

**ADDITIONAL FILE 1**

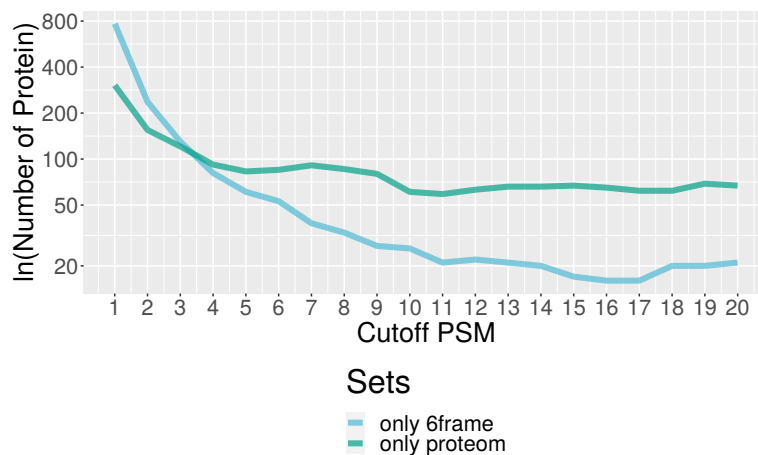
# SUPPLEMENTAL FIGURES AND TABLES

A workflow to identify novel proteins based on the direct mapping of Peptide-Spectrum-Matches to genomic locations

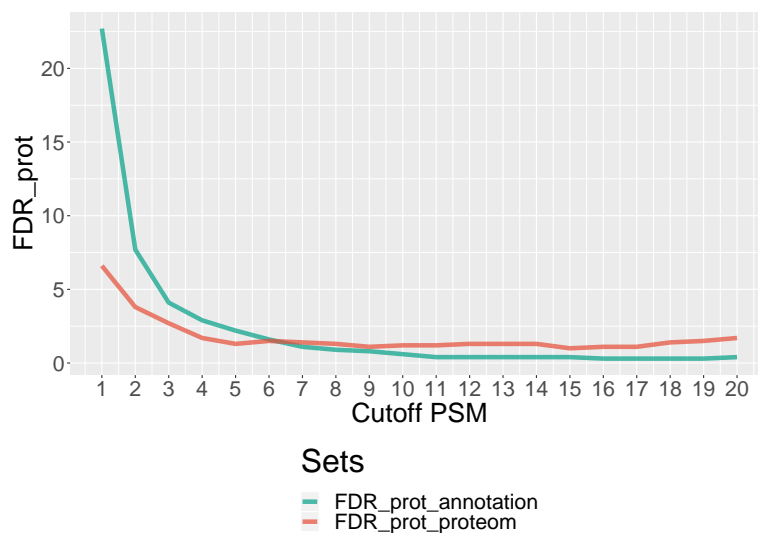
John Anders, Hannes Petruschke, Nico Jehmlich, Sven-Bastiaan Haange, Martin von Bergen and Peter F Stadler

### Supplemental Figures

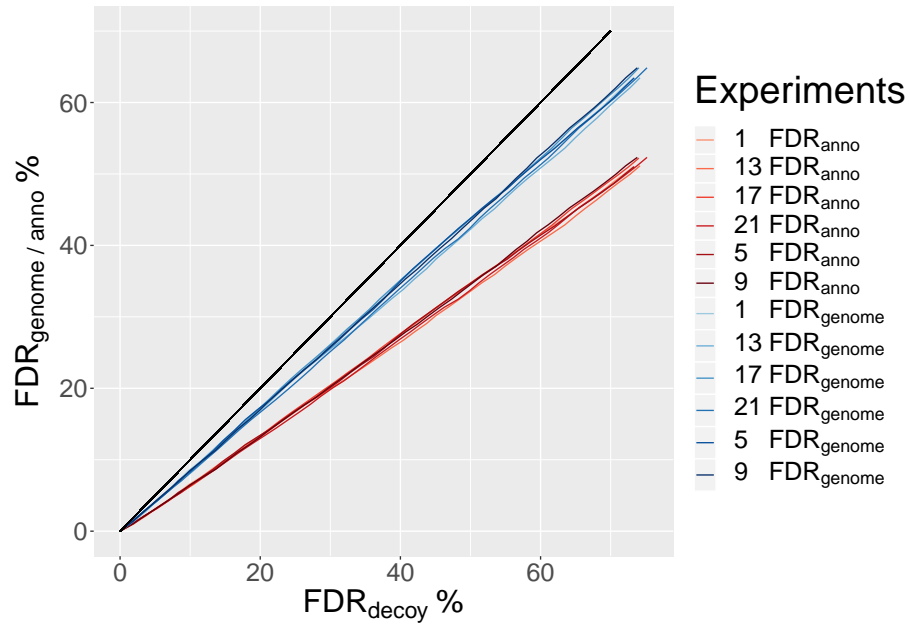
**Supplemental Figure 1.** The number of proteins that are identified only with the proteome and the 6frame databases for the *E. coli* data set, respectively, as a function of the minimal number  $k$  of PSMs required to call a protein. The graph summarizes the stacked bar plot corresponding to Fig. 1 of the the main text.



**Supplemental Figure 2.** False discovery rate (FDR) of proteins as a function of the minimum number of PSMs required to call a protein candidate of *E. coli*. The  $FDR_{prot}$  is estimated from the number of non-annotated candidates, assuming that the *E. coli* annotation is complete.

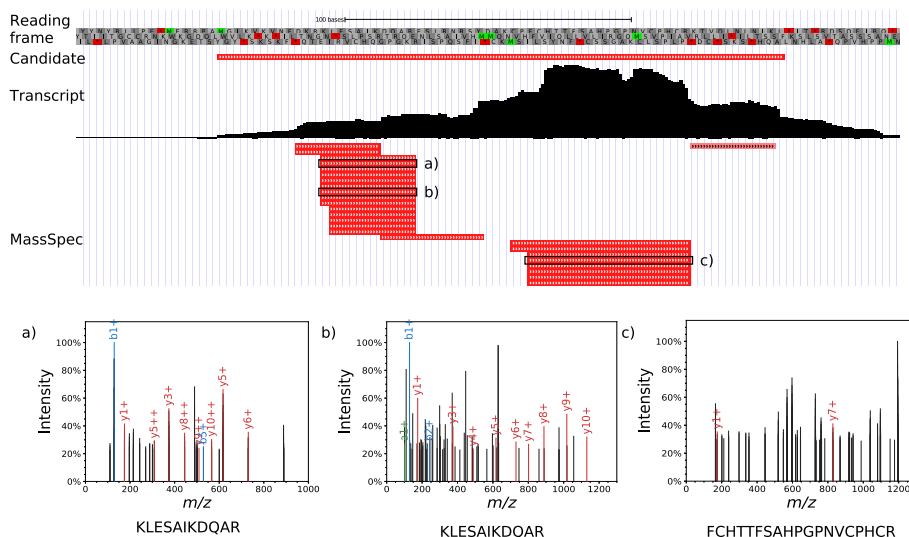


**Supplemental Figure 3.** The relation of the genomic (red) and annotation (blue) based  $FDR_{genome/anno}$  and the  $FDR_{decoy}$  inferred by the target decoy method. The black line represents a perfectly proportional relationship between  $FDR_{genome/anno}$  and  $FDR_{decoy}$ . The  $FDRs$  are plotted for all experimental replicas.



**Supplemental Figure 4.** Protein candidate 57 in *B. producta*, for which only hypothetical homologs can be found. The top of the figure shows a view in the UCSC Genome Browser. The first track show the reading frame of the genome on the negative strand. The second track is the protein candidate 5's predicted ORF. The next track shows a bar plot representation the reads per base mapping to protein candidate 5 from the transcriptomic data. At the bottom a list of the mapped PSMs can be found. The rightmost PSM is ambiguous and provides little evidence for the candidate. Below three mass spectra for top confidence PSMs and the corresponding peptides are shown.

nov 57



## Supplemental Tables

**Supplemental Table 1.** Comparison of predicted protein candidates between 6-frame database and a proteome database based on existing annotation including hypothetical protein.

10 PSM							
species	nov	hypo		known		$\Sigma$	
	6-frame	proteome	6-frame	proteome	6-frame	proteome	6-frame
<i>B. theta.</i>	37	1975	2079	248	254	2500	4706
<i>B. producta</i>	52	1138	1214	132	138	1559	2857
<i>E. coli</i>	26	150	136	988	1061	1370	2538
<i>E. ramosum</i>	10	355	373	53	62	541	944
<i>B. longum</i>	16	128	153	0	0	202	356
<i>A. caccae</i>	17	549	597	100	103	841	1456
<i>L. plantarum</i>	31	83	109	28	39	194	342
<i>C. butyricum</i>	14	135	181	32	37	303	422

6 PSM							
species	nov	hypo		known		$\Sigma$	
	6-frame	proteome	6-frame	proteome	6-frame	proteome	6-frame
<i>B. theta.</i>	72	2118	2238	256	262	2500	4706
<i>B. producta</i>	103	1289	1411	143	148	1559	2857
<i>E. coli</i>	65	182	183	1127	1187	1370	2538
<i>E. ramosum</i>	30	431	465	65	76	541	944
<i>B. longum</i>	42	170	202	0	0	202	356
<i>A. caccae</i>	39	632	721	119	120	841	1456
<i>L. plantarum</i>	48	116	147	36	47	194	342
<i>C. butyricum</i>	26	176	257	39	46	303	422

**Supplemental Table 2.** Full list of species in SIHUMix and the corresponding NCBI assembly and taxonomy ID.

full name	taxid	assembly id
<i>Anaerostipes caccae</i> DSM 14662	411490	GCA_014131675.1
<i>Bacteroides thetaiotaomicron</i> VPI5482	226186	GCA_014131755.1
<i>Bifidobacterium longum</i> NCC2705	206672	GCF_000007525.1
<i>Blautia producta</i> ATCC 27340 DSM 2950	1121114	GCA_014131715.1
<i>Clostridium butyricum</i> DSM 10702	1316931	GCA_014131795.1
<i>Escherichia coli</i> str K12 substr MG1655	511145	GCF_000005845.2
<i>Erysipelatoclostridium ramosum</i> DSM 1402	445974	GCA_014131695.1
<i>Lactobacillus plantarum</i> subsp <i>plantarum</i> ATCC 14917 JCM 1149 CGMCC 12437	525338	GCA_014131735.1