

Supplementary figures:

Figure S1

- 5 Peptide length distribution for the 'chlamyTotal' database.

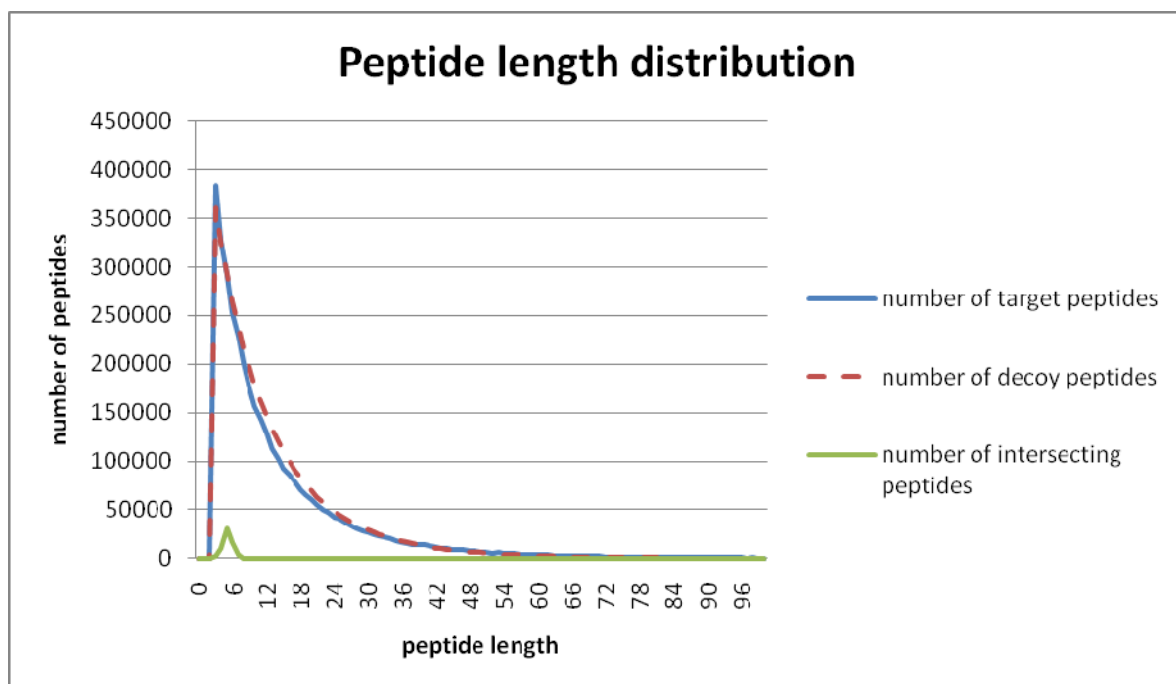
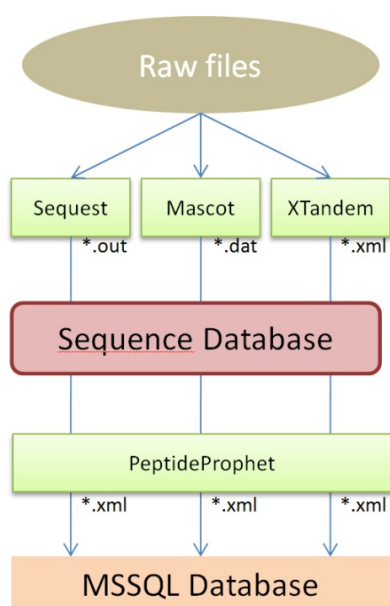


Figure S2

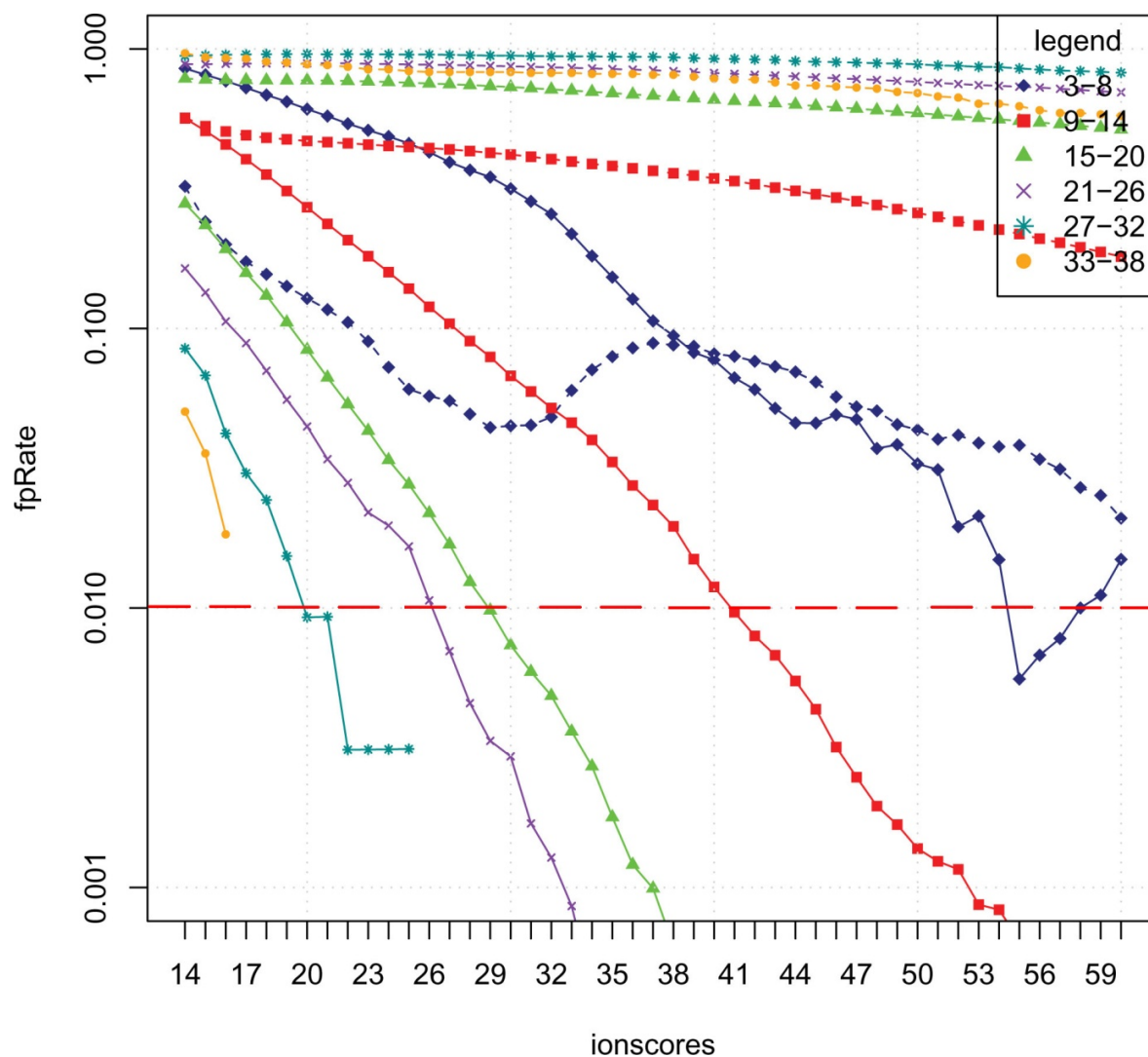
- 10 **Workflow: Raw files that are searched by different search algorithms against a sequence database. The output files are converted to one uniform format and then submitted to PeptideProphet. After that all information are stored in a MS-SQL database.**



15

Figure S3

Exemplary plot that shows how the thresholds for peptides of charge 2 identified by Mascot are defined.



30

Table S1

Peptide length dependent score thresholds for different search engines that were defined as thresholds to ensure a false positive rate lower than 1 %

Charge 1						
Peptide length (aa)	3 – 8	9 - 14	15 - 20	21 - 26	27 - 32	33 - 38
Mascot (ionscore)	-	40	45	-	-	-
Sequest (xcorr)	2.0	2.2	1.8	-	-	-
X!Tandem (expect)	-	10^{-2}	$10^{-1,75}$	-	-	-

Charge 2						
Peptide length (aa)	3 – 8	9 - 14	15 - 20	21 - 26	27 - 32	33 - 38
Mascot (ionscore)	-	41	29	27	20	17
Sequest (xcorr)	2.6	2.7	2.7	2.8	2.5	2.0
X!Tandem (expect)	-	10^{-2}	10^{-2}	$10^{-2,25}$	-	-

Charge 3						
Peptide length (aa)	3 - 8	9 - 14	15 - 20	21 - 26	27 - 32	33 - 38
Mascot (ionscore)	-	45	29	25	22	22
Sequest (xcorr)	-	3.1	2.9	3.3	3.2	-
X!Tandem (expect)	-	$10^{-2,5}$	$10^{-1,75}$	$10^{-2,75}$	$10^{-1,75}$	$10^{-1,5}$

35

Table S2
Information about peptides and single reaction monitoring transition data, retention times and ϵ values. CE = collision energy.

"Name"	peptide sequence	Precursor ion		Product ions		Precursor ion		Product ions		CE	RT	ε
		(m/z)	standard peptide	(m/z)		(m/z)	native peptide	(m/z)				
rbcl_1	DTDILAAFR		514.76	584.36	18		511.26	577.35	18	15.7	12997	
rbcl_2	LTYTPDYVVR		698.35	754.41	24		695.35	748.39	24	10.4	25758	
104827	DLGLPVTPLPDNLK		749.92	293.16	27		746.42	229.12	27	16.3	12667	
184810	FADGLNNGK		471.73	724.37	16		468.23	717.35	16	6.6	13074	
157731	NHYLVSATATK		606.32	252.11	28		602.82	252.11	28	6.3	20175	
148057_1	VSAASLLDVSTTTDK		757.89	979.49	25		754.39	979.49	25	11.5	13544	
148057_2	HQLIGATVGS DNK		673.85	791.39	28		670.35	791.39	28	6.4	16860	
127879	FALLPTTR		463.27	474.27	16		459.77	474.27	16	10.7	11997	
153656_1	IDADLDVFIQK		642.34	1055.58	22		638.84	1048.57	22	15.7	14997	
153656_2	FDLNTLASTK		558.79	854.51	17		555.29	847.49	17	11.0	13980	
130316_1	GDFLVPSYR		530.77	522.27	15		527.27	522.27	15	10.3	18292	
130316_2	GSSFDPK		429.21	244.17	19		425.71	244.17	19	7.2	11924	
104731_1	LLDQYLTK		500.78	774.41	17		497.28	767.39	17	7.0	12253	
104731_2	TLSAVLGVANNR		611.34	630.33	21		607.84	630.33	21	9.4	10863	
120177	IGQNVNPIK		494.78	875.50	19		491.78	896.48	19	6.6	8023	
76146	WVSVDQSGVR		526.28	847.43	19		523.28	847.43	19	6.3	8870	
193296	DALGNDIK		426.72	546.29	14		423.22	546.29	14	6.5	6897	
78348_1	VWNTGSPK		460.76	716.39	16		457.76	716.39	16	6.3	7818	
78348_2	IGLFGGAGVGK		491.78	692.37	17		488.28	692.37	17	10.7	14741	
134235_1	VLYGVNQR		478.26	736.37	17		474.76	736.37	17	6.4	12368	
134235_2	GLTVQYNK		465.25	752.39	16		461.75	752.39	16	6.6	12305	
195493_1	VDVAVTGSQK		505.27	619.34	20		502.27	619.34	20	6.2	8805	
196354	YADIWTTTHK		628.32	350.13	25		625.32	350.13	25	6.5	20113	

194461	FPTIGFDK	466.24	687.38	15	462.74	680.36	15	11.0	17097
155280	VSSDITHAEIK	603.81	554.29	23	600.31	550.78	23	6.4	14806
99751_1	YAAENDLGFEK	632.29	829.42	21	628.79	822.40	21	7.8	20326
99751_2	GVAEPTVPEVR	580.31	797.45	20	577.31	797.45	20	7.4	9771
191582	AGADALFEDPAR	620.29	343.21	34	616.79	343.21	34	9.3	15873
167973	TLAWFPENVSPPR	712.36	798.41	25	708.86	798.41	25	14.2	44974
130914	EIIIDVPLATK	609.86	536.35	32	606.36	529.33	32	14.2	9733
185915_1	LGADSGALEFVPK	655.84	244.17	24	652.34	244.17	24	11.7	16756
185915_2	DDYLNAPGETYSVK	789.86	394.12	30	786.36	394.12	30	8.9	23366
194541_1	VPEGVDWAADVVK	638.34	1176.63	34	635.34	1170.62	34	12.8	39667
194541_2	ASEEVVAVDNAAR	668.83	546.26	19	665.83	546.26	19	6.9	11819
196366	IGDPQWVFK	548.29	405.73	19	545.29	402.72	19	11.6	42014
126382_1	YAEIVEIR	500.27	636.42	22	496.77	629.40	22	8.2	12259
126382_2	GQVLEVDGTR	540.78	333.19	26	537.28	333.19	26	6.8	8901
54608	FEDPAEGEEVVR	692.32	392.15	21	688.82	392.15	21	13.4	15994
196298	FQSLGLNVK	506.79	650.43	45	503.29	643.41	45	5.0	13091
132210_1	ADLNVPLDK	496.27	472.28	15	492.77	472.28	15	7.9	7872
132210_2	ATLAITDDTR	542.28	720.35	28	538.78	720.35	28	7.4	8802
129019_2	VLITAPAK	410.26	315.20	19	406.76	315.20	19	6.8	6770
129019_3	AVSLVLPSTK	517.33	444.28	20	513.83	444.28	20	13.6	8699
55838	LQNIWGVPTSIR	651.88	573.34	23	648.88	573.34	23	10.5	10880
59760_2	SWWSIPHGPSIIAAR	755.43	727.45	38	752.43	727.45	38	9.1	18617
59760_3	ANSDAQQGGK	461.71	535.30	28	459.71	531.29	28	6.2	8022
19800_1	VDTGLQLPGDK	631.84	416.21	21	628.34	416.21	21	10.5	10743
19800_2	GILASDESNAATGK	685.83	591.31	25	682.33	591.31	25	8.2	12658
10441	TLLYGGIYGYPGDAK	797.9	487.25	25	794.4	487.25	25	13.0	29468
189186_1	GVFTNVTSPPTK	629.33	446.26	20	626.33	446.26	20	7.5	15865
189186_2	LINFYLGEK	552.3	333.18	25	548.8	333.18	25	12.9	18370
141319	VSTLIGYGSPNK	621.83	358.21	30	618.33	358.21	30	7.6	16019
135614	FIESQVAK	464.25	667.37	21	461.25	661.35	21	6.4	12076
128745	SLVDEQENVK	584.29	746.37	17	580.79	746.37	17	6.6	9005
102889	LAWEEPFGPVLPMR	874.46	502.28	28	870.96	502.28	28	19.8	48729
186676	GLLASDESTGTGK	678.35	907.44	24	674.85	907.44	24	7.8	12556

195910	IYLDISDDIK	601.31	925.49	18	597.81	918.48	18	13.6	14111
108283_1	AYVSNESAIR	558.28	689.36	17	555.28	689.36	17	7.2	14218
108283_2	LVAFDNQK	470.75	504.24	25	467.75	504.24	25	7.0	12158
189877	LGAADVADK	375.71	509.30	21	372.71	503.28	21	9.0	6765
196305	VHILTDGR	459.25	681.40	17	455.75	674.38	17	5.7	11941
83064	AVENINAIAPALK	721.92	428.29	19	718.92	428.29	19	14.2	12771
136854	IGNASVIK	404.24	523.35	21	401.24	517.33	21	6.8	6858
18029	FNYSGEPAPER	692.81	573.31	23	690.81	569.30	23	7.1	21405
135220	GALVSELPWQALK	745.41	677.39	18	741.91	670.38	18	17.1	41669
196624	KHFKGEADIK	590.32	767.44	37	586.82	760.42	37	13.5	18989
196443	AVNGNIIPSSTGAAK	703.38	718.37	12	700.38	718.37	12	6.9	13645
24263	TAEAGDTLSK	500.24	641.36	31	496.74	634.34	31	5.7	8730
137163	GADLIVIPAGVPR	642.88	596.35	15	639.38	596.35	15	13.6	11623
196612	ILTGPPQPIAK	551.33	428.29	26	547.83	428.29	26	7.0	9723
136279	IDDPAGLQDGLAK	660.34	488.78	21	656.84	485.27	21	8.2	11704
162449	TLGVVGLGAIGGR	588.85	707.43	26	585.35	700.41	26	12.2	11577
136037	TVLGIILGGGAGTR	646.38	575.29	22	642.88	575.29	22	17.4	12543
187891	ANDFGGEIIPSAK	698.85	473.27	21	695.35	473.27	21	9.6	17804
173725	APGLVGWVPAK	594.82	315.20	22	591.32	315.20	22	15.9	67625
136076	AVISDGVSGAK	505.27	417.22	15	502.27	417.22	15	6.2	9634
132263	DAALGNGLGR	504.26	258.11	12	500.76	258.11	12	6.3	9764
192398	VPVGTVEFEK	555.8	506.27	20	552.8	503.27	20	7.7	13925
140452	VPIADVDPYPAVAAK	753.4	197.13	40	750.4	197.13	40	10.3	18848
191662	AALEFLLK	456.27	520.35	15	452.77	520.35	15	14.5	11979
101528	LVEEAAAEAVK	568.3	459.26	13	565.3	459.26	13	7.0	9764
139269	IGEAHTAAGGR	504.27	360.20	17	501.27	360.20	17	6.0	9698
120516	GGAFLEAPVSGSK	613.82	574.32	21	610.32	574.32	21	6.7	16708
196431	NSNSPLAAAADPLDR	759.87	500.28	31	756.37	500.28	31	9.9	13758
195555	LEEEMPLNTYSNK	816.38	689.28	25	812.88	689.28	25	8.9	19117
173281	RPDADLEEAK	575.78	254.16	20	572.28	254.16	20	5.7	8861
153630	ALLAADQAAAR	503.78	408.22	14	500.28	408.22	14	6.7	8859
193810	LLLNNGVDPADLK	694.88	543.31	27	691.38	543.31	27	11.4	11811
196500	VDLATVAGTGNR	667.85	658.33	20	664.35	658.33	20	8.0	12665

57890	GLGLEAVGVNTDNR	711.36	775.37	34	707.86	775.37	34	9.6	12821
129025	TFAPGVPLK	468.77	364.27	23	465.27	357.25	23	8.4	12867
196044	YDGINLVTVR	578.81	474.30	19	575.31	474.30	19	10.8	14216
196833	ADPTHEGEDLLAVVR	814.91	721.89	23	811.41	718.38	23	12.1	18753
80866	IGGLPAPDVIR	557.83	599.35	25	554.33	599.35	25	10.6	9702
133318	APATDEALTELK	633.33	169.10	38	629.83	169.10	38	8.5	10706
128726	LGELLPSDR	503.77	474.23	18	500.27	474.23	18	8.2	7842
187367	FEDGSLPLPPAR	653.34	650.40	22	649.84	650.40	22	10.1	15858
163824	VPDSQILD FMR	664.33	453.23	30	660.83	453.23	30	16.2	15967
24120	IVDVLEM RPND AADR	860.43	432.22	40	857.43	432.22	40	10.1	14764
24552	IVGGVYDLATGK	600.33	305.18	35	596.83	305.18	35	9.4	15939
127317	DIVNILD TIK	575.83	476.27	16	572.33	476.27	16	19.2	8864
176233	ADLIAYLK	457.26	494.30	25	453.76	494.30	25	12.6	12134
185200	DGVTLP GIDGYFGVK	772.89	785.38	25	769.39	785.38	25	18.7	23985
194915	SNVPLHLR	471.77	321.71	14	468.27	318.20	14	6.6	12021
138059	IGLYEPVR	477.27	371.24	19	473.77	371.24	19	8.4	12200