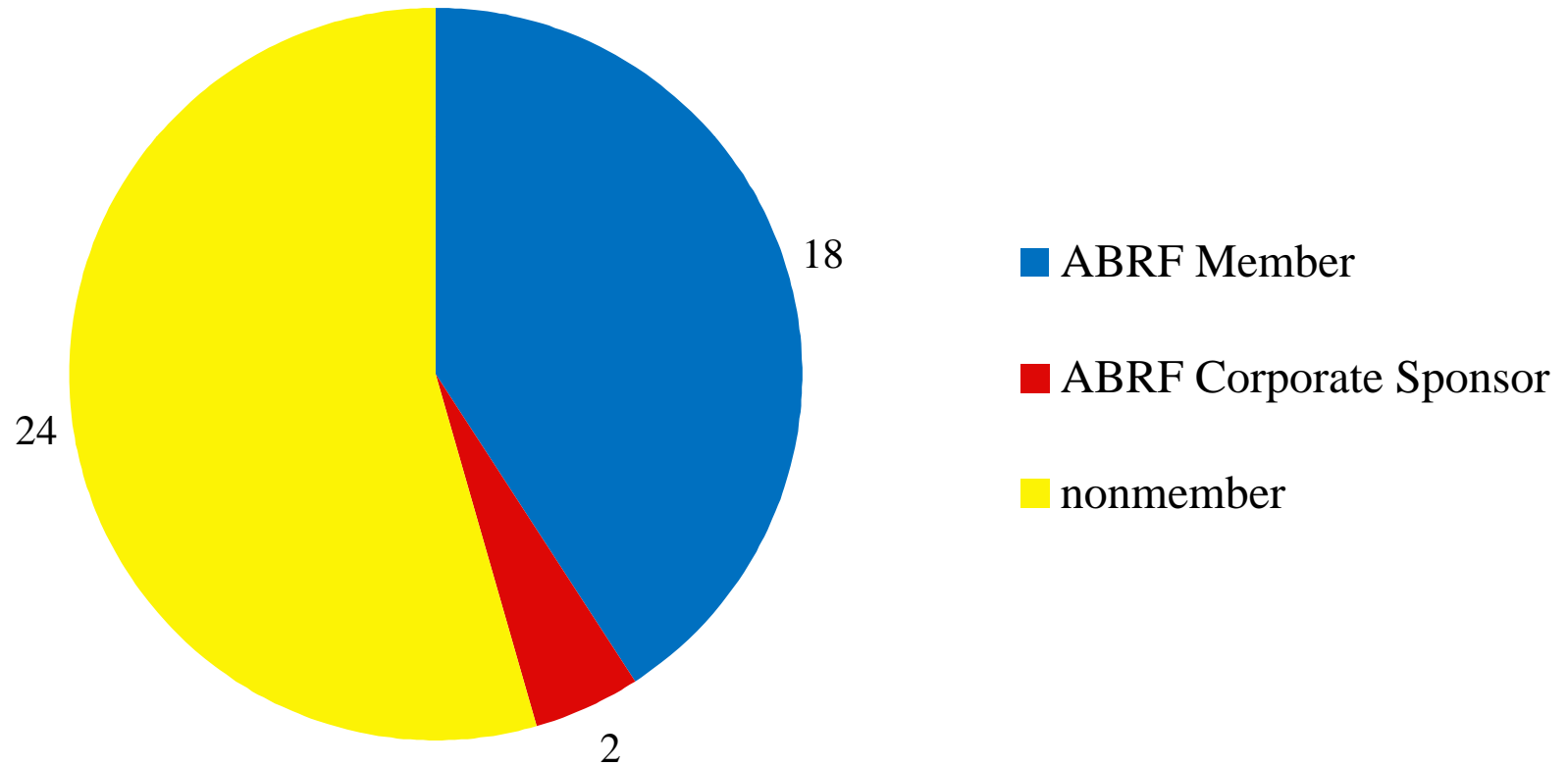
sPRG07 Demographics

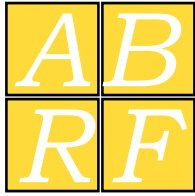




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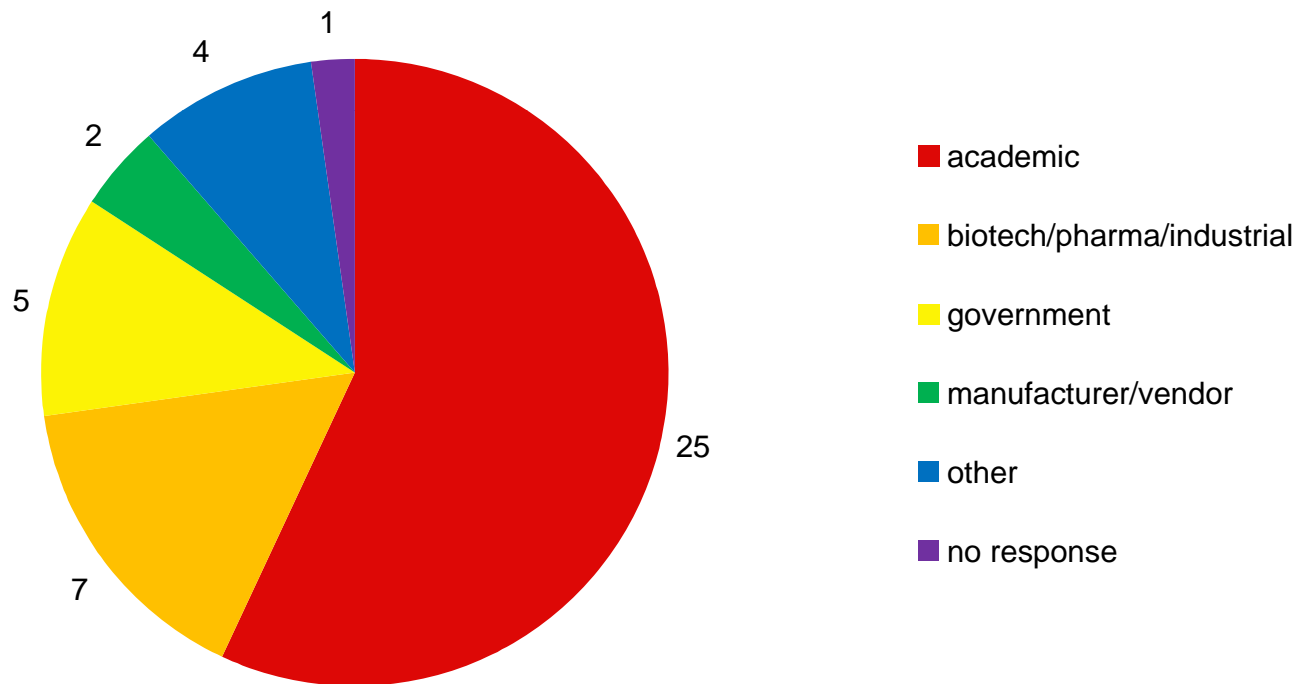
sPRG2007 ABRF Membership





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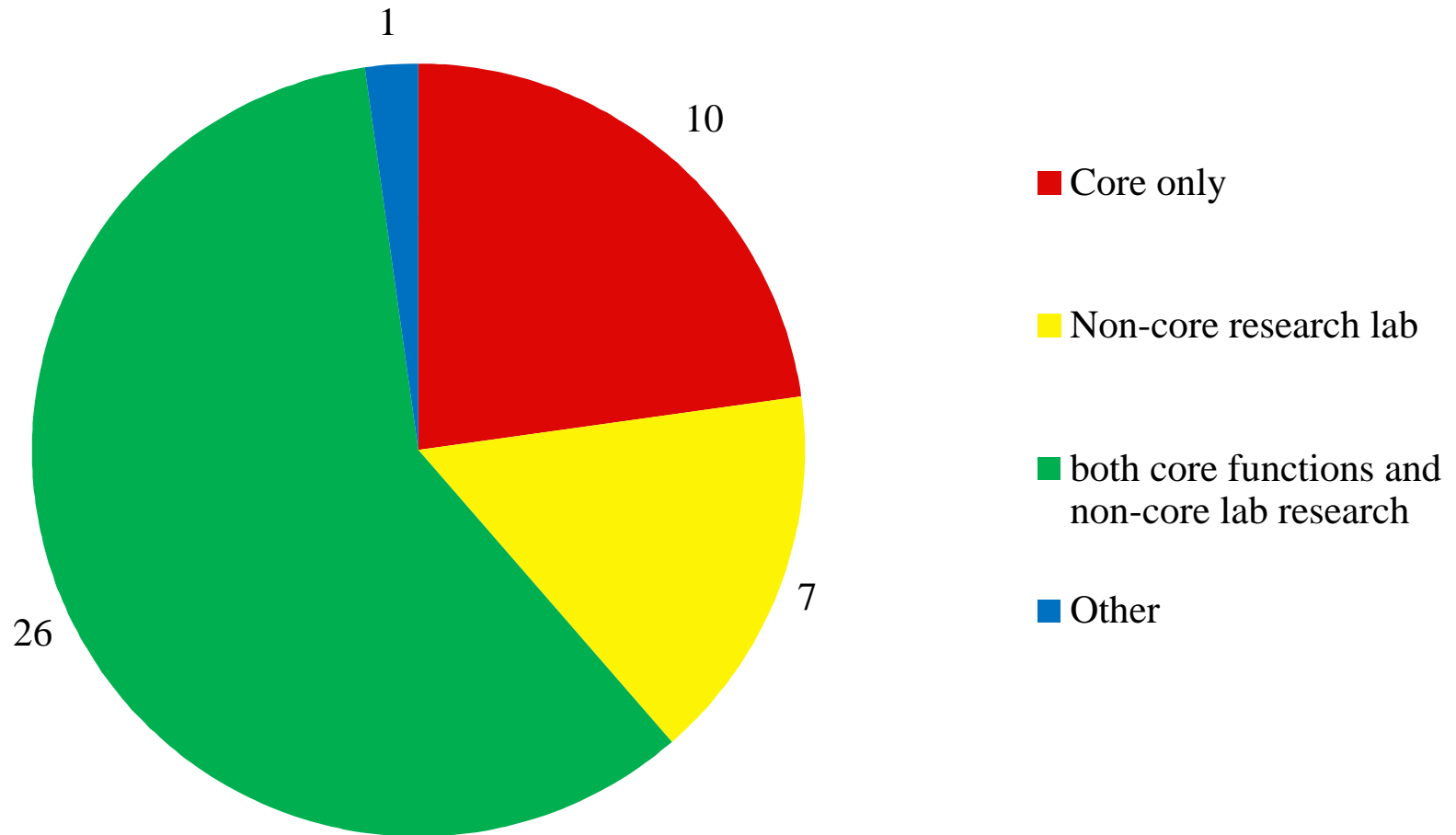
sPRG2007 Laboratories





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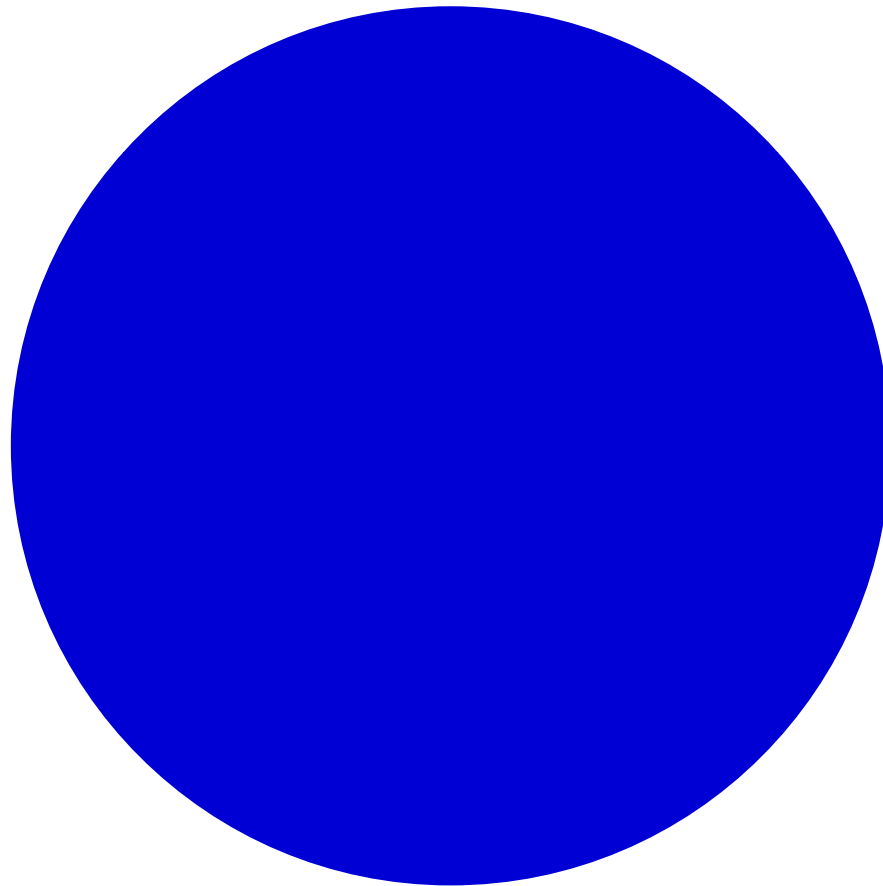
sPRG2007 Facility Type





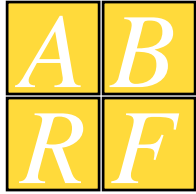
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Did you receive more than 1 sample?



■ No

■ Yes



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P02662 CASA1_BOVIN Alpha-S1-casein *Bos taurus*

10 20 30 40 50 60

MKLLILTCLV AVALARPKHP IKHQGLPQEV LNENLLRFFV APFPEVFGKE KVNELSKDIG

70 80 90 100 110 120

SESTEDQAME DIKQMEAESI SSSEEIVPNS VEQKHIQKED VPSERYLGYL EQLLRLKKYK

130 140 150 160 170 180

VPQLEIVPNS AEERLHSMKE GIHAQQKEPM IGVNQELAYF YPELFRQFYQ LDAYPSGAWY

190 200 210

YVPLGTQYTD APSFSDIPNP IGSENSEKTT MPLW

Known

56 Phosphoserine (1,0)

61 Phosphoserine (5,0)

63 Phosphoserine (3,1)

68 Phosphothreonine (variant D) (0,0)

79 Phosphoserine (0,0)

81 Phosphoserine (1,0)

82 Phosphoserine (1,0)

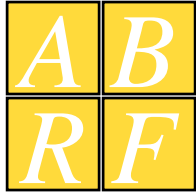
83 Phosphoserine (0,0)

90 Phosphoserine (2,0)

130 Phosphoserine (29,0)

Unverified

64 Phosphothreonine(1,1)



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P02663 CASA2_BOVIN Alpha-S2-casein *Bos taurus*

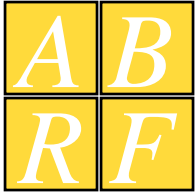
10	20	30	40	50	60
MKFFIFTCLL	AVALAKNTME	HVSSSEESII	SQETYKQEK	MAINPSKENL	CSTFCKEVVR
70	80	90	100	110	120
NANEEYSIG	SSSEESA EVA	TEEVKITVDD	KHYQKALNEI	NQFYQKFPQY	LQYLYQGPIV
130	140	150	160	170	180
LNPWDQVKN	AVPITPTLNR	EQLSTSEENS	KKTVDMESTE	VFTKTKLITE	EKNRLNFLK
190	200	210	220		
KISQRYQKFA	LPQYLKTVYQ	HQKAMKPWIQ	PKTKVIPYVR	YL	

Known

23 Phosphoserine (0,0)
24 Phosphoserine (0,0)
25 Phosphoserine (0,0)
31 Phosphoserine (0,0)
46 Phosphoserine (2,0)
71 Phosphoserine (0,0)
72 Phosphoserine (0,0)
73 Phosphoserine (0,0)
76 Phosphoserine (0,0)
144 Phosphoserine (0,1)
146 Phosphoserine (1,1)
158 Phosphoserine (2,0)

Unverified

145 Phosphothreonine (0,1)
150 Phosphoserine (0,1)

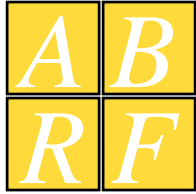


P04040 CATA_HUMAN Catalase *Homo sapiens*

10	20	30	40	50	60
MADSRDPASD	QMQHWKEQRA	AQKADVLTTG	AGNPVGDKLN	VITVGPRGPL	LVQDVVFTDE
70	80	90	100	110	120
MAHFDREIRP	ERVVHAKGAG	AFGYFEVTHD	ITKYSKAKVF	EHIGKKTPIA	VRFSTVAGES
130	140	150	160	170	180
GSADTVRDPR	GFAVKFYTED	GNWDLVGNNT	PIFFIRDPIL	FPSFIHSQKR	NPQTHLKDPD
190	200	210	220	230	240
MVWDFWSLRP	ESLHQVSFLF	SDRGIPDGHR	HMNGYGSHTF	KLVNANGEAV	YCKFHYKTDQ
250	260	270	280	290	300
GIKNLSVEDA	ARLSQEDPDY	GIRDLFNIAI	TGKYPSWTFY	IQVMTFNQAE	TFPFNPFDLT
310	320	330	340	350	360
KVWPHKDYPL	IPVGKLVVLR	NPVNYFAEVE	QIAFDPSNMP	PGIEASPKDM	LQGRLFAYPD
370	380	390	400	410	420
THRHRLLGPNY	LHIPVNCPIR	ARVANYQRDG	PMCMQDNQGG	APNYYPNSFG	APEQQPSALE
430	440	450	460	470	480
HSIQYSGEVR	RFNTANDDNV	TQVRAFVNVV	LNEEQRKRLC	ENIAGHLKDA	QIFIQKKAVK
490	500	510	520		
NFTEVHPDYG	SHIQALLDKY	NAEKPKNAIH	TFVQSGSHLA	AREKANL	

All Unverified

58 Phosphothreonine (1,0)	325 Phosphotyrosine (1,0)
84 Phosphotyrosine (0,1)	358 Phosphotyrosine (1,1)
88 Phosphothreonine (0,1)	361 Phosphothreonine (1,1)
92 Phosphoserine (0,1)	370 Phosphotyrosine (0,1)
138 Phosphothreonine (1,0)	379 Phosphotyrosine (0,1)
163 Phosphoserine (0,1)	483 Phosphothreonine (0,1)
167 Phosphoserine (0,1)	489 Phosphoserine (0,1)
	491 Phosphoserine (0,1)



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P01038 CYT_CHICK Cystatin *Gallus gallus*

```

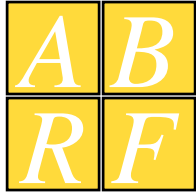
      10          20          30          40          50          60
MAGARGCVVL LAAALMLVGA VLGSEDRSRL LGAPVPVDEN DEGLQRALQF AMAEYNRASN
      70          80          90         100         110         120
DKYSSRVVRV ISAKRQLVSG IKYILQVEIG RTTCPKSSGD LQSCEFHDPEP EMAKYTTCTF
      130
VVYSIPWLNQ IKLLESKCQ
```

Known

103 Phosphoserine - partial (19,1)

Unverified

24 Phosphoserine (1,0)
28 Phosphoserine (1,0)
59 Phosphoserine (1,0)
92 Phosphothreonine (0,1)
93 Phosphothreonine (0,1)
97 Phosphoserine (1,3)
98 Phosphoserine (0,3)



P01012	OVAL_CHICK	Ovalbumin	<i>Gallus gallus</i>		
10	20	30	40	50	60
MGSIGAASME	FCFDVFKELK	VHHANENIFY	CPIAIMSALA	MVYLGAKDST	RTQINKVVRF
70	80	90	100	110	120
DKLPGFGD S I	EAQCGTSVNV	HSSLRDILNQ	ITKPNDVYSF	SLASRLYAEF	RYPILPEYLQ
130	140	150	160	170	180
CVKELYRGGI	EPINFQTAAD	QARELINSWV	ESQTNGIIRN	VLQPSSVDSQ	TAMVLVNAIV
190	200	210	220	230	240
FKGLWEKAFK	DEDTQAMPFR	VTEQESKPVQ	MMYQIGLFRV	ASMASEKMKI	LELPFASGTM
250	260	270	280	290	300
SMLVLLPDEV	SGLEQLESII	NFEKLTWETS	SNVMEERKIK	VYLPRMKMEE	KYNLTSVLMA
310	320	330	340	350	360
MGITDVFSSS	ANLSGISSAE	SLKISQAVHA	AHAEINEAGR	EVVGS S AEAGV	DAASVSEEFR
370	380				
ADHPFLFCIK	HIATNAVLFF	GRCVSP			

Known
 2 N-acetylglycine
 69 Phosphoserine (20,0)
 345 Phosphoserine (29,1)

Unverified
 75 Phosphotyrosine (1,1)
 92 Phosphothreonine (1,0)
 165 Phosphotyrosine (0,1)
 166 Phosphotyrosine (0,1)
 169 Phosphotyrosine (0,1)
 171 Phosphothreonine (0,1)
 325 Phosphotyrosine (2,0)
 354 Phosphoserine (0,4)
 356 Phosphoserine (0,4)

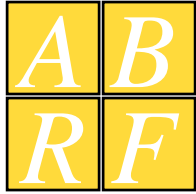
P31096 OSTP_BOVIN Osteopontin *Bos taurus*

```

      10          20          30          40          50          60
MRIA VICFCL LGIASALPVK PTSSGSSSEEK QLNNKYPDAV ATWLKPDPSQ KQTFLAPQNS
      70          80          90         100         110         120
VSSEETDDNK QNTLPSSKSNE SPEQTDDLDD DDDNSQDVNS NDSDDAETTD DPDHSDESHH
     130         140         150         160         170         180
SDESDEVDFP TDIPTIAVFT PFIPTESAND GRGDSVAYGL KRSRKKFRRS NVQSPDATEE
     190         200         210         220         230         240
DFTSHIESEE MHDAPKKTSQ LTDHSKETNS SELSKELTPK AKDKNKHSNL IESQENSKLS
     250         260         270
QEFHSLEDKL DLDHKSEEDK HLKIRISHEL DSASSEVN

```

Known	Known	Unverified
23 Phosphoserine (1,1)	210 Phosphoserine (10,5)	22 Phosphothreonine(0,2)
24 Phosphoserine (1,1)	233 Phosphoserine (24,0)	53 Phosphothreonine (0,1)
26 Phosphoserine (2,1)	240 Phosphoserine (6,1)	66 Phosphothreonine (0,1)
27 Phosphoserine (4,1)	245 Phosphoserine (9,0)	118 Phosphoserine (0,1)
60 Phosphoserine (7,2)	256 Phosphoserine (16,0)	155 Phosphoserine (5,0)
62 Phosphoserine (5,6)	267 Phosphoserine (31,0)	170 Phosphoserine (0,1)
63 Phosphoserine (5,5)	272 Phosphoserine (23,3)	183 Phosphothreonine (4,7)
76 Phosphoserine (1,2)	274 Phosphoserine (19,8)	198 Phosphothreonine (0,2)
78 Phosphoserine (1,0)		199 Phosphoserine (0,3)
81 Phosphoserine (2,0)		202 Phosphothreonine (0,3)
95 Phosphoserine ()		208 Phosphothreonine (7,7)
100 Phosphoserine ()		211 Phosphoserine (2,5)
103 Phosphoserine ()		214 Phosphoserine (2,4)
115 Phosphoserine ()		218 Phosphothreonine (0,1)
121 Phosphoserine (0,1)		228 Phosphoserine (2,2)
124 Phosphoserine (0,1)		237 Phosphoserine (1,3)
178 Phosphothreonine (13,2)		268 Phosphoserine (1,0)
184 Phosphoserine (9,7)		273 Phosphoserine (0,1)
188 Phosphoserine (12,4)		275 Phosphoserine (4,7)
205 Phosphoserine (14,2)		



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P45379 TNNT2_HUMAN Troponin T *Homo sapiens*

10	20	30	40	50	60
MSDIEEVVVEE	YEEEEQEEAA	VEEEDWRED	EDEQEEAAEE	DAEAEAETEE	TRAEEDEEEE
70	80	90	100	110	120
EAKEAEDGPM	EESKPKPRSF	MPNLVPPKIP	DGERVDFDDI	HRKRMEKDLN	ELQALIEAHF
130	140	150	160	170	180
ENRKKEEEEL	VSLKDRIERR	RAERAEQQRI	RNEREKERQN	RLAEERARRE	EEENRRKAED
190	200	210	220	230	240
EARKKKALSN	MMHFGGYIQK	QAQTERKSGK	RQTEREKKKK	ILAERRKVLV	IDHLNEDQLR
250	260	270	280	290	
EKAKELWQSI	YNLEAEKFDL	QEKFKQQKYE	INVLNRIND	NQKVSCTRKG	AKVTGRWK

Known

2 N-acetylserine - *By similarity*
2 Phosphoserine - *By similarity* (0,0)

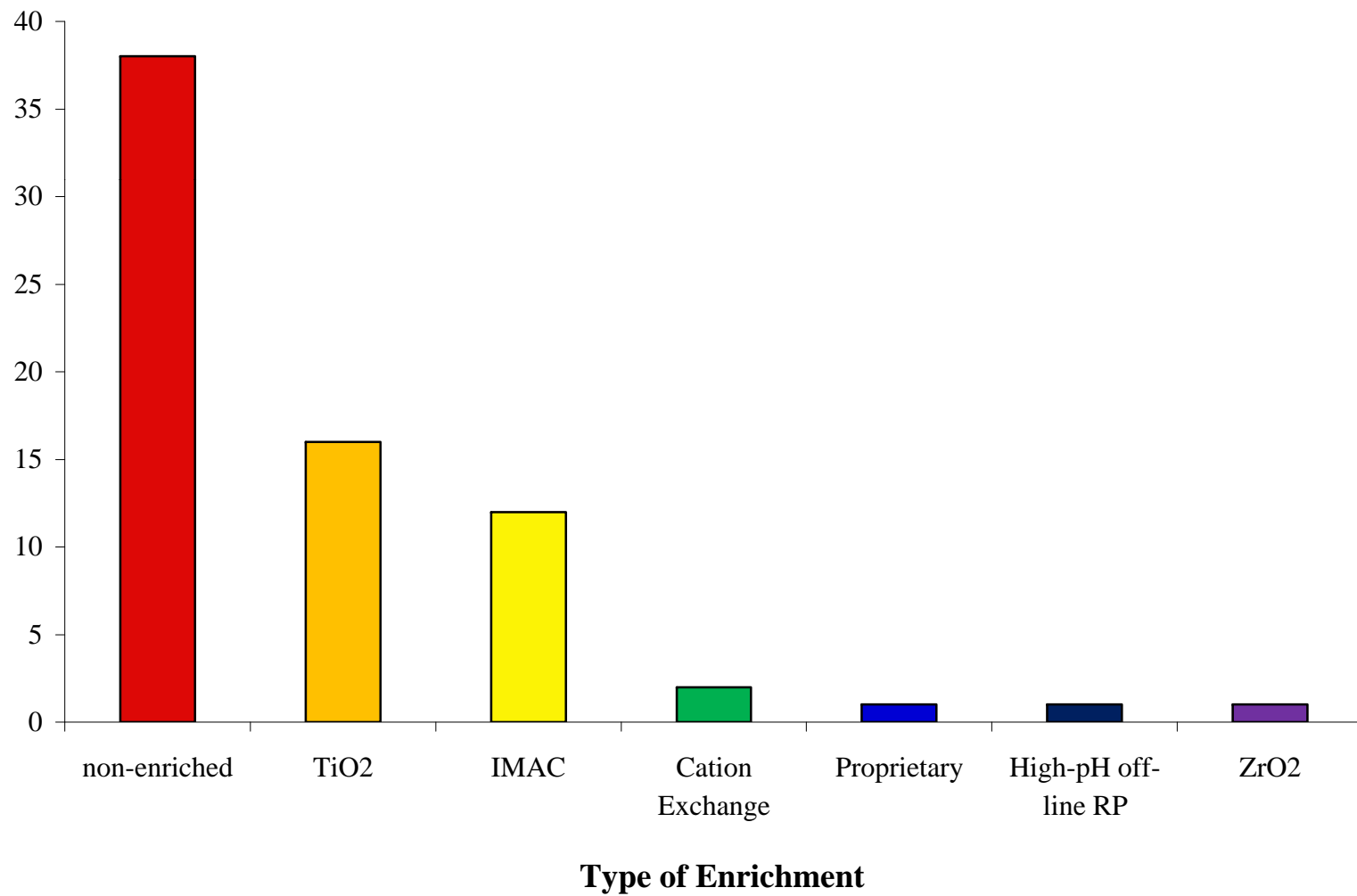
Unverified

73 Phosphoserine (0,1)
79 Phosphoserine (0,1)
189 Phosphoserine (1,1)
197 Phosphotyrosine (0,1)
204 Phosphothreonine (0,1)
287 Phosphothreonine (1,0)



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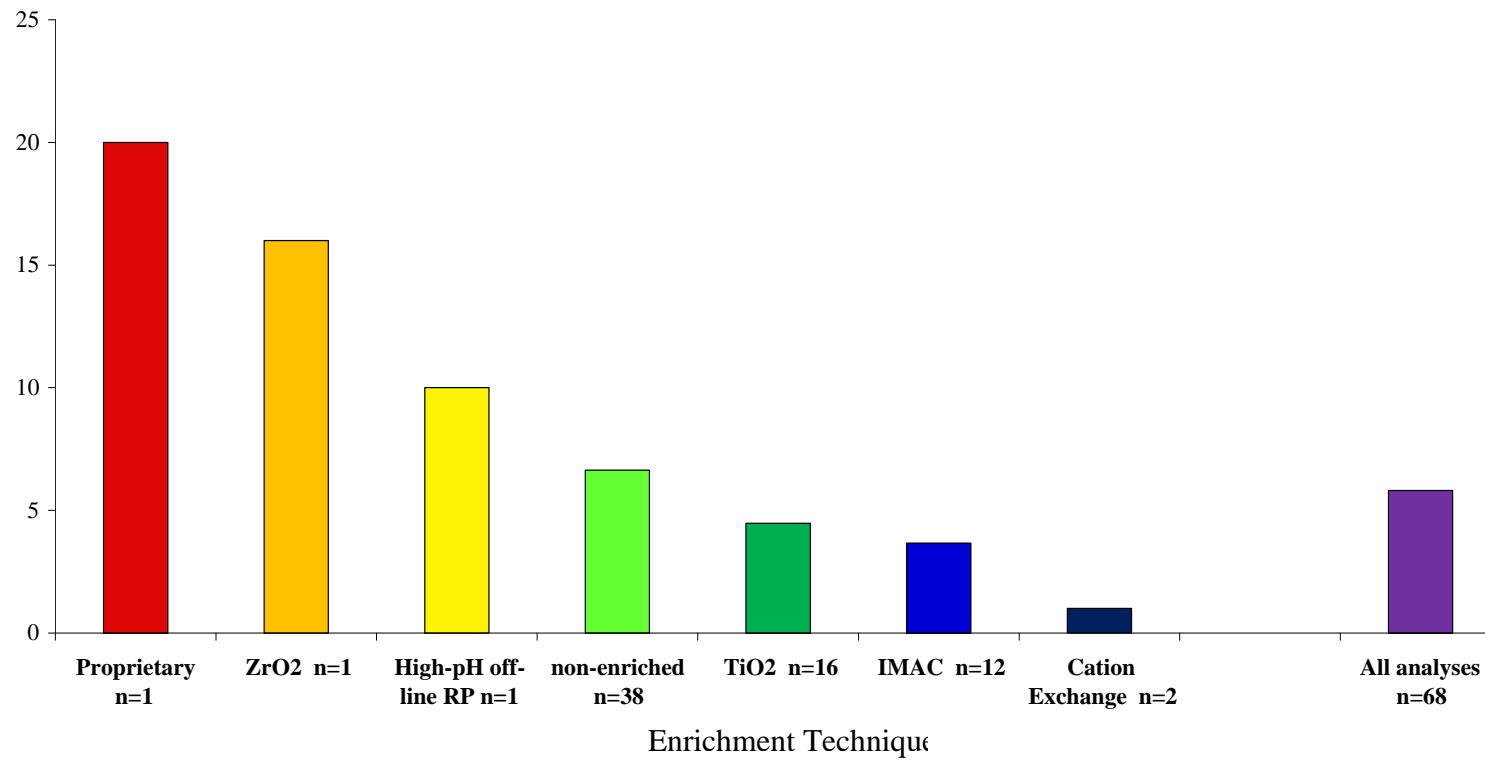
Enrichment Techniques Used in sPRG07 Study

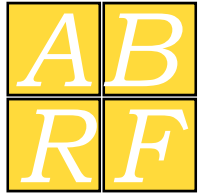




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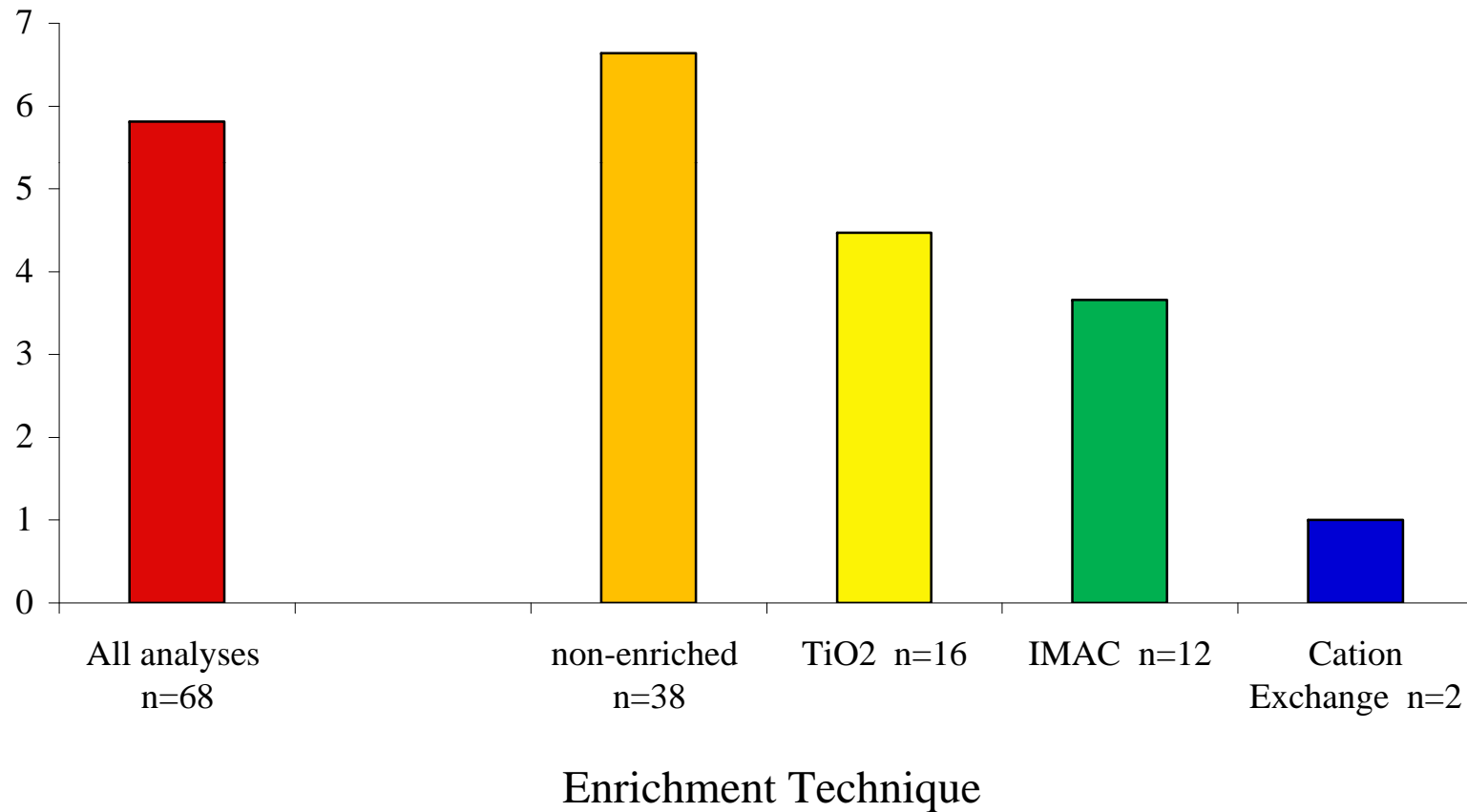
Phosphorylation Sites Identified Using Enrichment Technique

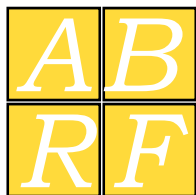




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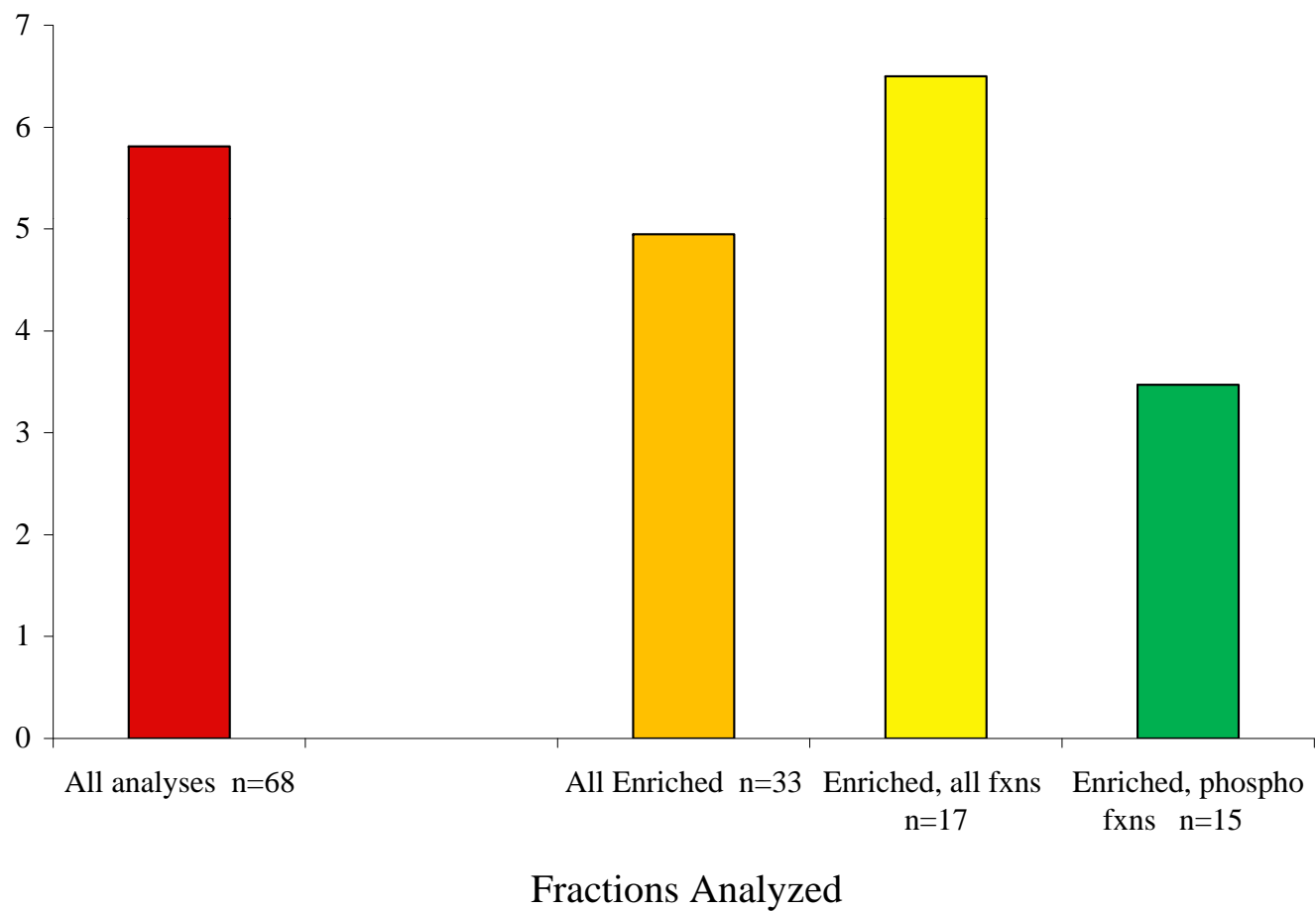
Phosphorylation Sites Identified Using Enrichment Techniques





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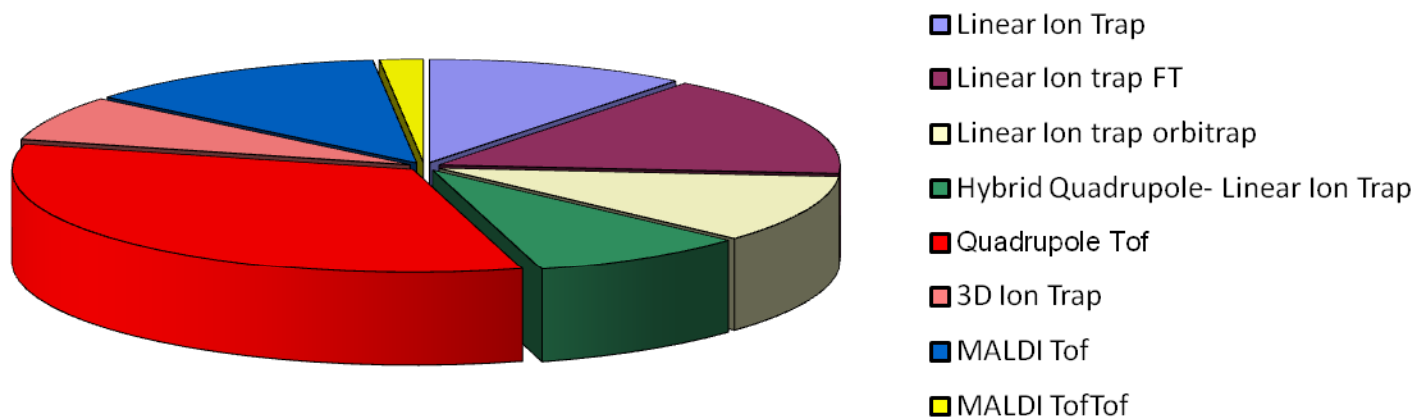
Analysis of Enriched Fractions





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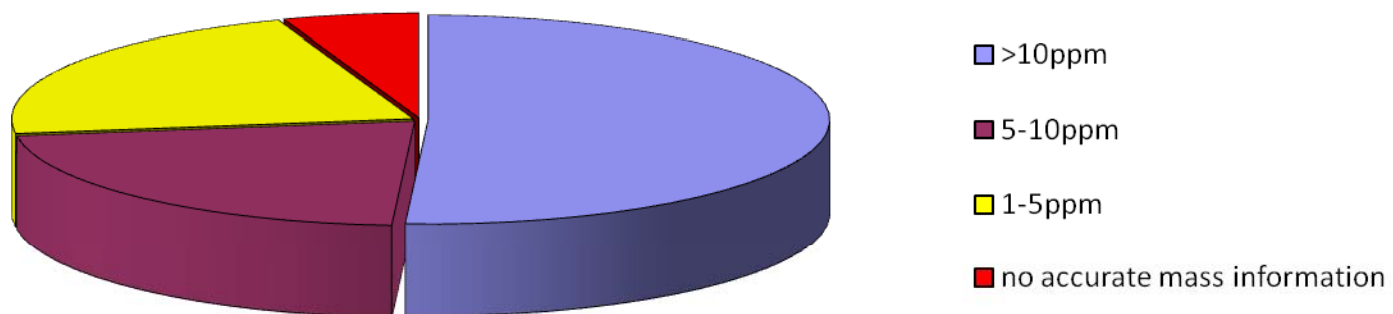
Types of mass spectrometric analysis carried out (sum of all analyses)





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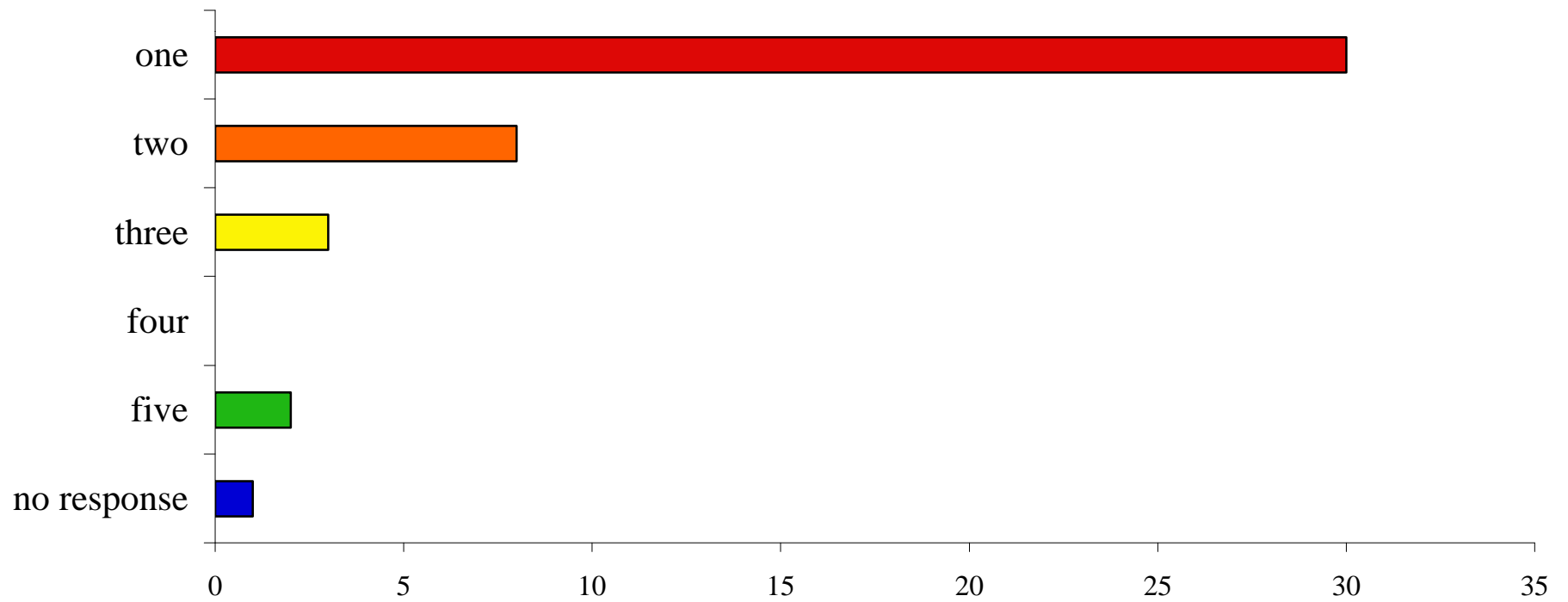
Mass Accuracy





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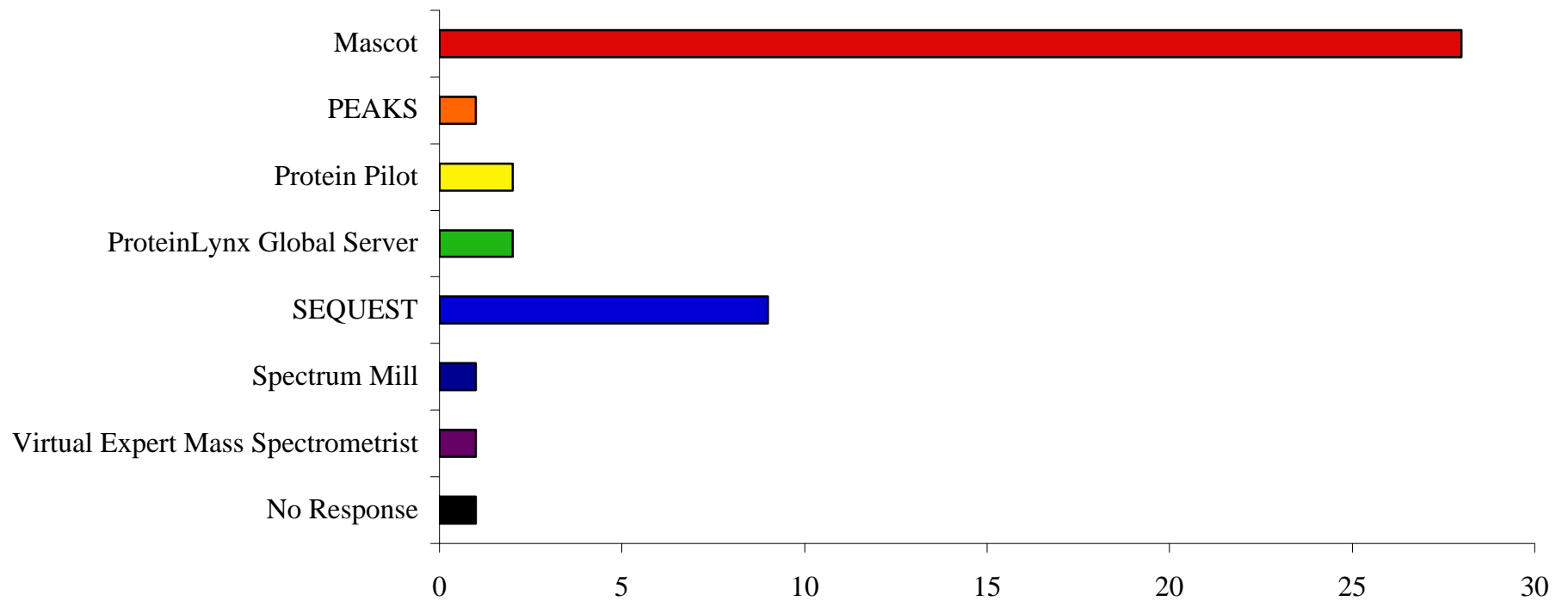
How Many Search Engines Did You Use?





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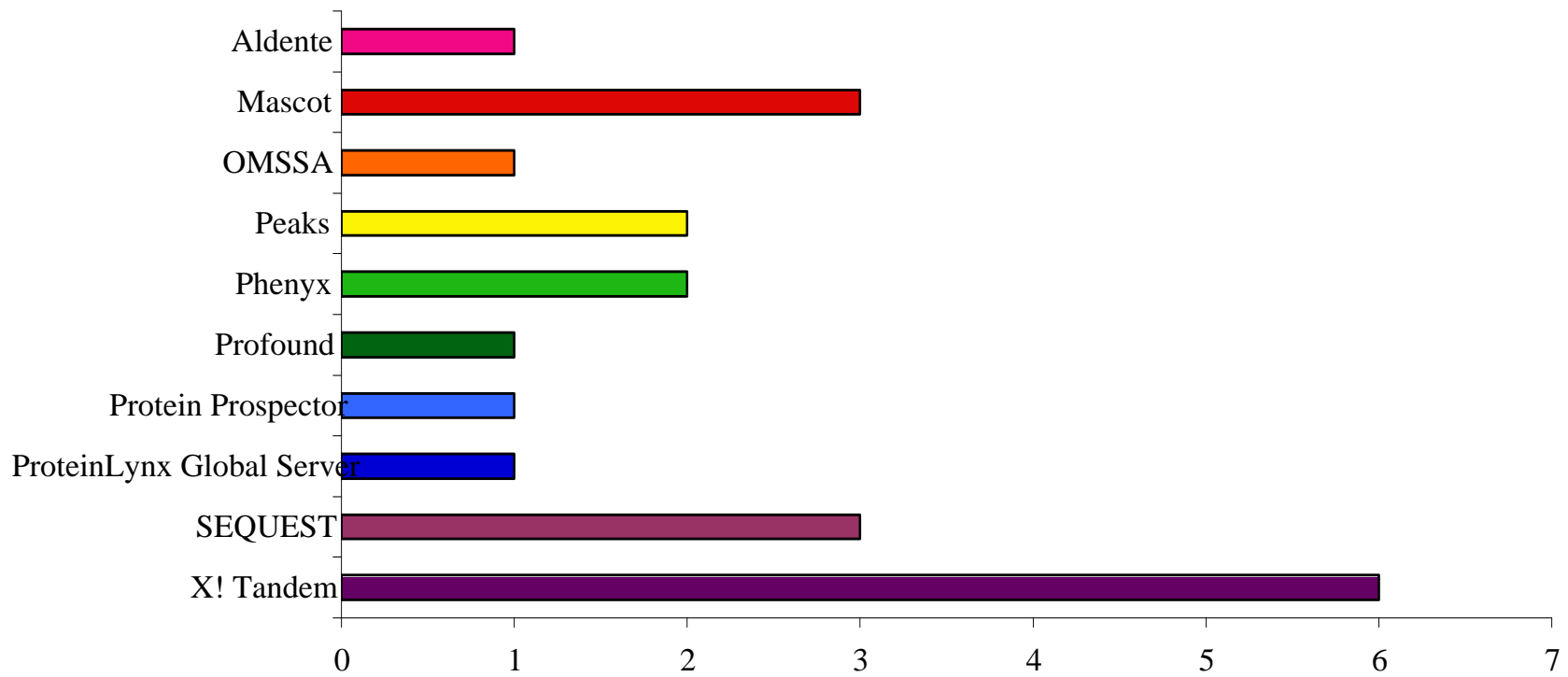
What Database Search Engine Did You Use?





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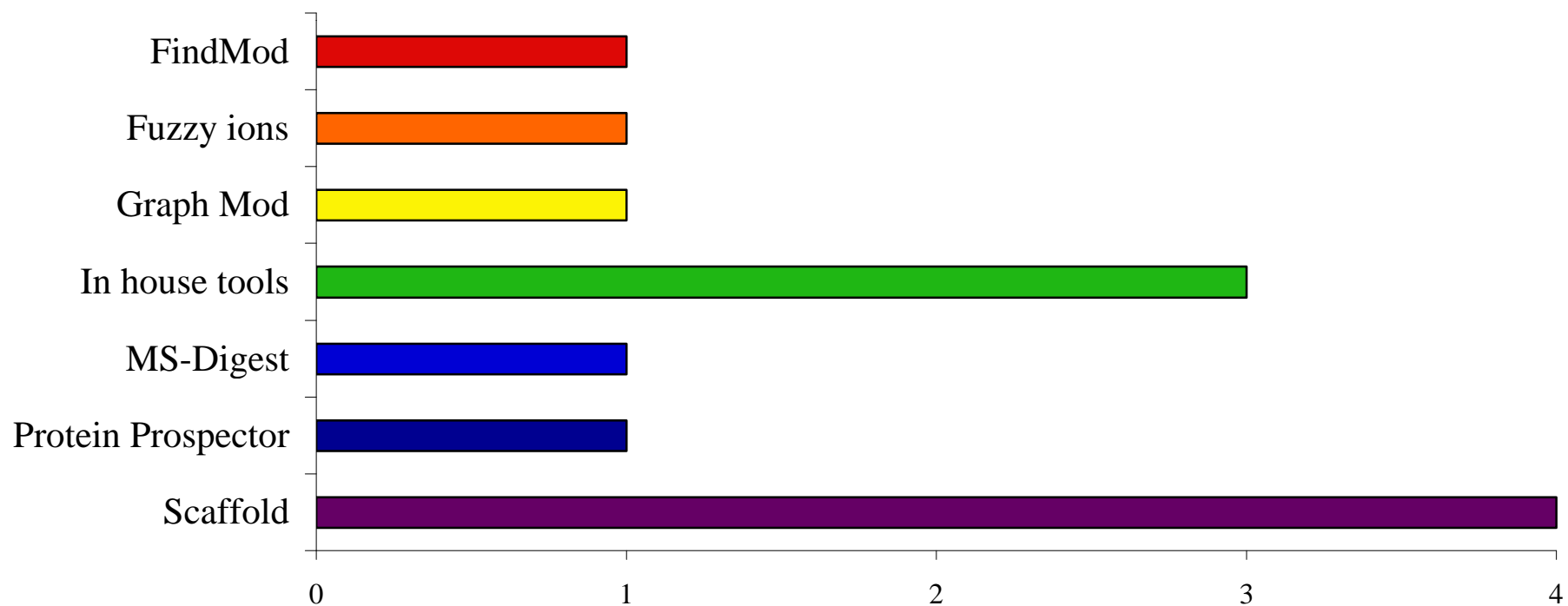
What Secondary Search Engine Did You Use?

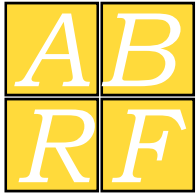




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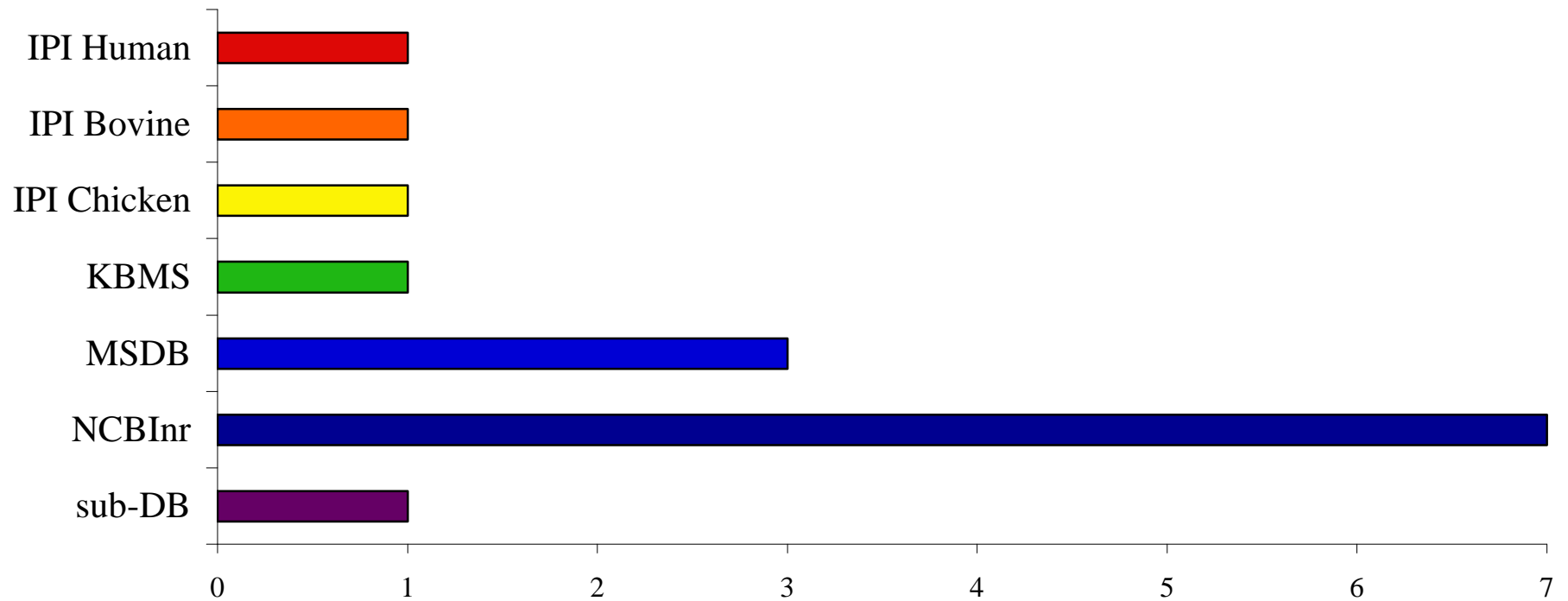
What Other Bioinformatics Tools Did You Use?





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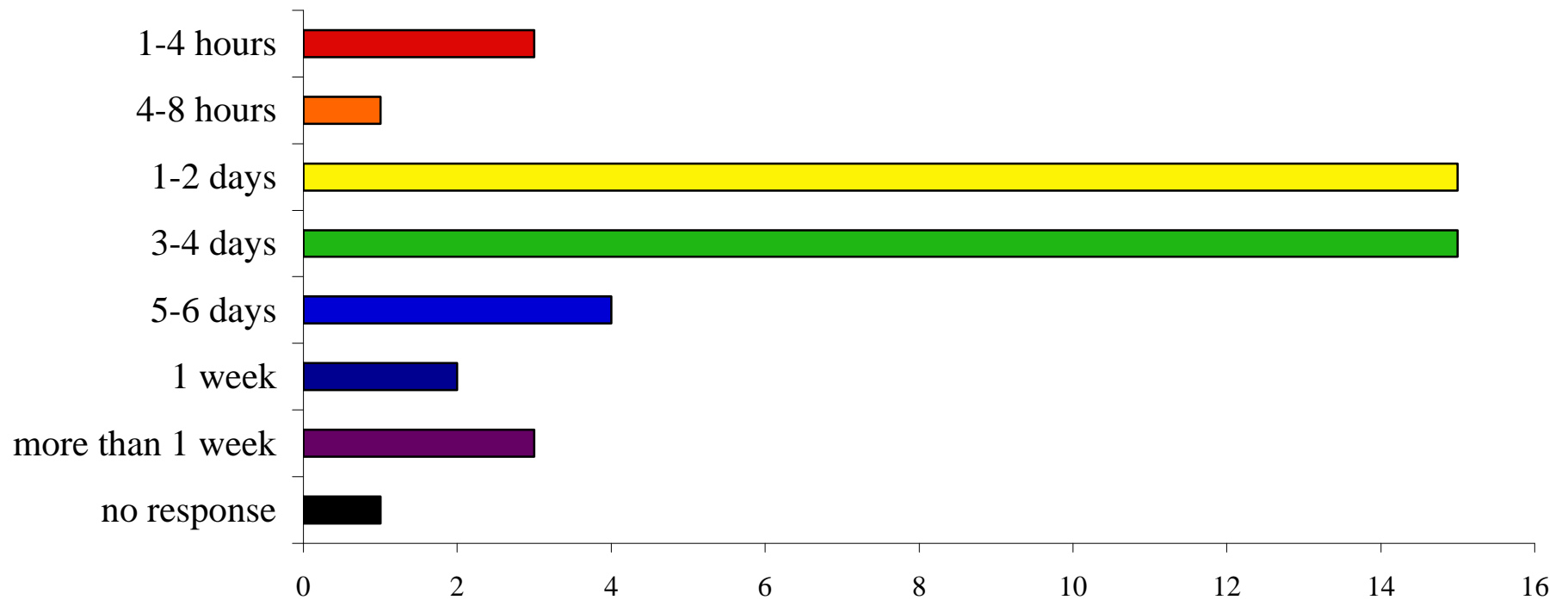
What Other Database(s) Did You Use?





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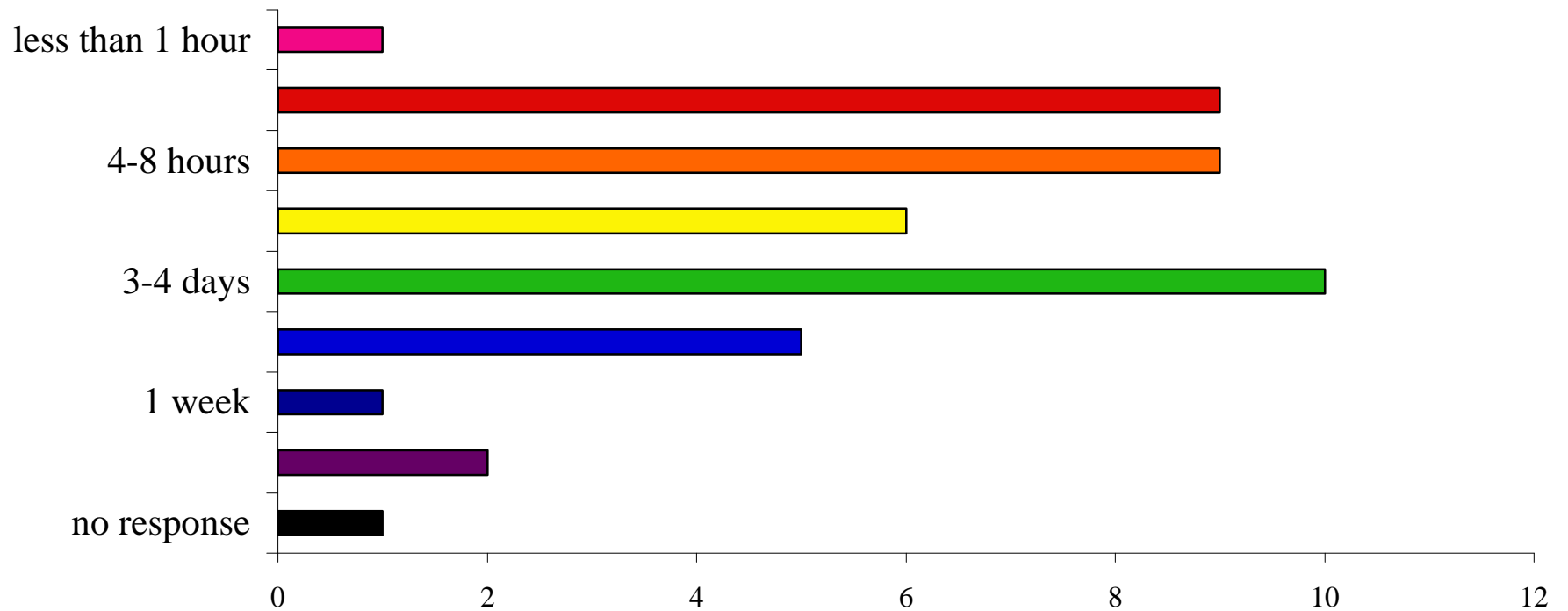
How Much Time Did It Take To Prepare And Analyze This Sample?





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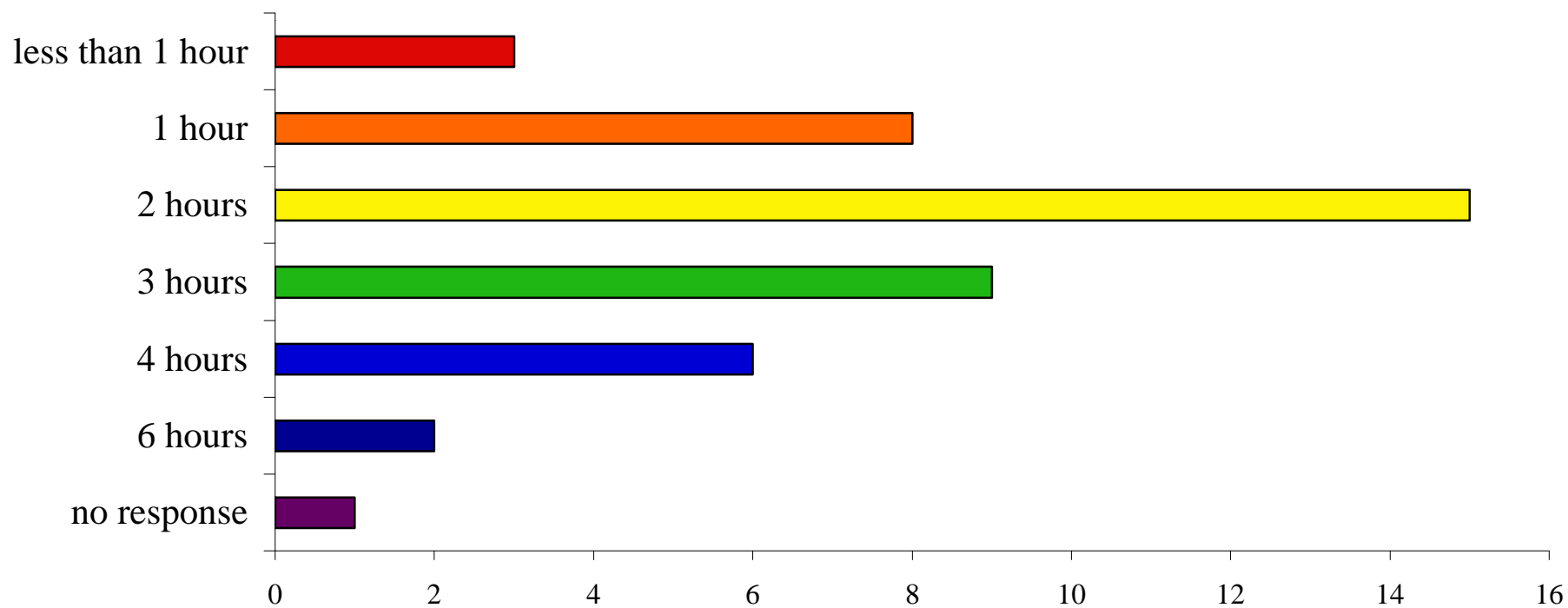
How Much Time Did It Take To Analyze The Data?

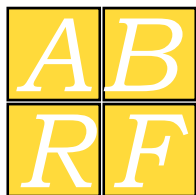




***Proteomics Standards
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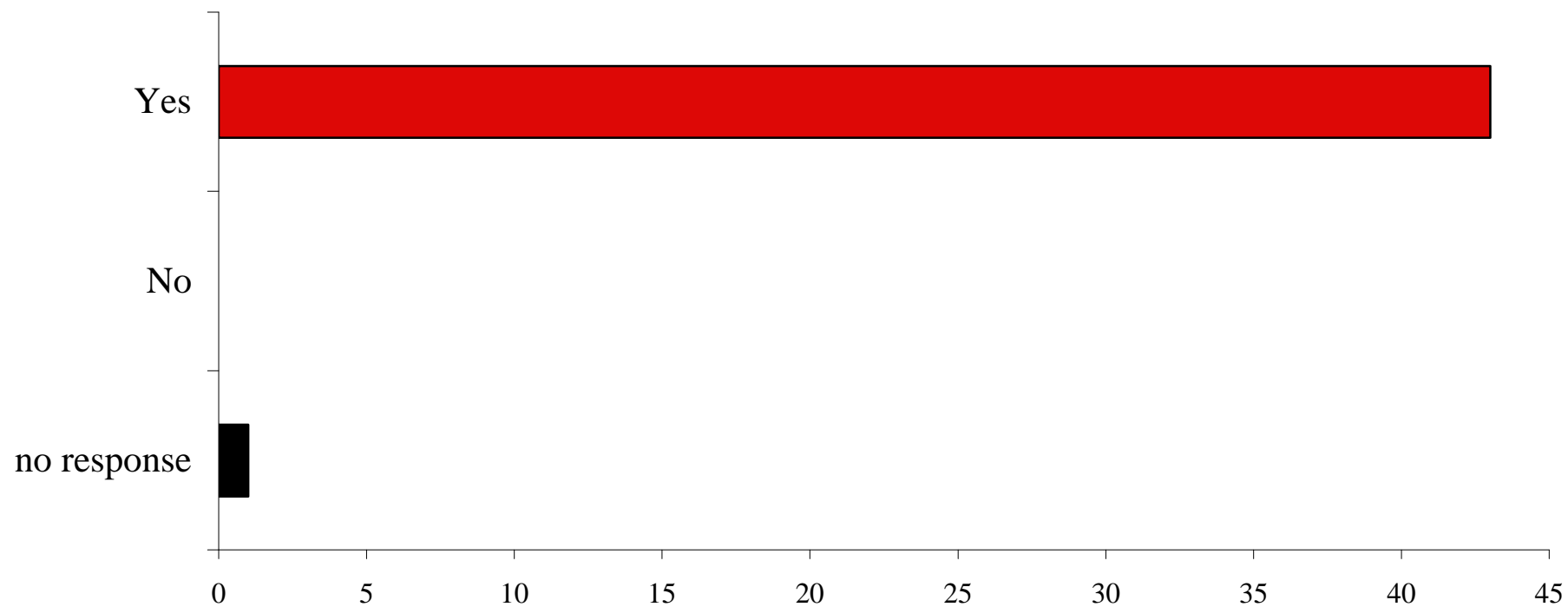
How Much Time Did It Take To Complete The Survey?





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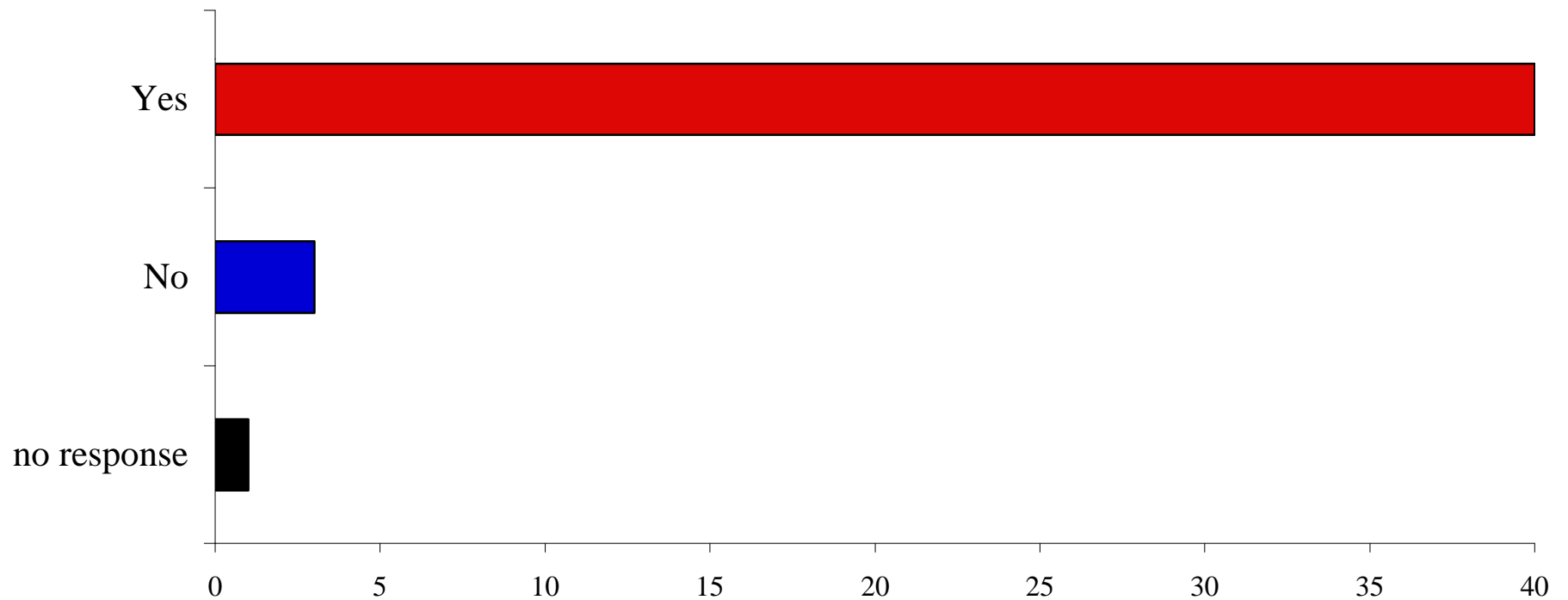
Do You Think This Type of Study Has Been Useful?





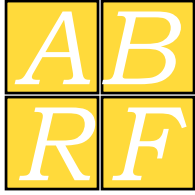
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Would This Standard be Helpful for Phosphorylation Studies?



Conclusions

- Qualitative phosphorylation site identification still presents a challenge for many labs. Some known sites were detected by >50% of labs, suggesting that these sites are readily detected mass spectrometric analysis of tryptic peptides. Other known sites were identified by few or no labs.
- On average, enrichment methods did not show an advantage over non-enrichment; however, results varied widely among labs using both approaches, with some obtaining excellent results and others performing poorly, with or without enrichment. This suggests the overwhelming importance of individual technique and experience.
- Comparing results between labs is hindered by lack of accepted statistical/spectral methods for identifying phosphopeptides. Examination of spectra underlying reported identifications indicated that identifications were not always made based on clear-cut spectral evidence.
- This study demonstrates the difficulty confronting the creation of a standard mix of phosphoproteins. This material in its present state can serve as a “standard” for comparison among labs, but it is insufficiently characterized for performance evaluation. A confident knowledge of the phosphorylated sites and their stoichiometry is essential for the latter.



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THANK YOU

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