

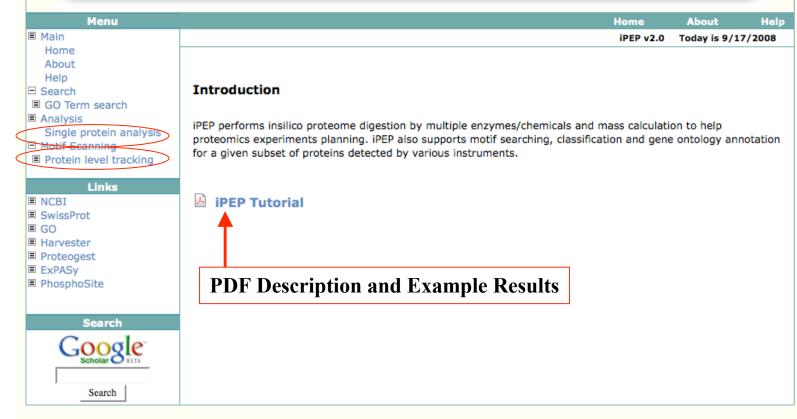
iPEP User's Guide

iPEP has been designed to aid in designing sample preparation techniques around the detection of specific sites or motifs in proteins and proteomes. The first application, single protein sequence analysis enables extensive analysis of the potential sites of post-translational modifications in a userselected proteins. The second application, proteome motif scanning, measures the occurrence of a specific sequence in the proteome and determines the efficacy of digestion and instrument selection on detecting the proteins that contain the motif.

iPEP Front Page http://ipep.moffitt.org

2

In Silico Examination of Proteolytic Peptides for Mass Spectrometry

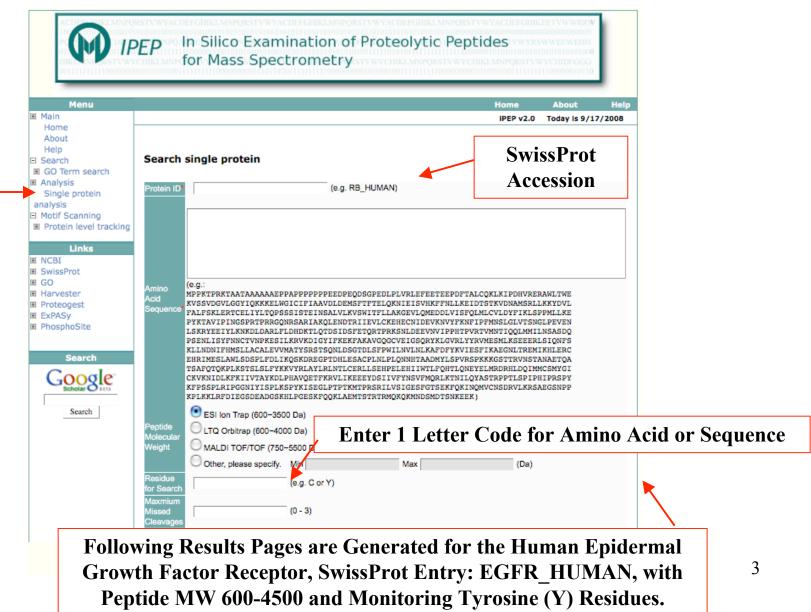


© Biomedical Informatics, H. Lee Moffitt Cancer Center and Research Institute All Rights Reserved

- 1. Examine Protein Sequence for Post-translational Modification Analysis (p. 3)
- 2. Monitor Detection of Proteins Containing Specific Motifs or Amino Acid Residues in Entire Proteomes to Design Peptide-based Proteomic Experiments (p. 12)

iPEP Protein Sequence Analysis:

Analysis of Potential Phosphotyrosine Sites in the Human Epidermal Growth Factor Receptor



iPEP Protein Sequence Analysis Results: EGFR Accession, Description, and Sequence

Search result for protein: EGFR_HUMAN

Click to Access Peptide Lists in Sequence Order for All Methods (p. 5)

Protein information (View all peptides per digest)

Accession	P00533	Links to SwissProt Entry
Entry Name	EGFR_HUMAN	
Description	Epidermal growth factor receptor precursor (EC 2.7.10.1) (F	eceptor tyrosine-protein kinase ErbB-1) - Homo sapiens (Human)
Sequence (1210 aa)	MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFED TIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALAVLSNYD SIQWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEEN TGPRESDCLVCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYS DGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSIS ITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNIT FGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSC ECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTL PKIPSIATGMVGALLLLLVVALGIGLFMRRRHIVRKRTLRRLLQER GAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKANKEILDEAYVM YVREHKDNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVK MALESILHRIYTHQSDVWSYGVTWELMTFGSKPYDGIPASEISSI FRELIIEFSKMARDPQRYLVIQGDERMHLPSPTDSNFYRALMDEED TSNNSTVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTF RDPHYQDPHSTAVGNPEYLNTVQPTCVNSTFDSPAHWAQKGSHQIS APQSSEFIGA	HFLSLQRMFNNCEVVLGNLEITYVQRNYDLSFLK ANKTGLKELPMRNLQEILHGAVRFSMNPALCNVE CQKLTKIICAQQCSGRCRGKSPSDCCHNQCAAGC FGATCVKKCPRNYVVTDHGSCVRACGADSYEMEE GDLHILPVAFRGDSFTHTPPLDPQELDILKTVKE SLGLRSLKEISDGDVIISGNKNLCYANTINWKKL RNVSRGRECVDKCNLLEGEPREFVENSECIQCHP VWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNG ELVEPLTPSGEAPNQALLRILKETEFKKIKVLGS ASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLD TPQHVKITDFGLAKLLGAEEKEYHAEGGKVPIKW LEKGERLPQPPICTIDVYMINVKCWMIDADSRPK MDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLSA LPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPS
Molecular Weight	600~4500 (Da)	

This panel shows the sequence of the selected SwissProt entry. Additional data, including sequence, residue, and peptide coverages, are also displayed as part of the same web page (pages 6, 8, and 10-12).

iPEP Protein Sequence Analysis Results: Peptide Lists (from p. 4)

Start	Stop	Peptide Sequence	Molecular Weight
		Digestion: Acid	
0	45	MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFED	4730.45562
46	74	HFLSLQRMFNNCEVVLGNLEITYVQRNYD	3514.71249
75	125	LSFLKTIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALAVLSNYD	5834.06433
126	165	ANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRD	4561.34333
166	170	IVSSD	519.25397
171	178	FLSNMSMD	943.37788
179	190	FQNHLGSCQKCD	1378.57574
191	229	PSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSD	4097.80558
230	246	CCHNQCAAGCTGPRESD	1750.62793
247	255	CLVCRKFRD	1138.57388
256	261	EATCKD	665.26897
262	277	TCPPLMLYNPTTYQMD	1886.82522
278	302	VNPEGKYSFGATCVKKCPRNYVVTD	2774.35191
303	313	HGSCVRACGAD	1074.43342
314	320	SYEMEED	901.30106
321	346	GVRKCKKCEGPCRKVCNGIGIGEFKD	2823.41274
347	367	SLSINATNIKHFKNCTSISGD	2249.11095
368	378	LHILPVAFRGD	1236.69779
379	387	SFTHTPPLD	1013.48173
388	392	PQELD	600.27543
393	415	ILKTVKEITGFLLIQAWPENRTD	2684.49002
416	457	LHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD	4689.57171
458	459	GD	190.05890

Continues for All Digestion Methods

iPEP Protein Sequence Analysis Results: Table of Proteolytic Peptide, Sequence, and Residue Coverage

Summary of peptide, sequence and residue coverage. (Click the link to view the residue coverage)

Digestion	Peptide Coverage	Sequence Coverage	Residue Coverage (Y)	Compare	
Trypsin	74/122	1054/1210	33/36		
Arg C	35/61	673/1210	20/36		User Can
Lys C	44/67	770/1210	20/36		Compare Digests
Lys N	44/67	770/1210	20/36		using Sequence
Acid	43/62	748/1210	25/36		Alignment
Asp N	43/62	749/1210	25/36		(see p. 7)
V8 phosphate	79/139	1016/1210	32/36		(see p. 7)
V8 bicarbonate	48/78	833/1210	27/36		
Pepsin (PH 1.3)	74/148	928/1210	32/36		
Pepsin (PH 2.0)	61/394	493/1210	7/36		
Proteinase K	63/407	461/1210	9/36		
Thermolysin	65/383	502/1210	20/36		
Chymo (F/Y/W/M/L)	60/82	1043/1210	27/36		
Chymo (F/Y/W)	88/238	856/1210	17/36		
CNBr	15/26	349/1210	18/36	Cyanoge	en bromide
IBA	4/1 4	100/1210	2/36	Iodosob	enzoic Acid
NTCB	28/61	395/1210	11/36	🗖 2-Nitro-	5-thiocyanatobenzoic Acid
v8_LysC	85/184	955/1210	31/36		
AspN_ nGlu	79/139	1015/1210	32/36		
AspN_LysC	81/125	1057/1210	28/36		
Trypsin_Chymo	91/204	922/1210	18/36		6
IBA_CNBr	22/39	487/1210	21/36		

iPEP Protein Sequence Analysis Results: Sequence Alignment of Trypsin and *S. aureus* V8 Protease Digests

(from p. 6)

Protein EntryEGFR_HUMANResidue(s)YMolecular Weight600~4500 (Da)

Coverage comparison (The blue indicates detectable peptide and the red is detectable residue)

EGFR_HUMAN Trypsin	MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEV		
V8 phosphate	MRPSGTAGAALLALLAALCPASRVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEV MRPSGTAGAALLALLAALCPASRALE-KKVCQGTSNKLTQLGTFE-HFLSLQRMFNNCEV	R	esidue
EGFR_HUMAN Trypsin V8 phosphate	VLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALA VLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALA VLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALA		racking
EGFR_HUMAN	VLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDF		Detectable
Trypsin V8 phosphate	VLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDF VLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDFLSNMSMDF		Sequence
EGFR_HUMAN Trypsin V8 phosphate	QNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSDCCHNQCAAGC QNHLGSCQKCDPSCPNGSCWGAGEENCQKIICAQQCSGRSPSDCCHNQCAAGC QNHLGSCQKCDPSCPNGSCWGAGE-NCQKLTKIICAQQCSGRCRGKSPSDCCHNQCAAGC		
EGFR_HUMAN Trypsin V8 phosphate	TGPRESDCLVCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYV TGPRESDCLVCRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYV TGPRECLVCRKFRDTCPPLMLYNPTTYQMDGKYSFGATCVKKCPRNYV		
EGFR_HUMAN Trypsin V8 phosphate	VTDHGSCVRACGADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFK VTDHGSCVRACGADSYEMEEDGVRCEGPCR-VCNGIGIGEFKDSLSINATNIKHFK VTDHGSCVRACGADGVRKCKKCEGPCRKVCNGIGIGESLSINATNIKHFK		7

iPEP Protein Sequence Analysis Results: Tyrosine Residue Detection in each Proteolytic Method

Summary of coverage of each residue in all enzymes. (Click the link to view all peptides containing the residue at postion selected)

Y) Position	Trypsin	Arg C	Lys C	Lys N	Acid	Asp N	V8 phosphate	V8 bicarbonate	Pepsin (PH 1.3)	Pepsin (PH 2.0)	Proteinase K	(Y) Position	Thermolysin	Chymo (F/Y/W/M/L)	Chymo (F/Y/W)	CNBr	IBA N	°CB ∨8_L	ysC AspN_nGlu	AspN_LysC	Trypsin_Chymo	IBA_CNE
8	1	1			1	1	a	a	a			68						1			V	
3		1			1	1	V		*			73		S	S			1	S			
7							V	V	S			87		V	V						V	
.11		1							a	V	V	111	a	a	V	1		1				1
112	V	1					V	V	V			112	a			1		1	V			1
116		1							a			116				1		1	V			
124		1					V		a			124	V	a		1		1	S		V	1
269	V		1	1	1	1			a			269	V	a		1	6	1		1	V	
274			1	1	1	1	V		S			274				1	6	1				
284			1	1	1	1			a		V	284		V	V	1	6	1				
298	V				1	1	V				V	298	V	a	V	1	6	1	S	1		1
815		1	1	1	1	1						315		*	V	1	6	1			V	1
¥70				1			V		*			470		a			6	1				
584					1	1	V					584				1	6	1	S		V	
509				1	1	1			*			609					6	1				1
525				1			V		*		*	625		V	V			1	V		V	1
726	2		1	1			V		*			726						1				
763		1			1	1			*			763		a				1	S		V	
300		1			1	1			V			800		V		1	6	1			V	1
312		1			1	1		a	a			812		*	V	1	6	1	S		V	1
326		1	1	1	V	1		a	V	a	a	826		a				1	V			
368			*	1					a		a	868	V	a	V							
390			1	1			V	a	S			890	V	a		1	1	1		1		
399					V	1	a	a	S			899	a			1	1	1		1		1
914			1	1	1	1	V	a	S			914				1		1	S		V	
943			s.	1		1		a	S	a		943		a	V	1	6	1	S		V	1
977					1		a	a	S		a	977		V	V	1		1	V			
97					V	1	V	a				997				1		1	S			
015					V	1	a		a			1015		*				1			V	
1068	V			1	V	1	V	a	S			1068	a					1				
1091	a						a		a			1091		V	V					V	V	
109	1	1			V	1			a			1109	V	a	V			1	V	1	V	
124						1			a	a	1	1124	a	a				1		1		
137	a				1	1			a			1137		a	V			1	a		V	
171				V						a		1171		a	V						V	
196			X	1		1			a			1196		V	A			1		1	S	

Links to Display of Detectable Peptides Containing Tyr 1068 (p. 9)

iPEP Protein Sequence Analysis Results: Peptides Containing Tyrosine 1068 (from p. 8)

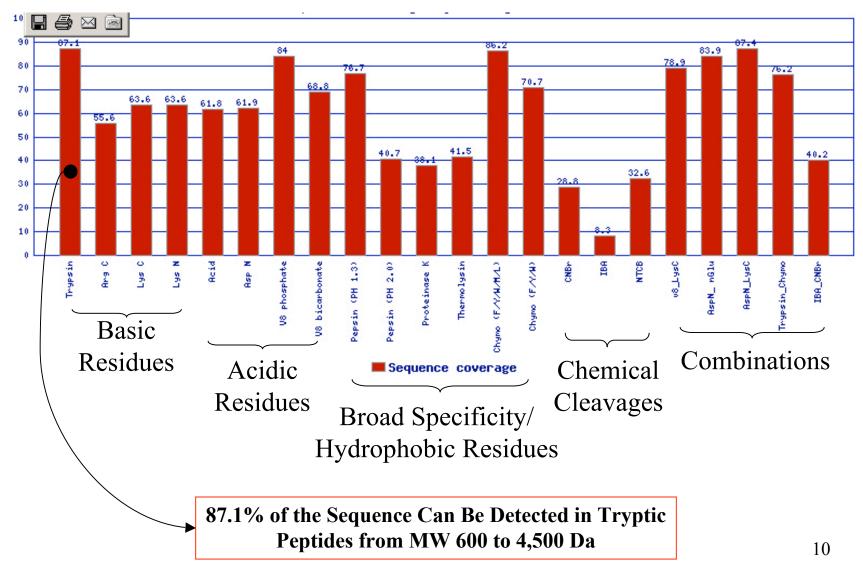
List of detectable peptides containing residue Y **EGFR_HUMAN Tyr 1068**

Digestion	Start	Stop	Peptide	Molecular Weight
Trypsin	1068	1098	YSSDPTGALTEDSIDDTFLPVPEYINQSVPK	3397.60881
Arg C	1068	1099	YSSDPTGALTEDSIDDTFLPVPEYINQSVPKR	3553.70992
Lys C	1061	1098	EDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPK	4273.02253
Lys N	1060	1097	KEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVP	4273.02253
Acid	1063	1071	SFLQRYSSD	1101.50902
Asp N	1062	1070	DSFLQR <mark>Y</mark> SS	1101.50902
V8 phosphate	1063	1071	SFLQRYSSD	1101.50902
V8 bicarbonate	1062	1078	DSFLQR <mark>Y</mark> SSDPTGALTE	1885.86930
Pepsin (PH 1.3)	1066	1076	QRYSSDPTGAL	1193.56759
Thermolysin	1065	1074	LQRYSSDPTG	1122.53048
v8_LysC	1063	1078	SFLQRYSSDPTGALTE	1770.84236
AspN_ nGlu	1062	1070	DSFLQR <mark>Y</mark> SS	1101.50902
AspN_LysC	1062	1070	DSFLQR <mark>Y</mark> SS	1101.50902

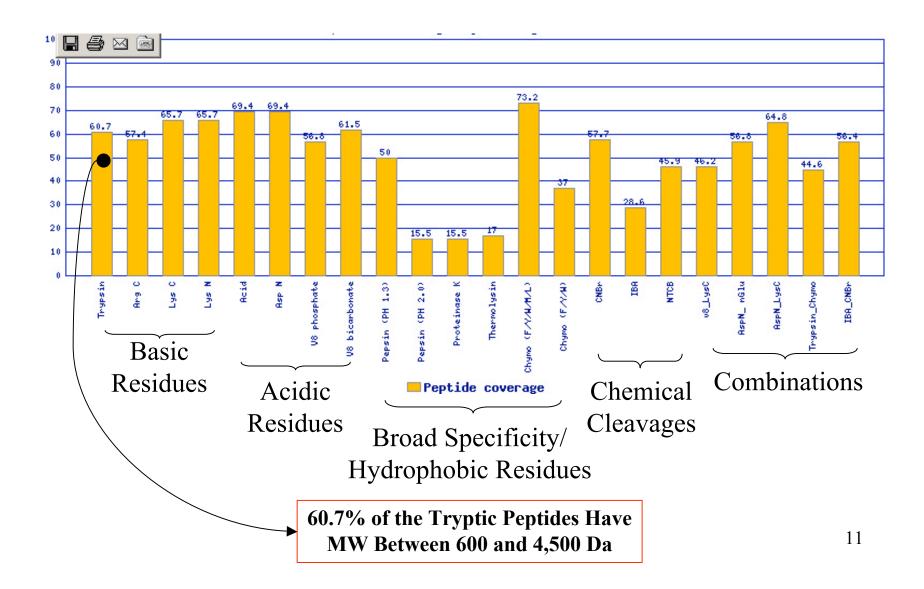
Enables Quantification using Specific Peptide Sequences

iPEP Protein Sequence Analysis Results:

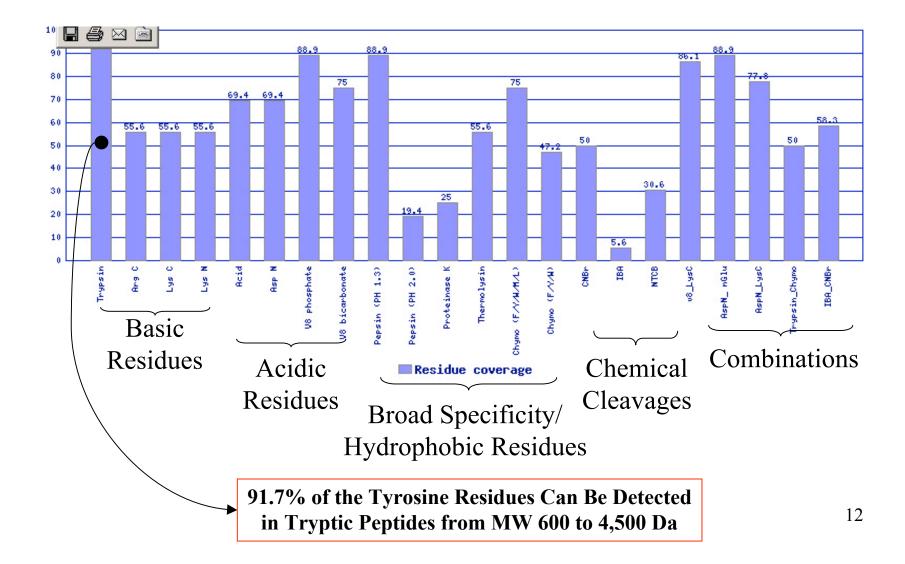
Graph of EGFR Sequence Coverage



iPEP Protein Sequence Analysis Results: Graph of EGFR Peptide Coverage



iPEP Protein Sequence Analysis Results: Graph of EGFR Residue Coverage



Proteome Motif Scanning

Search Parameter Page

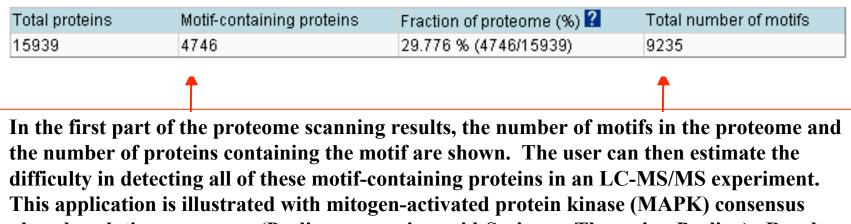
Search	About	Documentation	Help		
Go!				Τα	day is 9/26/2007
Menu Home About	Proteome motif	scanning			
I Help I Search	Data Source* 김	Please select	Select Dat	tabase	
Single protein analysis	Motif * 🞴 🛛 🔒			(Pro	osite: P-X-[ST]-P.)
Protein level		🗖 Trypsin	🗖 Arg C	🗆 Lys C	🗆 Lys N
		C Acid hydrolysis	🗖 Asp N	🗆 V8 Phosphate	□ V8 Bicarbonate
Links INCBI		🗖 Pepsin (PH 1.3)	🗖 Pepsin (PH 2.0)	🗖 Proteinase K	🗖 Thermolysin
■ SwissProt ■ GO	Digestion *(Choose 2)	Chymotrypsin high specificity	Chymotrypsin low specificity	CNBr	🗖 IBA
🗉 Harvester	Select 2 Proteolytic	ПИТСВ	🗖 V8/Lys C	□ Asp N/N- terminal Glu	Asp N/Lys C
	Methods	Trypsin/Chymotrypsin	IBA/CNBr		
	Molecular weight	 ESI Ion Trap (600-350) MALDI TOF/TOF (750-4) 	·	Filtering	
		O Other, please specify.	Min	Max	(Da)
					Submit Reset
	0	d codes. Use X as 10r J. N-terminus i			13

iPEP Proteome Motif Scan Results MAPK Sites in Human SwissProt Entries

Search criteria

Data source	SwissProt human protein data
Motif 김	P.[ST]P
Digest ?	Trypsin, Lys-C
Instrument	ESI (600~3500)

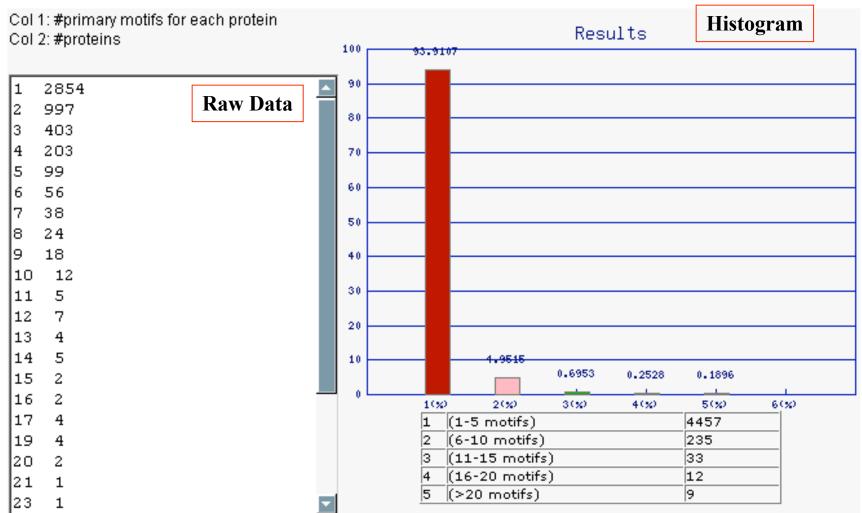
Complexity of proteome



phosphorylation sequences (Proline-any amino acid-Serine or Threonine-Proline). Results continue on p. 15-16.

iPEP Proteome Motif Scan Results:

Distribution of Motifs per Protein



Most proteins containing MAPK consensus phosphorylation sequences have a small number of motifs within their sequence.

15

iPEP Proteome Motif Scan Results:

Peptide Statistics and Detection

Trypsin

otal pro peptides 953610		Detectable peptie 488492	des Motif-containi 7510	ng peptio		Fraction 0.7875	n of peptidome	9 (%)	Determines if peptide fractionation techniques
Detecti	on Resi	ults						are necessary prior to LC-MS/MS.	
Motif	Filter	Peptides from motif- containing proteins	Motif-containing peptides	Motifs	Motif-contai proteins	ining	FASTA Sequence	Compare	
P.[ST] P	None	415027	7510	8409	4746		T	0	
P.[ST] P	MVV	213353	5088	5388	4740		0	0	
•C	oteolytic								
	oteolytic s	Detectable pepti	des Motif-containi	ng peptic	jes F	Fraction	n of peptidome	9 (%)	Compare Detection of
Fotal pro	S	Detectable peptic 283171	des Motif-containi 7574	ng peptio		Fraction	n of peptidome	9 (%)	Compare Detection of Pentides Motifs and
Fotal pro peptides 519240	S	283171		ng peptic			n of peptidome	9 (%)	Peptides, Motifs, and Motif-Containing Proteins
Fotal pro peptides 519240	S	283171		ng peptid Motifs		1.4587	of peptidome FASTA Sequence	e (%) Compare	Peptides, Motifs, and Motif-Containing Proteins to Select Ideal Digestion(s)
Fotal pro beptides 519240 Detecti	s on Resi	283171 JIts Peptides from motif-	7574 Motif-containing		1 Motif-contai	1.4587	FASTA		Peptides, Motifs, and Motif-Containing Proteins to Select Ideal Digestion(s) Trypsin will produce more detectable peptides than
Fotal pro peptides 519240 Detecti Motif P.(ST)	s on Resu Filter	283171 283171 JIts Peptides from motif- containing proteins	7574 Motif-containing peptides	Motifs	1 Motif-contai proteins	1.4587	FASTA Sequence	Compare	Peptides, Motifs, and Motif-Containing Protein to Select Ideal Digestion(s Trypsin will produce mor

iPEP Proteome Motif Scan Results:

Peptide Statistics and Detection

Trypsin

53610		488	3492	7510		0	.7875	Download	l Specif	fic Database
Detecti	on Resi	ults					1		-	ning Proteins
Motif	Filter		from motif- g proteins	Motif-containin peptides	g Motifs	Motif-contaiı proteins	ning FASTA Sequenc	e Compare		
P.[ST] P	None	415027		7510	8409	4746		0		
P.[ST]	MW	213353		5088	5388	4740	0			
P C								I		
c Total pro	oteolytic s	Dei	tectable peptid		ining pepti		raction of peptid	ome (%)		Compare Protei
c Total pro	oteolytic s	Dei	tectable peptid 3171	es Motif-conta 7574	ining pepti		raction of peptid .4587	ome (%)		Compare Protei Populations
c Total pro eptides 19240	oteolytic s	283			ining pepti			ome (%)		Populations
c Total pro eptides 19240	oteolytic s	283 ults Peptides					.4587	Compare		
c iotal pro eptides 19240 Detectio	oteolytic s on Resi	283 ults Peptides	3171 from motif-	7574 Motif-containin		1 Motif-contain	.4587 ning FASTA	Compare		Populations

Comparison of Proteins Detected: Extraction of Differences

Data source	SwissProt human protein data
Primary motif ?	P.[ST]P
Digest ໃ	Trypsin, Lys-C
Modified residue <table-cell></table-cell>	
Instrument	ESI (800~4000)

Result

Set	Difference	Analysis Result	Ç.	Select
\bigcirc	Union	4746 proteins in both digestion Trypsin or Lys-C	0	o
	Intersection	4709 proteins in digestion Trypsin and digestion Lys-C	0	0
	Set difference	32 proteins in digestion Trypsin	0	0
	Set difference	5 proteins in digestion Lys-C	0	0
0	Symmetric difference	37 proteins in digestion Trypsin or digestion Lys-C but not in both	0	0

Trypsin and Lys-C both produce peptides that enable detection of proteins that can not be observed in the other digest. Each set can be viewed in HTML or exported as a FASTA database (see p. 19).

View All GO Terms

Comparison of Proteins Detected: Extraction of Differences

Data source	SwissProt human protein data
Primary motif 김	P.(ST)P
Digest ?	Trypsin, Lys-C
Modified residue ໃ	
Instrument	ESI (800~4000)

Result

Set	Difference	Analysis Result	Çų S	Belect	
\bigcirc	Union	4746 proteins in both digestion Trypsin or Lys-C			
	Intersection	4709 proteins in digestion Trypsin and digestion Lys-C	0	0	
	Set difference	32 proteins in digestion Trypsin	0	0]
	Set difference	<mark>5</mark> proteins in digestion Lys-C	0	0	
0	Symmetric difference	37 proteins in digestion Trypsin or digestion Lys-C but not in both	0	0	
		Download Protein in FASTA View Proteins View A	II GO 1	Terms	

Comparison of Proteins Detected: Extraction of Gene Ontology Data

Data source	SwissProt human protein data
Primary motif ?	P.[ST]P
Digest ?	Trypsin, Lys-C
Modified residue <table-cell></table-cell>	
Instrument	ESI (800~4000)

Result

Set	Difference	Analysis Result	Ç.	Selec	t	
\bigcirc	Union	4746 proteins in both digestion Trypsin or Lys-C	0	0		
	Intersection	4709 proteins in digestion Trypsin and digestion Lys-C	0	0	-[1	
	Set difference	32 proteins in digestion Trypsin	0	0		
	Set difference	5 proteins in digestion Lys-C	0	0		The GO terms can be viewed for each set.
0	Symmetric difference	37 proteins in digestion Trypsin or digestion Lys-C but not in both	0	0		

Download Protein in FASTA View Proteins View All GO Terms