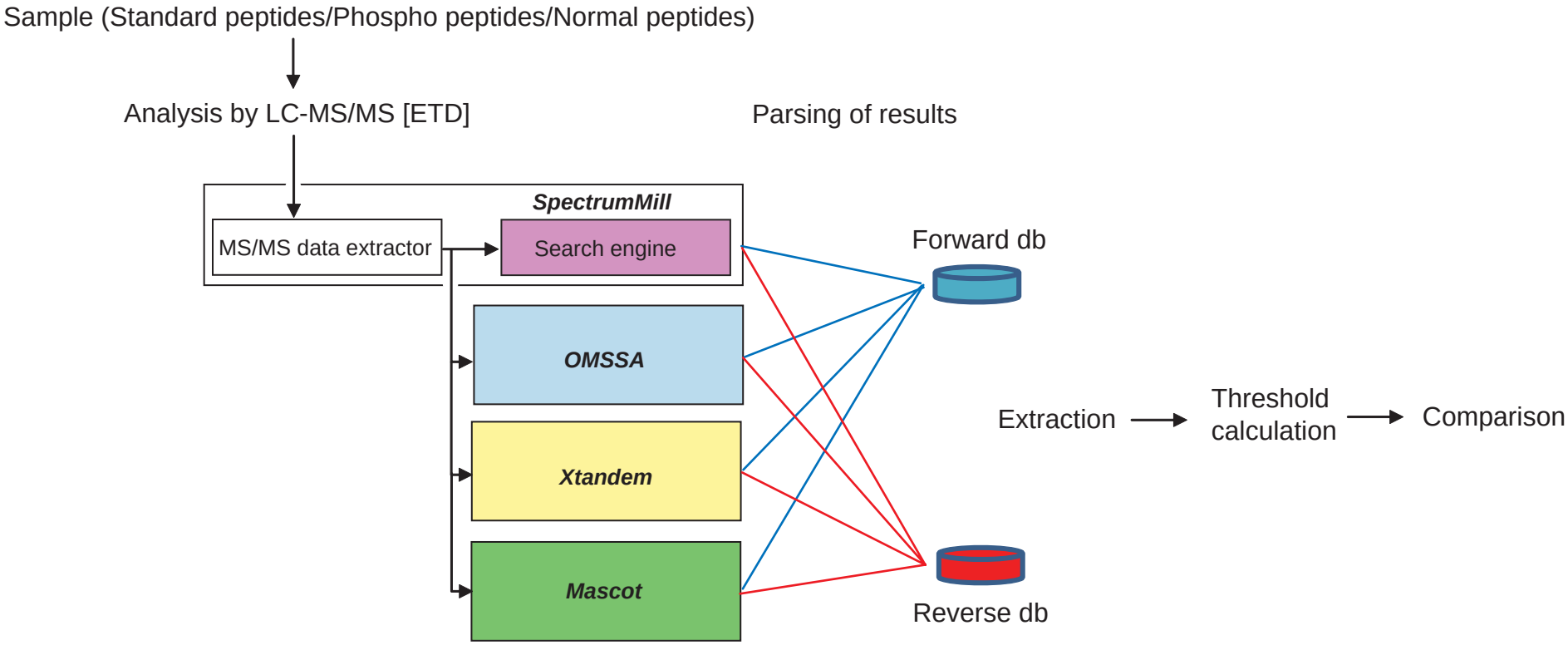
Supplementary Figure 1



Supplementary Table 2 (Venn diagram in a table)

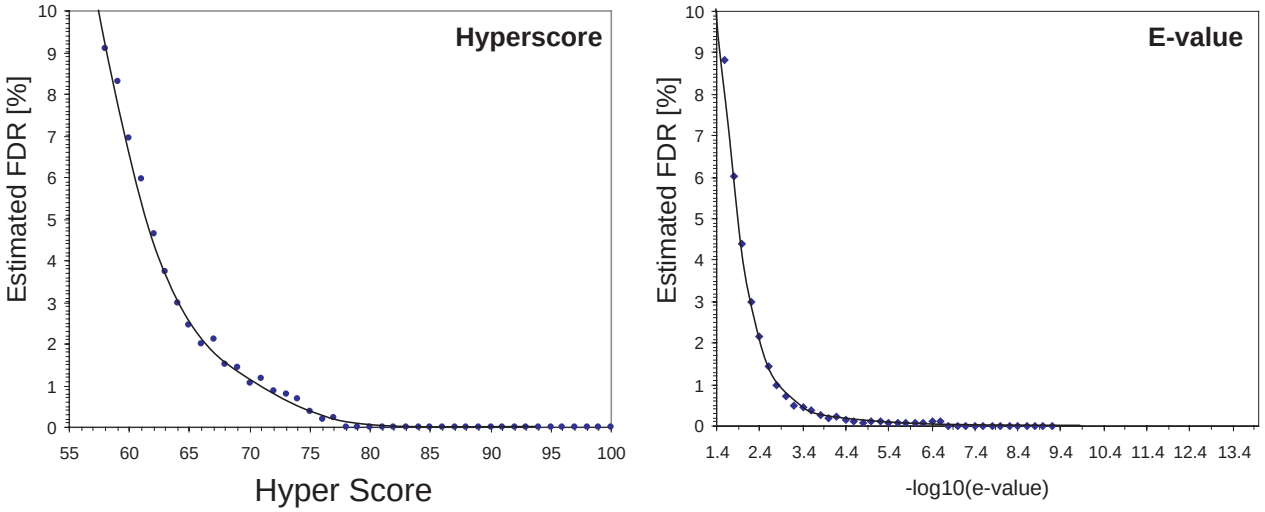
Venn Diagram Statistics for FDR 0.1%	
X!Tandem	2010
Mascot	3928
SpectrumMill	5126
OMSSA	2487
X!Tandem Only	269
Mascot Only	1356
SpectrumMill Only	2030
OMSSA Only	666
X!Tandem/Mascot	50
X!Tandem/SpectrumMill	250
X!Tandem/OMSSA	91
Mascot/SpectrumMill	953
Mascot/OMSSA	101
SpectrumMill/OMSSA	256
X!Tandem/Mascot/SpectrumMill	305
X!Tandem/Mascot/OMSSA	41
Mascot/SpectrumMill/OMSSA	328
SpectrumMill/OMSSA/X!Tandem	210
X!Tandem/Mascot/SpectrumMill/OMSSA	794

Venn Diagram Statistics for FDR 5%	
X!Tandem	7164
Mascot	8683
SpectrumMill	11439
OMSSA	6676
X!Tandem Only	1350
Mascot Only	2524
SpectrumMill Only	3311
OMSSA Only	1371
X!Tandem/Mascot	205
X!Tandem/SpectrumMill	800
X!Tandem/OMSSA	308
Mascot/SpectrumMill	1945
Mascot/OMSSA	112
SpectrumMill/OMSSA	623
X!Tandem/Mascot/SpectrumMill	639
X!Tandem/Mascot/OMSSA	141
Mascot/SpectrumMill/OMSSA	400
SpectrumMill/OMSSA/X!Tandem	1004
X!Tandem/Mascot/SpectrumMill/OMSSA	2717

Venn Diagram Statistics for FDR 1%	
X!Tandem	5130
Mascot	5529
SpectrumMill	7824
OMSSA	4491
X!Tandem Only	990
Mascot Only	1646
SpectrumMill Only	2361
OMSSA Only	849
X!Tandem/Mascot	117
X!Tandem/SpectrumMill	682
X!Tandem/OMSSA	235
Mascot/SpectrumMill	1022
Mascot/OMSSA	62
SpectrumMill/OMSSA	465
X!Tandem/Mascot/SpectrumMill	503
X!Tandem/Mascot/OMSSA	89
Mascot/SpectrumMill/OMSSA	277
SpectrumMill/OMSSA/X!Tandem	701
X!Tandem/Mascot/SpectrumMill/OMSSA	1813

Supplementary Figure 3

X!Tandem

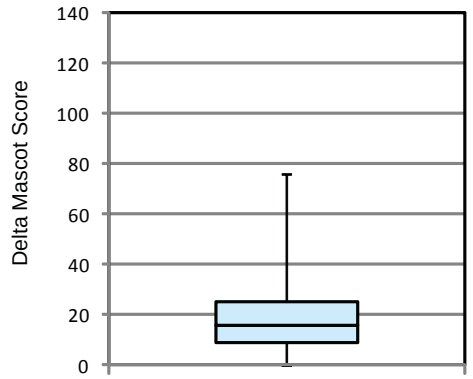


	FDR					
	0.1%		1%		5%	
X-Tandem	Unique	Total	Unique	Total	Unique	Total
[E value]	789	1926	1842	4786	2851	7164
[Hyperscore]	18	27	380	794	824	1714

Supplementary Figure 4

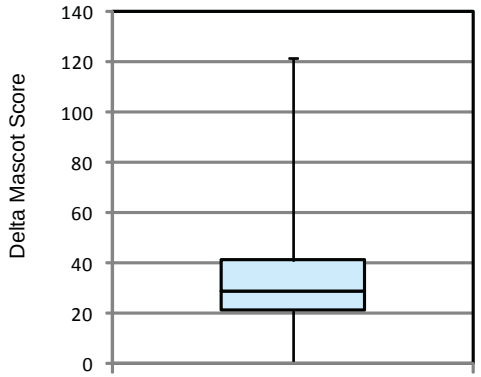
A

Mascot, Phospho peptides:
Difference between best match and 2nd best match



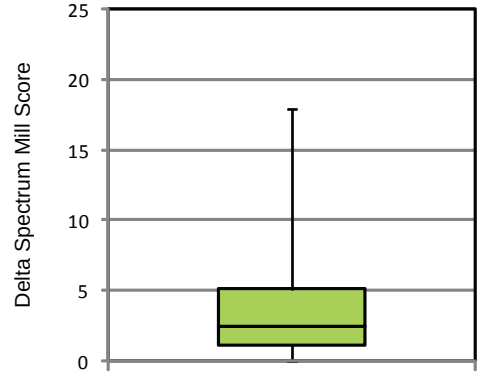
B

Mascot, Non-Phospho peptides:
Difference between best match and 2nd best match



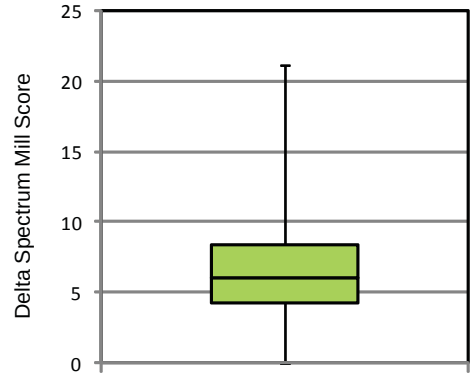
C

Spectrum Mill, Phospho peptides:
Difference between best match and 2nd best match



D

Spectrum Mill, Non-Phospho peptides:
Difference between best match and 2nd best match



A large phosphorylation focused Electron Transfer Dissociation data set analyzed by four different Tandem Mass Spectra Search Engines

Supplementary Table 1

Search parameters used in the comparison of four search engines using approximately 170.000 ETD generated fragmentation spectra

Search engine	**OMSSA**	**Mascot**	**X!Tandem**	**Spectrum Mill**
Version	2.1.0	2.2	2007.04.01.1	3.03.078
Database (forward and reverse versions)	RefSeq (Mar07, human)	RefSeq (Mar07, human)	RefSeq (Mar07, human)	RefSeq (Mar07, human)
Search 1;Precursor ion mass acq.	1.5 Da	1.5 Da	1.5 Da (a)	1.5 Da
Search 2;Precursor ion mass acq.	2.5 Da	2.5 Da	2.5 Da (a)	2.5 Da
Mass type	Monoisotopic	Monoisotopic	Monoisotopic	Monoisotopic
Search 1;Fragment ion mass acq.	0.5 Da	0.5 Da	0.5 Da	0.5 Da
Search 2;Fragment ion mass acq.	0.7 Da	0.7 Da	0.7 Da	0.7 Da
Mass type	Monoisotopic	Monoisotopic	Monoisotopic	Monoisotopic
Enzymatic specificity	FULL Trypsin or Lys-C	FULL Trypsin or Lys-C	FULL Trypsin or Lys-C	FULL Trypsin or Lys-C
Missed enzymatic events	3	3	3	3
Fixed Modifications	Carbamidomet. [C]	Carbamidomet. [C]	Carbamidomet. [C]	Carbamidomet. [C]
Variabel modifications	Phosphorylation [S,T,Y], Oxidation [M]	Phosphorylation [S,T,Y], Oxidation [M]	Phosphorylation [S,T,Y], Oxidation [M]	Phosphorylation [S,T,Y], Oxidation [M]
Maximum variable mod combinations searched per peptide	64 (i)			
Variabel mod. mass shift (max)	-	-	-	340
Submitted peak list format	.MGF	.MGF	.MGF (l)	.PKL (f)
Instrument	Search ETD MS/MS spectra	ETD-TRAP	-	Agilent ESI ino trap ETD
Fragment ions	c, z' (k)	c, z', z", y	c?, z?, y	c, c', z, z', y
Min. Charge state	2	-	2	N/A
Max. Charge state	5	5	5	5
Minimum parent M+H	-	-	400	-
Minimum fragment m/z	-	-	150	105
Minimum charge to start using multiply charged products	3	-	-	-
Minimum score peak intensity	-	-	-	50% (d)(j) , 40% (e)(j)
Hitlist length (max)	10	10	-	10
Number of results scored				
Fraction of product peaks below precursor to determine +1 precursor	0.95	-	-	-
Number of top intensity peaks in first pass	20	-	-	-
Minimum peaks	-	-	15	4 (h)
Maximum peaks	-	-	50	40
Dynamic peak thresholding	-	-	-	On
E-value cutof	0	-	1	-
Remove redundant	-	-	No	-
Protein, N-terminal residue modification mass	-	-	0	-
Protein, C-terminal residue modification mass	-	-	0	-
refinement specification	-	-	No	-
Cleavage C-terminal change	(c)	(c)	+17.002735 Da	(c)
Cleavage N-terminal change	(c)	(c)	+1.007825 Da	(c)
Isotope error allowed	-	-	No	-
Spectrum synthesish			No	

(a) X!Tandem, as the only of the four search engines allows to use an asymetrical precursor ion tolerance. In this comparison, this feature were not used.
(c) Not specified but only makes sense if this is the same value for the all the search engines
(d) Search 1
(e) Search 2
(f) The raw file is procesed into .PKL files. When searched, the .PKL fiels are deisotoped. The de-isotoping script were used to parse peaklists out for the four other search engines.
(h) Minimum number of peaks to be searched
(g) Report duplicate peptide matches
(i) Deafult value
(j) We have compared the use of 40% and 50% for a limited part of the data set. In those tests we did not find any difference neither in the number of identified spectra nor in the scores assigned to the identified peptides.
(k) According to Chi et al. {Chi, 2007 #303}
(l) X!Tandem does not read the generic .MGF format when it comes to ions with un-assigned charge states, here: 'CHARGE= 2+,3+,4+,5+'. Using this nomenclactur X!Tandem will treat the spectrum as a 2+ ion. To fix this problem we created an modified .MGF file where all peptide ions with un-assigned charge states were spilt into 4 spectra with charge states of 2+, 3+, 4+ and 5+ respectively. A similar approach as for .dta files.

Supplementary Figure 2.