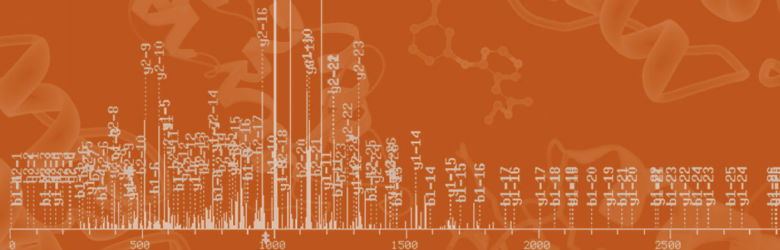
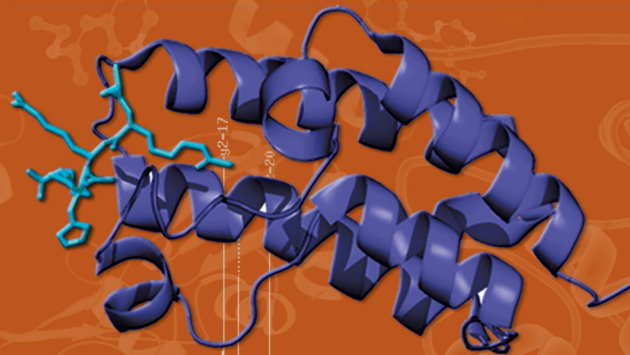


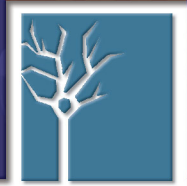
Arginine Mono-Methylation in Mouse Brain and Embryo

MethylScan(R-Me) Summary Report

May 16, 2012

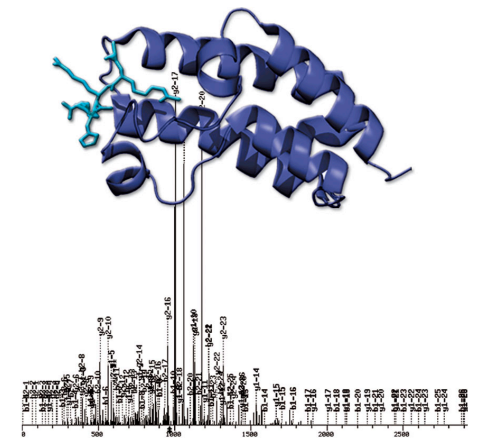


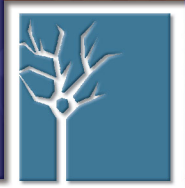
Cell Signaling
TECHNOLOGY®



MethylScan Deliverables

- Project Overview
- Methods
- MethylScan(R-Me) Results (**Table 1**)
 - Qualitative Results (Details tab)
 - Quantitative Results
 - Summary tab
 - Fold Change by Descending Order
 - Fold Change by Protein Type
- Overview of Quantitative Results
- Study Consultations





Method Summary

Methyl-Arg peptide enrichment using the following antibody:

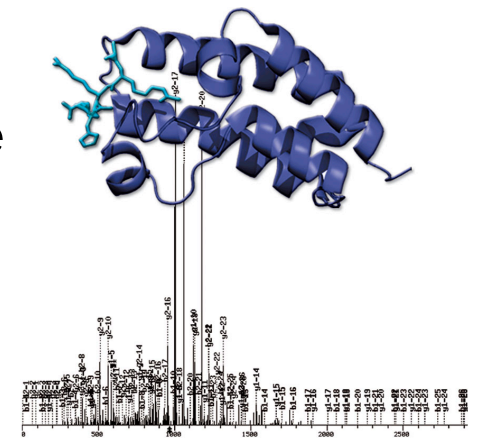
Mono-methyl Arg Antibody (#D5A12A3 & Me-R4-100)

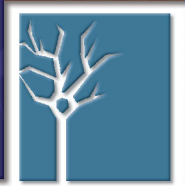
LC-MS/MS Analysis using LTQ-Orbitrap-Velos, ESI-CID

Sorcerer search results

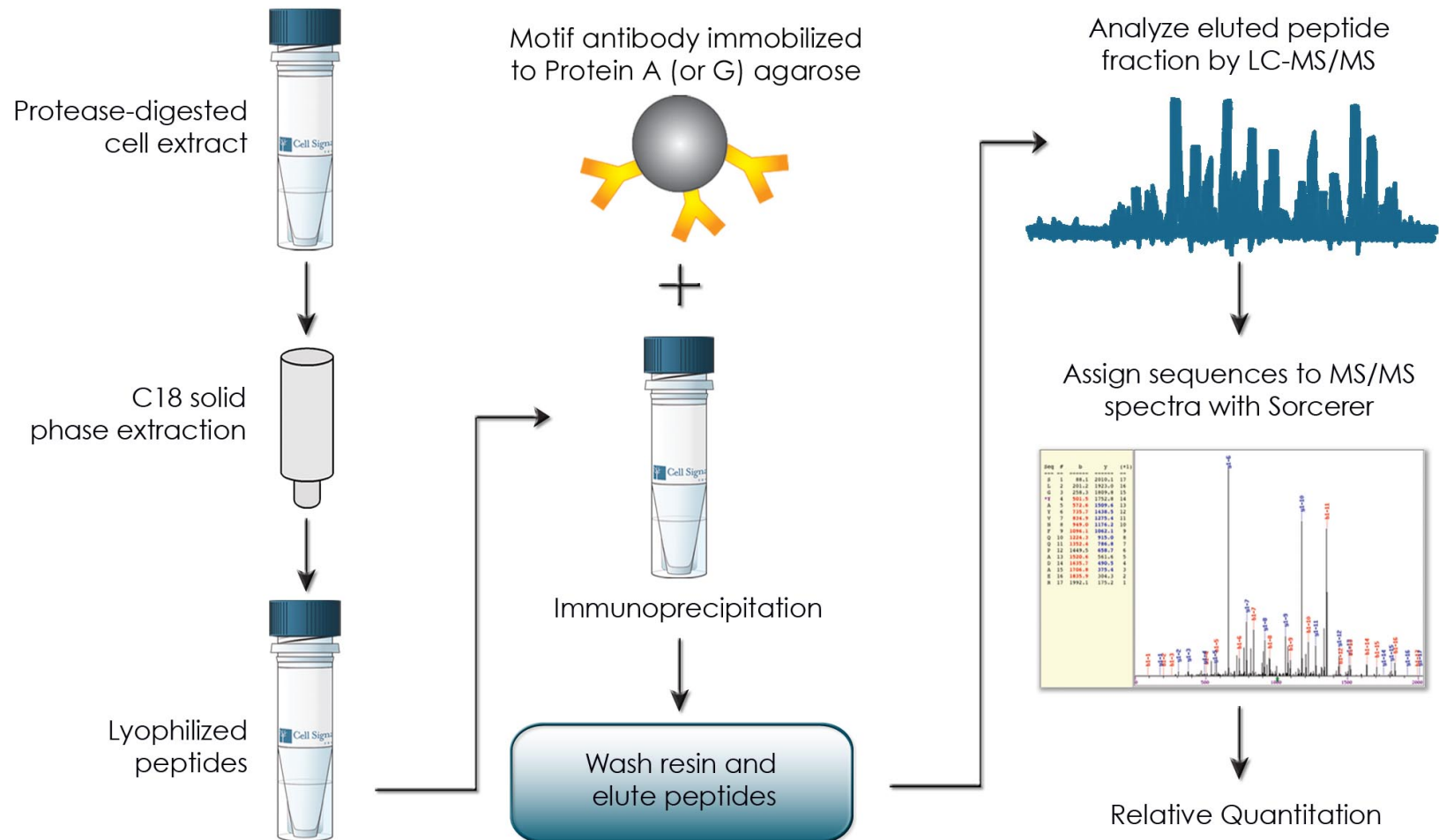
Label-free quantitation from Methyl-Arg peptide intensities (**Table 1**).

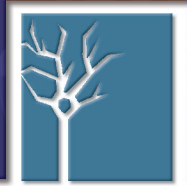
Informatics table (**Table 2**).





MethylScan Method



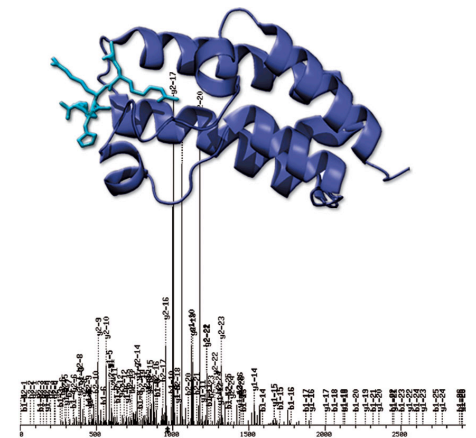


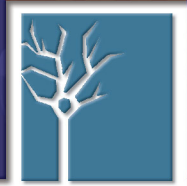
LC Methods

Peptides were loaded directly onto a 10 cm x 75 μm PicoFrit capillary column packed with Magic C18 AQ reversed-phase resin.

The column was developed with a 72-minute linear gradient of acetonitrile in 0.125% formic acid delivered at 280 nL/min.

MS Parameter Settings: MS Run Time 96 min, MS1 Scan Range (300.0 – 1500.00), Top 20 MS/MS (Min Signal 500, Isolation Width 2.0, Normalized Coll. Energy 35.0, Activation-Q 0.250, Activation Time 20.0, Lock Mass 371.101237, Charge State Rejection Enabled, Charge State 1+ Rejected, Dynamic Exclusion Enabled, Repeat Count 1, Repeat Duration 35.0, Exclusion List Size 500, Exclusion Duration 40.0, Exclusion Mass Width Relative to Mass, Exclusion Mass Width 10ppm)



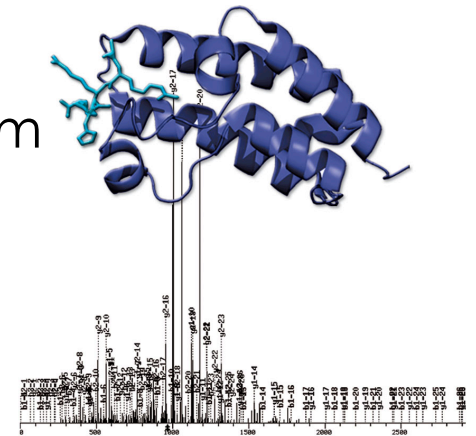


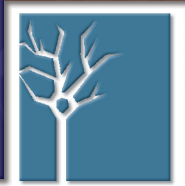
Informatics

MS/MS spectra were evaluated using SEQUEST 3G and the SORCERER 2 platform from Sage-N Research (v4.0, Milpitas CA) (Lundgren et al., *Curr Protoc Bioinformatics*, 2009).

Searches were performed against the most recent update of the NCBI *mus musculus* database with mass accuracy of +/-50 ppm for precursor ions and 1 Da for product ions.

Results were filtered with mass accuracy of +/- 5 ppm on precursor ions and presence of the intended motif.





CST Products for Subsequent Validation

Gene Name	Protein Name	Site
Cfl1	cofilin 1	§112
Elmo1	ELMO1	9
Exoc7	EXOC7	423, 438
Ezr	ezrin	§79
Krt10	K10	556, 566
Lmna; Lmna	lamin A/C; lamin A/C iso.	§417; §305
Lmnb1	Lamin B1	112
Mapre2	RP1	125
Msn	moesin	§139
Msn	moesin	§162
Msn	moesin	§165
Msn	moesin	§352
Msn	moesin	79
Pfn1	profilin 1	§126
Pfn1	profilin 1	127
Pfn1	profilin 1	§54
Pfn1	profilin 1	§70
Pfn1	profilin 1	§91
Plec; Rps10	plectin 1; RPS10	28; 24
Rdx	radixin	§526
Rdx	radixin	§79

Gene Name (**siRNA Product Link**)

SignalSilence® Akt siRNA I #6211

PhosphoSitePlus® protein, site, and accession data: [Akt1](#)

◀ pathway ▼ more info ▼ application references ■ datasheet PDF ■ MSDS PDF ■ protocols

Protein Name (**Total Antibody Product Link**)

Akt (5G3) Mouse mAb #2966

PhosphoSitePlus® protein, site, and accession data: [Akt1](#)

◀ pathway ▼ more info ▼ application references ■ datasheet PDF ■ MSDS PDF ▼ protocols

Site (**Site Specific Antibody Product Link**)

Phospho-Akt (Ser473) (587F11) Mouse mAb #4051

Have you tried your application using our **XP® monoclonal antibodies**? Try product: [4060](#)

PhosphoSitePlus® protein, site, and accession data: [Akt1](#)

◀ pathway ▼ more info ▼ application references ■ datasheet PDF ■ MSDS PDF ▼ protocols

Blue text = hyperlink to CST product pages for siRNA products (Gene Name column), total antibodies (Protein Name column), and site-specific antibodies (Site column).



Experimental Flow Diagram

Cells

Treatment(s)

Curation Sets

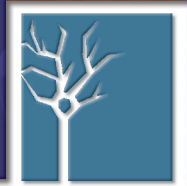
Analysis

Mouse Brain → Untreated → CS 13444, 13445

→ LTQ-Orbitrap
Velos
LC-MS/MS
ESI-CID

Mouse Embryo → Untreated → CS 13450, 13451

2 samples, duplicate injections = 4 LC-MS/MS experiments



Qualitative Results

Experiment	Tissue Type	Treatment	Antibody	CS	Protease	Methyl-Peptide Assignments	
						Redundant	Non-Redundant
1	mouse brain	untreated	Mono-methyl	13444	Trypsin	925	597
2	mouse brain	untreated	Mono-methyl	13445	Trypsin	967	612
3	mouse embryo	untreated	Mono-methyl	13450	Trypsin	1108	665
4	mouse embryo	untreated	Mono-methyl	13451	Trypsin	1203	689

A total of **1,549** redundant methyl-peptide assignments (**Table 1**, Details tab) to **713** non-redundant methyl-peptides (**Table 1**, Summary tab) for the Mono-methyl antibody (D5A12A3 & Me-R4-100).

A 5% default false positive rate was used to filter the SORCERER results.

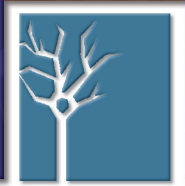


Table Overview

Each PTMScan table consists of 5 sheets:

- Column Definitions tab

- Details (qualitative) tab

- Summary (quantitative) tab

- Fold Change by Descending Order tab (quantitative)

- Fold Change by Protein Type tab (quantitative)

Tables will open to the Fold Change by Descending Order tab.

Other tabs are accessed by clicking on sheet names at bottom of window (**blue boxes** in following slides).



Table Overview: Column Definitions

Column Name	Definition	In Details	In Summary
Accession	is the UniProt accession number for the assigned protein	Y	Y
Calculated m/z	"is the calculated, accurate mass-to-charge ratio of the identified peptide from the primary amino acid sequence and associated modifications"	Y	Y
Charge	is the observed charge state for the identified peptide	Y	Y
Count in Details	"is the number of times the designated modification site is represented from other peptide assignments in the Details tab. These redundant assignments can be as a result of multiple charge states, presence of oxidized methionine and overlapping sequence."		Y
Delta Cn	"is the delta Xcorr value. In general, a Delta Cn of 0.1 or greater is good"	Y	
Description	is the FASTA database description of the protein entry	Y	Y
Fold Change	is the relative fold-change between the integrated peak area of the heavy (numerator) and light (denominator) conditions. A negative value indicates the peptide is more abundant in the light condition		Y
Gene Name	"is the gene name for the assigned protein. In some cases, the Gene Name is hyperlinked (when in blue text) when there are protein-specific antibodies available at CST"	Y	Y
kD	is the translated molecular weight of the assigned protein	Y	
Mass Accuracy (ppm)	is the mass measurement error obtained between the observed and theoretical mass of the assigned peptide	Y	
MS2 Spectrum Number	is the spectrum number of the MS2 channel of the raw LC-MS/MS data file	Y	
uDeltaCn	is the delta Xcorr value excluding peptides of the same sequence with phosphates localized to different residues.	Y	
P value	"is the probability score which is generated from a neural network of MS/MS spectra (CID only, parent ions of z=2 or 3) to indicate the likelihood that a sequence can be correctly assigned. A value of 0 is indicates a high false-positive assignment"	Y	
Peak Apex MS Spectrum Number	is the spectrum number that is referenced for the corresponding apex peak intensity measurement of the parent ion from the MS channel of the raw LC-MS/MS data file	Y	
Peptide	is the amino acid sequence for the peptide assignment	Y	Y
Raw Intensity	is the parent ion intensity observed during the MS at its chromatographic apex. A blank intensity value indicates that the peptide was not analyzed by MS/MS for that particular sample and does not suggest that the peptide was absent from the sample		Y
Retention Time	is the corresponding apex retention time of the parent ion from the MS channel of the raw LC-MS/MS data file		Y
Site	"is the site of modification for the peptide assignment. In some cases, the site is hyperlinked (when in blue text) when there are site-specific antibodies available at CST"	Y	Y
Species	indicates the origin(s) of the identified peptide	Y	
IIDL	is the IIDL address for the PhoenixSitePlus web site for the protein assignment	Y	Y

Column Definitions tab contains description of column headers in data tables.



Table Overview: Details Tab

MethylScan (Mono-Methyl-Arginine)_RESULTS

Table #1: Mouse Brain and Embryo; Trypsin Digest; Mono-Methyl-Arg antibody

Samples: Sample 1 = Mouse Brain = CS13444, 13445; Sample 2 = Mouse Embryo = CS.

Legend: * - methylation, # - oxidized methionine, § - published site, **Blue Text** - CST a

Row Index	Protein Type	Gene Name	Protein Name	Site
Activator protein				
1	Activator protein	6720463M24Rik	BORA	316
2	Activator protein	6720463M24Rik	BORA	316
3	Activator protein	6720463M24Rik	BORA	316
Adaptor/scaffold				
4	Adaptor/scaffold	Abi1; Abi1	Abi-1; Abi-1 iso4	189; 184
5	Adaptor/scaffold	Akap8	AKAP8	109
6	Adaptor/scaffold	Akap8	AKAP8	109
7	Adaptor/scaffold	Akap8	AKAP8	109
8	Adaptor/scaffold	Akap8	AKAP8	109
9	Adaptor/scaffold	Akap8	AKAP8	188
10	Adaptor/scaffold	Akap8l	HAP95	172
11	Adaptor/scaffold	Akap8l	HAP95	172, 180
12	Adaptor/scaffold	Akap8l	HAP95	238
13	Adaptor/scaffold	Akap8l	HAP95	238
14	Adaptor/scaffold	Akap8l	HAP95	238
15	Adaptor/scaffold	Ank1; Ank1	ANK1; ANK1 iso2	1684; 1670
16	Adaptor/scaffold	Ank3; Ank3; Ank3; Ank3	ANK3; ANK3 iso6; ANK3	293; 293; 293; 293
17	Adaptor/scaffold	Caskin1	CASKIN1	1020
18	Adaptor/scaffold	Cblb	Cbl-b	13
19	Adaptor/scaffold	Crk	Crk	68

Column Definition | **Details** | Summary | Fold change by descending order | Fold change by protein type | +

Details Tab contains a redundant list of all methyl-peptides identified in the study with their accompanying SORCERER metrics.



Table Overview: Summary Tab

MethylScan (Mono-Methyl-Arginine)_RESULTS

Table #1: Mouse Brain and Embryo; Trypsin Digest; Mono-Methyl-Arg antibody

Samples: Sample 1 = Mouse Brain = CS13444, 13445; Sample 2 = Mouse Embryo = CS13444, 13445

Legend: * - methylation, # - oxidized methionine, § - published site, **Blue Text** - CST at

		Fold Change			
Index	Index in Detail	Brain:Embryo	Gene Name	Protein Name	Site
1	Activator protein				
2	2	-23.2	6720463M24Rik	BORA	316
3	Adaptor/scaffold				
4	4	Brain	Abi1; Abi1	Abi-1; Abi-1 iso4	189; 184
5	6	-4.6	Akap8	AKAP8	109
6	9	Embryo	Akap8	AKAP8	188
7	10	1.0	Akap8l	HAP95	172
8	11	1.2	Akap8l	HAP95	172, 180
9	12	Embryo	Akap8l	HAP95	238
10	15	Brain	Ank1; Ank1	ANK1; ANK1 iso2	1684; 1670
11	16	-6.7	Ank3; Ank3; Ank3; Ank3	ANK3; ANK3 iso6; ANK3	293; 293; 293; 293
12	18	3.4	Cblb	Cbl-b	13
13	19	Embryo	Crk	Crk	68
14	23	21.4	Dlgap3	SAPAP3	922
15	24	17.8	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	132; 132
16	26	17.6	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	132, 138; 132, 138
17	27	Brain	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	290; 290
18	28	-7.5	Dnmbp	DNMBP	1097
19	29	-22.3	G3bp1	G3BP-1	427
20	31	-14.5	G3bp1	G3BP-1	427, 433
21	35	-5.1	G3bp1	G3BP-1	433

Summary | Column Definition | Details | Summary | Fold change by descending order | Fold change by protein type

Summary Tab (quantitative results) is a non-redundant list of sites quantified in the study (**BLUE BOX**). The table header (**BLACK BOX**) contains information about the study and legend text.



Table Overview: Fold Change by Descending Order

MethylScan (Mono-Methyl-Arginine)_RESULTS

Table #1: Mouse Brain and Embryo; Trypsin Digest; Mono-Methyl-Arg antibody

Samples: Sample 1 = Mouse Brain = CS13444, 13445; Sample 2 = Mouse Embryo = CS1

Legend: * - methylation, # - oxidized methionine, § - published site, **Blue Text** - CST a

		Fold Change			
Index	Index in Detail	Brain:Embryo	Gene Name	Protein Name	Site
1	4	Brain	Abi1; Abi1	Abi-1; Abi-1 iso4	189; 184
2	15	Brain	Ank1; Ank1	ANK1; ANK1 iso2	1684; 1670
3	27	Brain	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	290; 290
4	95	Brain	Rims1; Rims1	RIMS1; RIMS1 iso7	1146; 1160
5	109	Brain	Shank1	SHANK1	1059
6	112	Brain	Shank1	SHANK1	1091
7	113	Brain	Shank1	SHANK1	1416
8	115	Brain	Shank1	SHANK1	1447
9	117	Brain	Shank1	SHANK1	1901
10	122	Brain	Shank1	SHANK1	493
11	124	Brain	Shank1	SHANK1	504
12	129	Brain	Shank1	SHANK1	958
13	134	Brain	Shank2; Shank2	SHANK2; SHANK2 iso2	93; 93
14	136	Brain	Shank3	SHANK3	926
15	201	Brain	Wipf2	WIRE	292, 316
16	205	Brain	Wipf2	WIRE	356
17	224	Brain	8430408G22Rik	Depp	62, 65, 68, 73
18	235	Brain	Atp1a4	ATP1A4	237
19	240	Brain	Cenpv	CENPV	39
20	249	Brain	Cpeb3	CPEB3	206

Excel files will open to **Fold Change Descending Order Tab**. It is a non-redundant list of sites quantified in the study organized by maximum fold change across samples. Other sheets can be accessed from tabs at bottom of window (**blue box and arrow**). Table headers (**black box**) contain information about the study and legend text



Table Overview: Fold Change by Protein Type Tab

MethylScan (Mono-Methyl-Arginine)_RESULTS

Table #1: Mouse Brain and Embryo; Trypsin Digest; Mono-Methyl-Arg antibody

Samples: Sample 1 = Mouse Brain = CS13444, 13445; Sample 2 = Mouse Embryo = CS1

Legend: * - methylation, # - oxidized methionine, § - published site, **Blue Text** - CST a

		Fold Change			
Index	Index in Detail	Brain:Embryo	Gene Name	Protein Name	Site
1	Activator protein				
2	2	-23.2	6720463M24Rik	BORA	316
3	Adaptor/scaffold				
4	4	Brain	Abi1; Abi1	Abi-1; Abi-1 iso4	189; 184
5	6	-4.6	Akap8	AKAP8	109
6	9	Embryo	Akap8	AKAP8	188
7	12	Embryo	Akap8l	HAP95	238
8	15	Brain	Ank1; Ank1	ANK1; ANK1 iso2	1684; 1670
9	16	-6.7	Ank3; Ank3; Ank3; Ank3	ANK3; ANK3 iso6; ANK3	293; 293; 293; 293
10	18	3.4	Cblb	Cbl-b	13
11	19	Embryo	Crk	Crk	68
12	23	21.4	Dlgap3	SAPAP3	922
13	24	17.8	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	132; 132
14	26	17.6	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	132, 138; 132, 138
15	27	Brain	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	290; 290
16	28	-7.5	Dnmbp	DNMBP	1097
17	29	-22.3	G3bp1	G3BP-1	427
18	31	-14.5	G3bp1	G3BP-1	427, 433
19	35	-6.1	G3bp1	G3BP-1	433
20	48	-9.7	G3bp1	G3BP-1	445
21	54	-23.8	G3bp1	G3BP-1	445, 458

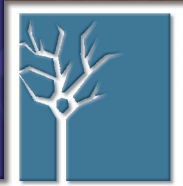
Fold Change by Protein Type tab provides the overall quantitative results sorted by protein type containing only those sites that increase above 2.5-fold (**GREEN**) and those sites that decrease below -2.5-fold (**RED**).



Table Overview: Summary Tab

MethylScan (Mono-Methyl-Arginine)_RESULTS					
Table #1: Mouse Brain and Embryo; Trypsin Digest; Mono-Methyl-Arg antibody					
Samples: Sample 1 = Mouse Brain = CS13444, 13445; Sample 2 = Mouse Embryo = CS13444, 13445					
Legend: * - methylation, # - oxidized methionine, § - published site, Blue Text - CST a					
		Fold Change			
Index	Index in Detail	Brain:Embryo	Gene Name	Protein Name	Site
1	Activator protein				
2	2	-23.2	6720463M24Rik	BORA	316
3	Adaptor/scaffold				
4	4	Brain	Abi1; Abi1	Abi-1; Abi-1 iso4	189; 184
5	6	-4.6	Akap8	AKAP8	109
6	9	Embryo	Akap8	AKAP8	188
7	10	1.0	Akap8l	HAP95	172
8	11	1.2	Akap8l	HAP95	172, 180
9	12	Embryo	Akap8l	HAP95	238
10	15	Brain	Ank1; Ank1	ANK1; ANK1 iso2	1684; 1670
11	16	-6.7	Ank3; Ank3; Ank3; Ank3	ANK3; ANK3 iso6; ANK3	293; 293; 293; 293
12	18	3.4	Cblb	Cbl-b	13
13	19	Embryo	Crk	Crk	68
14	23	21.4	Dlgap3	SAPAP3	922
15	24	17.8	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	132; 132
16	26	17.6	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	132, 138; 132, 138
17	27	Brain	Dlgap4; Dlgap4	SAPAP4; SAPAP4 iso2	290; 290
18	28	-7.5	Dnmbp	DNMBP	1097
19	29	-22.3	G3bp1	G3BP-1	427
20	31	-14.5	G3bp1	G3BP-1	427, 433
21	35	-5.1	G3bp1	G3BP-1	433

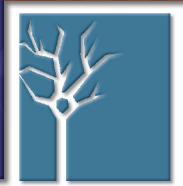
Summary Tab (quantitative results) is a non-redundant list of sites quantified in the study (**BLUE BOX**). The table header (**BLACK BOX**) contains information about the study and legend text.



Quantitative Table: Summary Tab

Index	Index in Detail	Fold Change		Gene Name	Protein Name	Site
		Brain:Embryo				
81	209	43.2		Wipf2	WIRE	430
82	Adhesion or extracellular matrix protein					
83	210	17.6		1110038F14Rik	C8orf33	136
84	211	Embryo		1110059M19Rik	CXorf64	155
85	212	-23.8		2310014H01Rik	KIAA1949	23
86	213	5.5		2310057M21Rik	C10orf88	218
87	214	Embryo		2500003M10Rik	C1orf77	98
88	216	-5.6		2700081O15Rik	MGC3032	15
89	217	-3.8		2700081O15Rik	MGC3032	15, 26
90	218	-6.6		2700081O15Rik	MGC3032	15, 26, 28
91	219	-1.4		2700081O15Rik	MGC3032	228
92	221	-4.5		4833420G17Rik	C5orf34	300
93	223	-3.8		4931428F04Rik	KIAA0895L	101
94	224	Brain		8430408G22Rik	Depp	62, 65, 68, 73
95	225	5.8		9030409G11Rik; 903040	KAZ; RP1-21O18.1 iso2	398; 387
96	226	-3.7		Aimp2	JTV1	26
97	227	1.1		Ankrd44	ANKRD44	735, 737, 746
98	232	4.2		Arhgef33	Arhgef33	766
99	234	Embryo		Ash1l	ASH1L	971, 982
100	235	Brain		Atp1a4	ATP1A4	237
101	236	1.5		BC068157	LOC73072	812
102	239	-13.8		Cdc42ep5	CDC42EP5	38
103	240	Brain		Cenpv	CENPV	39
104	245	-5.9		Cnbp	Cnbp	32, 34
105	246	-4.0		Cnbp	Cnbp	72

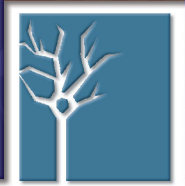
Redundancy is eliminated from the Details Table to provide a summary of the quantitative results (**black box**). The table is indexed for easy re-sorting to original order (**blue box**), and index number from the Details tab is included for easy lookup of peptides between tabs ("Index in Detail", **red box**)



Quantitative Table: Summary Tab

Index	Index in Detail	Fold Change		Gene Name	Protein Name	Site
		Brain:Embryo				
646	1434	-6.0		Elavl1	HuR	206
647	1438	-4.1		Elavl1	HuR	206, §217
648	1444	-1.0		Elavl1	HuR	§217
649	1447	2.7		Elavl4	ELAVL4	237, §248
650	1451	-1.7		Fxr2	FXR2	393
651	1453	1.2		Fxr2	FXR2	399
652	1460	-2.3		Fxr2	FXR2	434, 436
653	1461	-1.8		Fxr2	Fxr2	484, 490
654	1462	-4.1		Fxr2	Fxr2	490
655	1463	-9.0		Hnrnpa0	hnRNP A0	139
656	1465	Brain		Hnrnpa0	hnRNP A0	286
657	1467	-5.5		Hnrnpa0	hnRNP A0	293
658	1471	-6.4		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	194; 194
659	1474	-1.0		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	194, 196; 194, 196
660	1475	-8.9		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	206; 206
661	1478	-5.8		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	8, 225, 232; 206, 218, 2
662	1479	-9.5		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	8, 225, 232; 218, 225, 2
663	1481	-8.0		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	225; 225
664	1486	-3.3		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	284; 337
665	1489	-3.9		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	300; 353
666	1492	2.3		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	300, 318; 353, 371
667	1494	-8.8		Hnrnpa1; Hnrnpa1	hnRNP A1; hnRNP A1 iso	318; 371

The Summary table contains Gene Name, Protein Name, and methylation Site for each identified peptide. siRNA products are available for Gene Names in blue text (**GREEN BOX**). Total antibodies or methylation site-specific antibodies are available for Protein Names or Sites in blue text (**BLUE BOX**). Published (§, **RED BOX**) and novel sites (**BLACK BOX**) of methylation.



Quantitative Table: Summary Tab



Protein Name	Site	Description	Accession	URL
Sam68	325	KH domain-containing, RNA-binding, signal transduction-associated protein 1	Q60749	http://www.phosphosite.
Sam68	325, 331	KH domain-containing, RNA-binding, signal transduction-associated protein 1	Q60749	http://www.phosphosite.
Sam68	331	KH domain-containing, RNA-binding, signal transduction-associated protein 1	Q60749	http://www.phosphosite.
Sam68	331, 340	KH domain-containing, RNA-binding, signal transduction-associated protein 1	Q60749	http://www.phosphosite.
Sam68	36, 45, 52	KH domain-containing, RNA-binding, signal transduction-associated protein 1	Q60749	http://www.phosphosite.
Sam68	36, 52	KH domain-containing, RNA-binding, signal transduction-associated protein 1	Q60749	http://www.phosphosite.
Sam68; KHDRBS2	442; 348	KH domain-containing, RNA-binding, signal transduction-associated protein 1	Q60749 ; Q9WU01	http://www.phosphosite.
KHSRP	412	far upstream element-binding protein 2	Q3U0V1	http://www.phosphosite.
KHSRP	412, 414	far upstream element-binding protein 2	Q3U0V1	http://www.phosphosite.
KHSRP	412, 414, 416	far upstream element-binding protein 2	Q3U0V1	http://www.phosphosite.
KHSRP	412, 416	far upstream element-binding protein 2	Q3U0V1	http://www.phosphosite.
KHSRP	414, 416	far upstream element-binding protein 2	Q3U0V1	http://www.phosphosite.
KHSRP	416	far upstream element-binding protein 2	Q3U0V1	http://www.phosphosite.
KHSRP	443	far upstream element-binding protein 2	Q3U0V1	http://www.phosphosite.
LARP	213	la-related protein 1	Q6ZQ58	http://www.phosphosite.
LARP4	445	la-related protein 4 isoform 1	Q8BWW4	http://www.phosphosite.
LARP5	495	la-related protein 4B	Q6A0A2	http://www.phosphosite.
LSM11	322	U7 snRNA-associated Sm-like protein LSm11	Q8BUV6	http://www.phosphosite.
MSI1	197	RNA-binding protein Musashi homolog 1	Q61474	http://www.phosphosite.
MSI1	197, 199	RNA-binding protein Musashi homolog 1	Q61474	http://www.phosphosite.
NCBP2	146	nuclear cap-binding protein subunit 2	Q9CQ49	http://www.phosphosite.
CFIM25	15	cleavage and polyadenylation specificity factor subunit 5	Q9CQF3	http://www.phosphosite.

Multiple isoforms for a single peptide are separated by semicolon (**RED BOX**).

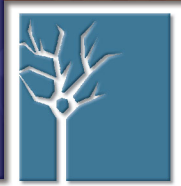
A link to the PhosphoSitePlus page for each protein is given (URL, **BLUE ARROW**).



Quantitative Table: Summary Tab

Protein Name	Site	Peptide	Charge	Calc. m/z
RBMXRT; RBMXRT iso3	122, 141; 122, 141	GPPSR*GGYMDDGGYSMNFMSSSR*GPLPVK	4	798.1192
RBMXRT; RBMXRT iso3	161, 169; 161, 169	R*STPSGPVR*SSSGMGGR	3	568.6250
RBMXRT; RBMXRT iso3	169; 169	STPSGPVR*SSSGMGGR	2	767.3755
RBMXRT; RBMXRT iso3	182; 182	TPVSR*GR	2	393.7327
Refbp2; THOC4	42; 72	NR*PAPYSRPK	3	400.5596
SAMD4	566	NR*GFGQSNLPTASSVSGMGR	3	727.6870
SFRS2IP	1168	SGWTSASSWAVR*K	3	479.5809
RBM16	1117	GLHEER*GR	3	323.1738
PAI-RBP1	216	R*GGSGSHNWGTVKDELTESPK	4	564.7805
PAI-RBP1; PAI-RBP1 iso2	366; 351	KPANDITSQLLEINFGDLGRPGR*GGR	4	671.3572
PAI-RBP1; PAI-RBP1 iso2	369; 354	KPANDITSQLLEINFGDLGRPGRGGR*	5	537.2872
SF1 iso2	537	SLPAAAMR*	2	451.2498
SF3B1	614	M#ISTM#R*PDIDNMDEYVR	4	533.7419
SF3B2	205	TPLGPR*VAAPVGPVVPTPTVLPMGAPVPR	4	778.4560
SF3B2	205, 228	TPLGPR*VAAPVGPVVPTPTVLPMGAPVPR*PR	4	781.9600
SF3B2	205, 228, 230	TPLGPR*VAAPVGPVVPTPTVLPMGAPVPR*PR*	4	785.4639
SF3B2	205, 230	TPLGPR*VAAPVGPVVPTPTVLPM#GAPVPRPR*	5	628.9684
SF3B2	228	VAAPVGPVVPTPTVLPMGAPVPR*PR	3	830.4857
snRNP 70	201	R*GGADVNIIR	2	486.2727

Table includes protein/peptide/site information (Peptide, Charge, Calc. m/z, Count in Details). Methylation (*, **RED ARROW**), oxidized methionine (#, **BLUE ARROW**).



Quantitative Table: Summary Tab

Protein Name	Site	Avg. RT	H or A	Raw Intensity			
				(CS13444)	(CS13445)	(CS13450)	(CS13451)
ADCY1	1078	22.78	H	502276	487888	-	-
ADCY8	55	16.74	H	77066	93287	-	-
ADRA2A	353	15.33	H	152104	206861	-	-
TMEM16H	999	64.77	A	10526122	10487250	2532809	2501649
APOB48R	919	53.88	A	-	-	3150790	4556241
ARMCX2	286	62.76	H	-	-	164705	197365
ASTN2	402	30.79	H	382040	352014	86255	157854
PMCA1; ATP2B2; ATP2B3	1087, 1091; 1099, 1106	52.18	A	86779082	83851091	-	-
PMCA1; ATP2B2; ATP2B3	1109; 1087; 1106; 1042	56.76	A	1879455941	205615711	4179995	4558210
PMCA1; ATP2B2; ATP2B3	1087, 1091; 1106, 1110	54.69	A	37614130	40874955	-	-
PMCA1; ATP2B2; ATP2B3	1113; 1091; 1110; 1046	51.84	A	550885115	554820347	1111224	1297106
ATP2B2	632	60.14	H	628094	498487	472666	468713
PMCA4; PMCA4 iso3	1096; 28	50.62	H	4582290	3024311	-	-
ATP5A1	207	40.93	H	890833	814348	265157	250153
ataxin-2L; ataxin-2L iso2	359; 359	15.67	A	691961	880861	5605430	4649580
ataxin-2L; ataxin-2L iso2	371; 371	29.72	H	71840	52104	119503	64743
ataxin-2L; ataxin-2L iso2	415; 415	13.26	H	1077130	861704	2410180	2283830
AVL9	589	18.14	H	599131	778870	235265	267656
BAMBI	174	86.36	A	-	-	97155	338931
CACNA1A	2278	26.48	H	453825	326754	-	-
CACNA1A	39	38.05	H	3174460	2095144	-	-
CACNA1B; CACNA1B iso2	1897; 1918; 1858	37.82	H	946083	817428	75337	70256
CACNA1B; CACNA1B iso2	22; 22; 22	25.25	H	1955710	1949450	-	-
CACNB4	505	11.66	H	292109	350531	-	-
GPIP137	606	27.41	H	176101	140881	679203	735154
GPIP137	631	47.56	A	75700057	84552789	381407003	461616908
GPIP137	631, 638	48.21	H	151809	133256	1193679	871575

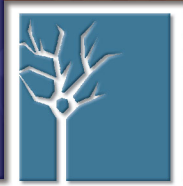
Table includes Average Retention Time of each peptide (**BLACK BOX**), whether measurement is height (H) or area (A) (**RED BOX**), and intensities (**BLUE BOX**). **Bold values** = intensities manually reviewed



Quantitative Table: Summary Tab

Protein Name	Site	Average Intensity		Ratio
		Brain	Embryo	Brain : Embryo
Arpp19	25	-	41321	-
Bex1	50	79623	24383	3.27
BEX2	51	-	323705	-
BEX2	54	-	180547	-
CEP170	428	36342	-	-
CLASP1 iso3	661	282587	116776	2.42
CUL1	63	1032586	-	-
MRE11A	603	76464	454805	0.17
NUDE1; NUDE1 iso3	293; 293	1460000	422166	3.46
PEG10	844, 857	-	1076901	-
PEG10	857	78449	3721115	0.02
BAT2D1	243	149490	719584	0.21
BAT2D1	256	1424871	352493	4.04
BAT2D1	2671	109898	1345330	0.08
BAT2D1	2747	524612	356595	1.47
BAT2D1	2766, 2775	-	128462	-
BAT2D1	280, 282	224457	2608321	0.09
BAT2D1	723	281705	322870	0.87
UHRF2	105	9517	246669	0.04

Table includes Average Intensity (**RED BOX**), Raw Ratio (**BLUE BOX**).



Quantitative Table: Summary Tab

Protein Name	Site	Fold Change	
		Brain : Embryo	Species
Arpp19	25	-	mouse
Bex1	50	3.27	mouse
BEX2	51	-	mouse
BEX2	54	-	mouse
CEP170	428	-	mouse
CLASP1 iso3	661	2.42	mouse
CUL1	63	-	mouse
MRE11A	603	-5.95	mouse
NUDE1; NUDE1 iso3	293; 293	3.46	mouse
PEG10	844, 857	-	mouse
PEG10	857	-47.43	mouse
BAT2D1	243	-4.81	mouse
BAT2D1	256	4.04	mouse
BAT2D1	2671	-12.24	mouse
BAT2D1	2747	1.47	mouse
BAT2D1	2766, 2775	-	mouse
BAT2D1	280, 282	-11.62	mouse

Table includes raw Fold Change (**BLUE BOX**). Species of tissue profiled is shown (**green box**).



Table Overview: Details Tab

MethylScan (Mono-Methyl-Arginine)_RESULTS

Table #1: Mouse Brain and Embryo; Trypsin Digest; Mono-Methyl-Arg antibody

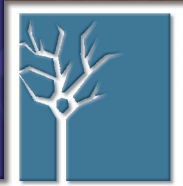
Samples: Sample 1 = Mouse Brain = CS13444, 13445; Sample 2 = Mouse Embryo = CS.

Legend: * - methylation, # - oxidized methionine, § - published site, **Blue Text** - CST a

Row Index	Protein Type	Gene Name	Protein Name	Site
Activator protein				
1	Activator protein	6720463M24Rik	BORA	316
2	Activator protein	6720463M24Rik	BORA	316
3	Activator protein	6720463M24Rik	BORA	316
Adaptor/scaffold				
4	Adaptor/scaffold	Abi1; Abi1	Abi-1; Abi-1 iso4	189; 184
5	Adaptor/scaffold	Akap8	AKAP8	109
6	Adaptor/scaffold	Akap8	AKAP8	109
7	Adaptor/scaffold	Akap8	AKAP8	109
8	Adaptor/scaffold	Akap8	AKAP8	109
9	Adaptor/scaffold	Akap8	AKAP8	188
10	Adaptor/scaffold	Akap8l	HAP95	172
11	Adaptor/scaffold	Akap8l	HAP95	172, 180
12	Adaptor/scaffold	Akap8l	HAP95	238
13	Adaptor/scaffold	Akap8l	HAP95	238
14	Adaptor/scaffold	Akap8l	HAP95	238
15	Adaptor/scaffold	Ank1; Ank1	ANK1; ANK1 iso2	1684; 1670
16	Adaptor/scaffold	Ank3; Ank3; Ank3; Ank3	ANK3; ANK3 iso6; ANK3	293; 293; 293; 293
17	Adaptor/scaffold	Caskin1	CASKIN1	1020
18	Adaptor/scaffold	Cblb	Cbl-b	13
19	Adaptor/scaffold	Crk	Crk	68

Column Definition | **Details** | Summary | Fold change by descending order | Fold change by protein type | +

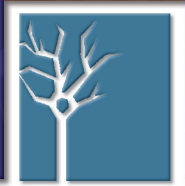
Details Tab contains a redundant list of all methyl-peptides identified in the study with their accompanying SORCERER metrics.



Qualitative Table: Details Tab

Protein Name	Site	kD	-7/+7 peptide
HAP95	238	71	YGMFQGMrGGGAFSG
HAP95	238	71	YGMFQGMrGGGAFSG
ANK1; ANK1 iso2	1684; 1670	204; 203	SVRQVLDrSQARTLD; SVRQVLDrSQ
ANK3; ANK3 iso6; ANK3	293; 293; 293; 293	212; 193; 188; 212	TPLHCGArSGHEQVV; TPLHCGArSG
CASKIN1	1020	151	SSIGGGGrAIRRPPE
Cbl-b	13	109	NGRNPGGrGGNPRKG
Crk	68	34	IINSSGPrPPVPPSP
Crk	68	34	IINSSGPrPPVPPSP
SAPAP3	46	106	EAFSTPrFCAPRAG
SAPAP3	922	106	PKKPSRGrGVPVKER
SAPAP3	922	106	PKKPSRGrGVPVKER
SAPAP4; SAPAP4 iso2	132; 132	108; 108	PRGEAKArGESPGRi; PRGEAKArGE
SAPAP4; SAPAP4 iso2	132, 138; 132, 138	108; 108	PRGEAKArGESPGRi, ARGESPrIRH
SAPAP4; SAPAP4 iso2	132, 138; 132, 138	108; 108	PRGEAKArGESPGRi, ARGESPrIRH
SAPAP4; SAPAP4 iso2	290; 290	108; 108	TDTNYVkrGSWSTLT; TDTNYVkrGS
DNMBP	1097	177	ISDQLFTrFKENTER
G3BP-1	427	52	DRRDNLrGPGGPRG
G3BP-1	427	52	DRRDNLrGPGGPRG
G3BP-1	427, 433	52	DRRDNLrGPGGPRG, LRGPGGPrGC
G3BP-1	427, 433	52	DRRDNLrGPGGPRG, LRGPGGPrGC

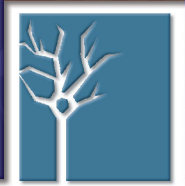
- The Details Tab includes the peptide sequence 7 amino acids to each side of the modified residue (**BLUE BOX**), the molecular weight of the protein from which the peptide is derived (**BLACK BOX**).



Qualitative Table: Details Tab

Protein Name	Site	MS2 Spectrum Number				Peak Apex MS Spectrum Number			
		(CS13444)	(CS13445)	(CS13450)	(CS13451)	(CS13444)	(CS13445)	(CS13450)	(CS13451)
TRIP6	25								
UBE2M	169	15677		15736	15948	15736		15839	16027
UBE2M	169	15914							
UBE2M	169	15881		15710	16131	15715		15822	16027
UBE2M	169	15653		15922	15912	15715		15822	16027
UBE2M	169	15711				14641			
UBE2M	169	14601		14699	14913	14662		14791	14985
UBE2M	169	14828		14895	15119	14662			
UBE2M	169	15657							
WASP	381			8849	8981			8908	8998
N-WASP	304	5728		5908	5575				5612
N-WASP	304	5526		5508					
N-WASP	304	5339							

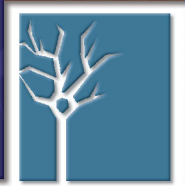
The Details Tab includes the MS/MS (or MS2, **BLUE BOX**) and MS (**RED BOX**) scan numbers for each peptide assignment. Experimental details that are mainly used as a reference to the raw data.



Qualitative Table: Details Tab

Protein Name	Site	Retention Time				Xcorr			
		(CS13444)	(CS13445)	(CS13450)	(CS13451)	(CS13444)	(CS13445)	(CS13450)	(CS13451)
BAG4	54	53.24	0.00	53.04	53.02				5.92
BAG4	54	53.24	53.22	52.97	52.93	3.15			3.20
CCAR1	281	0.00	0.00	49.24	49.06				
KLHDC10	13	21.29	21.44	21.33	21.04	5.63		5.56	5.80
KLHDC10	13	21.29	21.44	21.33	21.04				
KLHDC10	13	21.33	21.44	21.33	21.04	4.05		4.00	4.43
KLHDC10	13	21.33	21.44	21.33	21.04				
KLHDC10	13	18.97	19.00	18.93	18.83			3.44	

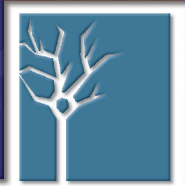
The Details Tab includes the Retention Time (**black box**), and Xcorr values (or Sorcerer cross-correlation value, **red box**) for each peptide assignment. Experimental details are used as a reference and measure of confidence for the peptide assignment.



Qualitative Table: Details Tab

Protein Name	Site	Mass Accuracy (ppm)				DeltaCN			
		(CS13444)	(CS13445)	(CS13450)	(CS13451)	(CS13444)	(CS13445)	(CS13450)	(CS13451)
CDC42EP5	38			-0.83	-0.59			0.18	0.17
CDC42EP5	38			-0.25				0.11	
CENPV	39	-1.53				0.33			
CENPV	39	-1.52				0.34			
Cic	30								
Cnbp	32, 34			-1.24	0.11			0.37	0.31
Cnbp	32, 34			-1.17	0.09			0.25	0.34
Cnbp	32, 34				0.41				0.00
Cnbp	72								
COG5	278	-1.10		-1.46	-1.76	0.12		0.00	0.10
COG5	278								
CPEB3	206								
CPEB3	309								
CRLF1	85	0.91				0.00			
Csdc2	37	0.15				0.09			
Csdc2	56								
CTTNBP2	495								
FAM104A	3	-1.83				0.07			
FAM104A	4								
FAM104A	4								
FAM104A	4	1.05			-1.12	0.13			0.09

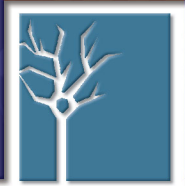
The Details Tab includes the measured peptide mass accuracy (**BLACK BOX**), and DeltaCN value (**BLUE BOX**) for each peptide assignment. Experimental details that are used as a reference and measure of confidence to the peptide assignment.



Qualitative Table: Details Tab

Protein Name	Site	Rsp				PP Probability			
		(CS13444)	(CS13445)	(CS13450)	(CS13451)	(CS13444)	(CS13445)	(CS13450)	(CS13451)
CDC42EP5	38			2	1			0.99	0.99
CDC42EP5	38			1				1.00	
CENPV	39	6				0.98			
CENPV	39	1				0.98			
Cic	30								
Cnbp	32, 34			1	1			1.00	1.00
Cnbp	32, 34			1	1			1.00	1.00
Cnbp	32, 34				96				0.62
Cnbp	72								
COG5	278	12		8	23	0.92		0.64	0.82
COG5	278								
CPEB3	206								
CPEB3	309								
CRLF1	85	99				0.85			
Csdc2	37	15				0.93			
Csdc2	56								
CTTNBP2	495								
FAM104A	3	1				0.23			
FAM104A	4								
FAM104A	4								
FAM104A	4	2			1	0.99			0.99

The Details Tab includes the Rsp value (**BLUE BOX**), the Peptide Prophet Probability (**RED BOX**) for each peptide assignment. Experimental details that are used as a reference and measure of confidence to the peptide assignment.



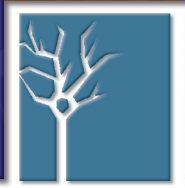
Qualitative Table: MS/MS Assignments

Protein Name	Site	Peptide	Charge	Calc. m/z	MS2 Spectrum Number			
					(CS13444)	(CS13445)	(CS13450)	(CS13451)
snRNP A	157	AVQGGAAAPVVGAVQPVPGMPPMPQAPR*	3	888.8086			20845	21352
snRNP A	157	AVQGGAAAPVVGAVQPVPGMPPMPQAPR*	3	888.8086				21372
snRNP A	157	AVQGGAAAPVVGAVQPVPGM#PPM#PQAPR*	3	899.4719				
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPMPQAPR*	3	931.5069	19263		19012	19419
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPMPQAPR*	3	931.5069	19199		19266	19639
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPMPQAPR*	3	931.5069	19221		19331	
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPMPQAPR*	3	931.5069			19226	
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPMPQAPR*	3	931.5069				
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPMPQAPR*	4	698.8820			19029	19403
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPMPQAPR*	4	698.8820				19595
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPM#PQAPR*	3	936.8386			16497	16739
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPM#PQAPR*	3	936.8386				
snRNP A	157	KAVQGGAAAPVVGAVQPVPGMPPM#PQAPR*	4	702.8807				
snRNP A	157	KAVQGGAAAPVVGAVQPVPGM#PPMPQAPR*	3	936.8386			16477	16634
snRNP A	157	KAVQGGAAAPVVGAVQPVPGM#PPMPQAPR*	3	936.8386				16652
snRNP A	157	KAVQGGAAAPVVGAVQPVPGM#PPM#PQAPR*	3	942.1702				
snRNP A	157	KAVQGGAAAPVVGAVQPVPGM#PPM#PQAPR*	4	706.8795				

The site, R157, is assigned 20 times in the 4 curation sets (**RED BOX**, count number of MS2 entries).

The site is represented in 6 overlapping sequences (incomplete digestion and methionine oxidation, **red arrow**).

The methylated peptide assignments are seen in multiple charge states (**blue arrow**).



Cell Signaling Technology Consultation

Study Design

Discuss details of study, samples, sample preparation.

Final Results

Provide final results, report, highlight sites of interest.

Follow-up

Review final results, discuss additional experiments.