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Abstract

Background: The functions of proteins are strongly related to their localization in cell compartments (for example the cytoplasm or membranes) but the experimental determination of the sub-cellular localization of proteomes is laborious and expensive. A fast and low-cost alternative approach is *in silico* prediction, based on features of the protein primary sequences. However, biologists are confronted with a very large number of computational tools that use different methods that address various localization features with diverse specificities and sensitivities. As a result, exploiting these computer resources to predict protein localization accurately involves querying all tools and comparing every prediction output; this is a painstaking task. Therefore, we developed a comprehensive database, called CoBaltDB, that gathers all prediction outputs concerning complete prokaryotic proteomes.

Description: The current version of CoBaltDB integrates the results of 43 localization predictors for 784 complete bacterial and archaeal proteomes (***2.548.292 proteins in total***). CoBaltDB supplies a simple user-friendly interface for retrieving and exploring relevant information about predicted features (such as signal peptide cleavage sites and transmembrane segments). Data are organized into three work-sets ("*specialized tools*", "*meta-tools*" and "*additional tools*"). The database can be queried using the organism name, a locus tag or a list of locus tags and may be browsed using numerous graphical and text displays.

Conclusions: With its new functionalities, CoBaltDB is a novel powerful platform that provides easy access to the results of multiple localization tools and support for predicting prokaryotic protein localizations with higher confidence than previously possible. CoBaltDB is available at http://www.umr6026.univ-rennes1.fr/english/home/research/basic/software/cobalten.

Background

Determining the subcellular localization of proteins is essential for the functional annotation of proteomes [1,2]. Bacterial proteins can exist in soluble (*i.e* free) forms in cellular spaces (cytoplasm in both monoderm and diderm bacteria and periplasm in diderms), anchored to membranes (cytoplasm membrane in monoderms, inner- or outer membrane in diderms) or cell wall (in monoderms). They can also be released into the extracellular environment or directly translocated into host cells [3]. All protein synthesis takes place in the cytoplasm, so all non-cytoplasmic proteins must pass through one or two lipid bilayers by a mechanism commonly called "secretion". Protein secretion is involved in various processes including plant-microbe interactions [4,5]), biofilm formation [6,7] and virulence of plant and human pathogens [8-10]. Two main systems are involved in protein translocation across the cytoplasmic membrane, namely the essential and universal Sec (Secretion) pathway and the Tat (Twin-arginine translocation) pathway found in some prokaryotes (monoderms and diderms) and eukaryotes alike [11-16]. The Sec machinery recognizes an N-terminal hydrophobic signal sequence and translocates unfolded proteins [12], whereas the Tat machinery recognizes a basic-rich N-terminal motif (SRR-x-FLK) and transports fully folded proteins [13,14]). In addition to these systems, diderm bacteria have six further systems that secrete proteins using a contiguous channel spanning the two

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membranes (T1SS, [17,18], T3SS, T4SS and T6SS [19-24]) or in two steps, the first being Sec- or Tat-dependent export into the periplasmic and the second being translocation across the outer membrane (T2SS, [25-27] and T5SS, [28,29]). Other diderm protein secretion systems exist: they include the chaperone-usher system (CU or T7SS, [30,31]) and the extracellular nucleation-precipitation mechanism (ENP or T8SS, [32]). It is worth mentioning that the terminology T7SS has also been proposed to describe a completely different protein secretion system, namely the ESAT-6 protein secretion (ESX) in Mycobacteria, now considered as diderm bacteria [33]. Beside Sec and Tat pathways, monoderm bacteria have additional secretion systems for protein translocation across the cytoplasmic membrane, namely the flagella export apparatus (FEA [34]), the fimbrilin-protein exporter (FPE, [35,36]) and the WXG100 secretion system (Wss, [37,38]).

Establishing whole proteome subcellular localization by biochemical experiments is possible but arduous, time consuming and expensive. Data concerning predicted proteins (from whole genome sequences) is continuously increasing. High-throughput *in silico* analysis is required for fast and accurate prediction of additional attributes based solely on their amino acid sequences. There are large numbers of global (that yield final localization) and specialized (that predict features) tools for computer-assisted prediction of protein localizations. Most specialized tools tend to detect the presence of N-terminal signal peptides (SP). Prediction of Sec-sorting signals has a long history as the first methods, based on weight matrices, were published about fifteen years ago [39-41]. Numerous machine learning-based methods are now available [42-50]. The distinction between Tat- and Sec- sorting signals is essentially based on the recognition, in the n/h regions edge, of the twin-arginine motif [51], using regular expressions combined with hydrophobicity measures [52] or machine learning [53]. Prelipoproteins SP have the same n- and h- regions as Sec SP but contain, in the c-region, a well-conserved lipobox [54], recognized for cleavage by the type II signal peptidase [55]. Lipoprotein prediction tools use regular expression patterns to detect this lipobox [56,57], combined with Hidden Markov Models (HMM) [58] or Neural Networks (NN) [59]. Other attributes predicted by specialized tools are α-helices and β-barrel transmembrane segments. In 1982, Kyte and Doolittle proposed a hydropathy-based method to predict transmembrane (TM) helices in a protein sequence. This approach was enhanced by combining discriminant analysis [60], hydrophobicity scales [61-63] amino acid properties [64,65]. Complex algorithms are also available and employ statistics [66], multiple sequence alignments [67] and machine learning approaches [68-73]. β-barrel segments, embedded in outer membrane proteins, are harder to predict than α-helical segments, mostly because they are shorter; nevertheless, many methods are available based on similar strategies [74-87].

This plethora of protein localization predictors and databases [88-91] constitutes an important resource but requires time and expertise for efficient exploitation. Some of the tools require computing skills, as they have to be locally installed; others are difficult to use (numerous parameters) or to interpret (large quantities of graphics and output data). Web tools are disseminated and need numerous manual requests. Additionally, researchers have to decide which of these numerous tools are the most pertinent for their purposes, and selection is problematic without appropriate training sets. Recent work shows that the best strategy for exploiting the various tools is to compare them [92-94].

Here, we describe CoBaltDB, the first public database that displays the results obtained by 43 localization predictor tools for 776 complete prokaryotic proteomes. CoBaltDB will help microbiologists explore and analyze subcellular localization predictions for all proteins predicted from a complete genome; it should thereby facilitate and enhance the understanding of protein function.

Construction and content

Data sources

The major challenge for CoBaltDB is to collect and integrate into a centralized open-access reference database, non-redundant subcellular prediction features for complete prokaryotic orfeomes. Our initial dataset contained 784 complete genomes (731 bacteria and 53 Archaea), downloaded with all plasmids and chromosomes (1468 replicons in total), from the NCBI ftp server ftp://ftp.ncbi.nih.gov/genomes/Bacteria in mid-December 2008. This dataset contains 2,548,292 predicted non-redundant proteins (Additional file 1).

The CoBaltDB database was designed to associate results from disconnected resources. It contains three main types of data: *i*) CoBaltDB pre-computed prediction using 23 feature-based localization tools (Table 1), *ii*) CoBaltDB pre-computed prediction obtained using 5 localization meta-tools (Table 2) and *iii*) data collected from 20 public databases with both predicted and experimentally determined subcellular protein localizations (Table 3).

These data were organized in five "*boxes*" with regard to the features predicted: three boxes correspond to signal peptide detection (Lipoprotein, Tat- and Sec- dependent targeting signals); one box for the prediction of alpha-transmembrane segments (TM-Box); and one box, only available for diderms (Gram-negatives), for outer membrane localization through prediction of beta-barrels.

Table 1 A summary of CoBaltDB precomputed features-tools

Program	Reference	Analytical method	CoBaltDB features prediction group(s)				
LipoP 1.0 Server	[59]	HMM + NN	LIPO		SEC		
DOLOP	[57]	RE	LIPO				
LIPO	[56]	RE	LIPO				
TatP 1.0	[53]	RE + NN		TAT			
TATFIND 1.4	[52]	RE		TAT			
PrediSi	[112]	Position weight matrix			SEC		
SignalP 3.0 Server	[45-47]	HMM + NN			SEC		
SOSUIsignal	[113]	Multi-programs			SEC		
SIG-Pred	J.R. Bradford	Matrix			SEC		
RPSP	[44]	NN			SEC		
Phobius	[48,49]	HMM			SEC	αTMB	
HMMTOP	[71]	HMM				αTMB	
TMHMM Server v.2.0	[70]	HMM				αTMB	
TM-Finder	[65]	AA FEATURES				αTMB	
SOSUI	[114]	AA FEATURES				αTMB	
SVMtm	[73]	SVM				αTMB	
SPLIT 4.0 Server	[115]	AA FEATURES				αTMB	
MCMBB	[116]	HMM					βBarrel
TMBETADISC:	[117]						
_COMP		AA FEATURES					βBarrel
_DIPEPTIDE		Dipeptide composition					βBarrel
_MOTIF		Motif(s)					βBarrel
TMB-Hunt2	[118]	SVM					βBarrel

HMM: Hidden Markov Model, NN: Neural Network, RE: Regular Expression, AA: Amino Acid, SVM: Support Vector Machine

Table 2 A summary of CoBaltDB precomputed meta-tools

Program	Reference	Analytical method	Localizations
Subcell Specialization Server 2.5	[119]	Multiple classifiers	5 diderms/3 monoderms
SLP-Local	[120]	SVM	3 with no distinction
SubLoc v1.0	[121]	SVM	3 with no distinction
Subcell (Adaboost method)	[122]	AdaBoost algorithm	3 with no distinction
SOSUIGramN	[123]	Physico-chemical parameters	5 diderms/no monoderm

SVM: Support Vector Machine

Data generation

There is a great diversity of web and stand-alone resources for the prediction of protein subcellular location. We retrieved and tested 99 currently (in 2009) available specialized and global tools (software resources) that use various amino acid features and diverse methods: algorithms, HMM, NN, Support Vector Machine (SVM), software suites and others), to predict protein subcellular localization (Additional file 2). All tools were evaluated: some are included in CoBaltDB, some may be launched directly from the platform (Table 4), and others were excluded because of redundancy or processing reasons or both (Table 5). Some tools are specific to Gram-negative or Gram-positive bacteria. Many prediction methods applicable to both Gram categories have different parameters for the two groups of bacteria. For these reasons, each NCBI complete bacterial and archaeal genome implemented in CoBaltDB was registered as "monoderm" or "diderm", on the basis of information in the literature and phylogeny (Additional file 3). Monoderms and diderms were considered as Gram-negative and Gram-positive, respectively. All archaea were classified as monoderm prokaryotes since their cells are bounded by a single cell membrane and possess a cell envelope [3,95]. An exception was made for *Ignicoccus hospitalis* as it owns an outer sheath resembling the outer membrane of gram-negative bacteria [96].

Currently, CoBaltDB contains pre-computed results obtained with 48 tools and databases, and additionally provides pre-filled access to 50 publicly available tools that could not be pre-computed or that provide new

Table 3 A summary of CoBaltDB integrated databases and tools features.

Databases	Reference	Features predicted	Genome(s)	Protein numbers
EchoLOCATION	[124]	Subcellular-location (EXP)	*E. coli K-12*	4330 (506 exp)
Ecce	_	Subcellular-location	*E. coli K-12*	306
LocateP DataBase	[89]	Subcellular-location	178 MD	542788
cPSORTdb	[91]	Subcellular-location	140 BA	1634278
ePSORTdb	[91]	Subcellular-location (EXP)		2165
THGS	[125]	Transmembrane Helices	689 PROK	465411
Augur	[88]	Subcellular-location	126 MD	111223
CW-PRED	[126]	Cell-anchored (surface)	94 MD	954
PROFtmb	[78]	Beta-barrel (OM)	78 DD/19 MD	2152
HHomp	[127]	Beta-barrel (OM)		12495
PRED-LIPO	[58]	Lipoprotein SPs	179 MD	895
SPdb	[90]	Signal peptides (SPs)	855 PROK	7062
ExProt	[128]	Signal peptides (SPs)	23 AR/61 MD/115DD	
Signal Peptide Website	_	Signal peptides (SPs)	384 BA	1161 (EXP)
PRED-SIGNAL	[129]	Signal peptides (SPs)	48 AR	9437
TMPDB	[130]	Alpha Helices & Beta-barrel		188
DTTSS	Shandong Univ.	Type III secretion system		1035
TOPDB	[131]	Transmembrane Proteins	755 BA/16 AR	
TMBC-Database	Andrew Garrow	Transmembrane Beta-barrel		1219
Swissprot signal testset	[132]	Signal peptides (SPs) (EXP)	176 SP+/122 SP-	

MD = monoderm, DD = diderm. AR (Archeae), BA (Bacteria), PROK (Prokaryotes) include both bacteria and Archaee, EXP = Experimental database

information (tools dedicated to a special phylum, consensus tools or tools predicting proteins secreted via other pathways). The data pre-computing process is illustrated in Figure 1; web-based and stand-alone tools were used separately. Web-based localization prediction tools were requested via a Web automat, a python automatic submission workflow using both "httplib" and "urllib" libraries. A different script was created for each tool. For web-tools with no equivalent (such as "TatP" for Tat-BOX and "LIPO" for Lipoprotein-BOX) and incompatible with automatic requests, we collected results manually. CoBaltDB also provides a platform with automatically pre-filled forms for additional submissions to a selection of fifty recent or specific web tools (Table 4). The stand-alone tools were installed on a Unix platform (unique common compatible platform) and included in a global python pipeline with the HTTP request scripts. We selected information from a up-to-date collection of 20 databases and integrated this data within CoBaltDB; these databases were retrieved by simple downloading or creating an appropriate script which navigates on the web databases to collect all protein information. The global python pipeline used multithreading to speed up the pre-computation of the 784 proteomes.

Database Creation and Architecture

For each protein, every output collected (a HTML page for web tools and a text file for standalone applications) was parsed and selected items were stored in a particular format: binary "marshal" files. The object structure obtained by parsing tool output was directly saved into a marshal file, allowing a quick and easy opening by directly restoring the initial parsing object. Another script then creates the CoBaltDB repository, by reading and analysing all marshal files to generate a specific formatted file (".cbt") for each replicon. These files contain all the required protein information and a simplified representation of the tools' results. Some initialization files containing information about phylogeny or genome features are also used.

The repository is used by the Graphical User Interface (GUI) to display CoBaltDB information. For raw data from tools, the GUI accesses the marshal file directory.

Accessing the CoBaltDB Repository and Raw Data

The CoBaltDB platform has been developed as a client-server application. The server is installed at the Genouest Bioinformatics platform http://www.genouest.org/?lang=en. The client is a Java application that needs to be locally downloaded by the users. Queries are submitted to the server-side CoBaltDB repository using a locally installed client GUI that provides tabular and graphical representations of the data. The repository is accessed through SOAP-based web services (Simple Object Access Protocol), implemented in Java 5 using the Apache Axis 1.4 toolkit and deployed on the servlet engine Tomcat 5.5.20. CoBaltDB integrates: an

Table 4 Tools available using CoBaltDB "post" window

Program	Reference	Analytical method	CoBaltDB features prediction group(s)			
LipPred	[133]	Naive Bayesian Network	LIPO			
PRED-LIPO	[58]	HMM	LIPO		(only Monoderm)	
SPEPLip	[134]	NN	LIPO	SEC		
SecretomeP	[135]	Pattern & NN		ΔSEC_SP		
Signal-3L	[136]	Multi-modules		SEC		
Signal-CF	[137]	Multi-modules		SEC		
Signal-Blast	[138]	BlastP		SEC		
Sigcleave	EMBOSS	Von Heijne method		SEC		
PRED-SIGNAL	[129]	HMM		SEC	(only Archae)	
Flafind	[139]	AA features		T3SS Archae + T4SS Bacteria		
T3SS_prediction	[110]	SVM & NN		T3SS		
EffectiveT3	[111]	Machine learning		T3SS		
NtraC Signal Analysis	[140]	Pattern model		SEC (long SP)		
Philius	[141]	HMM		SEC	αTMB	
(SP)OCTOPUS	[142,143]	Blast Homology, NN, HMM		SEC	αTMB	
MemBrain	[144]	Machine learning		SEC	αTMB	
DAS	[145]	Dense Alignment Surface			αTMB	
HMM-TM	[146]	HMM			αTMB	
SVMtop Server 1.0	[147]	SVM			αTMB	
UMDHMM_TMHP	[148]	HMM			αTMB	
waveTM	[149]	Hydropathy signals algorithm			αTMB	
PRED-TMR	[150]	AA features			αTMB	
TMAP	[67]	AA features			αTMB	
igTM	[151]	Grammatical Inference			αTMB	
TOPCONS	[152]	Tools Consensus			αTMB	
TUPS	[153]	Tools Consensus			αTMB	
ConPred II	[154]	Tools Consensus			αTMB	
MEMSAT3	[66,155]	NN			αTMB	
SABLE	[156]	NN			αTMB	
TM-Pro	[64,157]	AA features			αTMB	
ProspRef	_	Knowledge-based method			αTMB	
PSIPRED	[158,159]	NN, PSSM			αTMB	
NPS@	[160]	Tools Consensus			αTMB	
SAM-T08	[161]	HMM			αTMB	
PORTER	[162]	NN			αTMB	
TMPred	EMBnet	Weight-matrices			αTMB	
TMMOD	[163]	HMM			αTMB	
TopPred II	[61]	G. von Heijne algorithm			αTMB	
YASPIN	[164]	Hidden Neural Network			αTMB	
MemType-2L	[165]	PseudoPSSM, classifier			Membrane Type	
BOMP	[84]	AA features				βBarrel
TMBETADISC-RBF	[87]	RBF network, PSSM				βBarrel
TMBETA-NET	[117]	AA features				βBarrel
PRED-TMBB	[85]	HMM				βBarrel
ConBBPred	[76]	Tools Consensus				βBarrel
CW-PRED (submit)	[126]	HMM		Cell-Wall (only Monoderm)		
ProtCompB	SoftBerry	Multi-methods	Localization			
CELLO	[166]	SVM	Localization			
PSL101	[167]	SVM, structure homology	Localization			
PSLpred	[168]	SVM	Localization			
GPLoc-neg	[169]	Basic classifier	Localization		(only Diderm)	

Table 4: Tools available using CoBaltDB "post" window *(Continued)*

GPLoc-pos	[170]	Basic classifier	Localization	(only Monoderm)
LOCtree	[171]	SVM	Localization	
PSORTb	[91]	Multi-modules	Localization	
SLPS	[172]	Nearest Neighbor on domain	Localization	
Couple-subloc v1.0	Jian Guo	AA features	Localization	
TBPRED	[173]	SVM	Localization	(only *Mycobacterium*)

HMM: Hidden Markov Model, NN: Neural Network, AA: Amino Acid, SVM: Support Vector Machine, PSSM: Position Specific Scoring Matrix, T3SS: Type III Secretion System, RBF: Radial Basis Function

Table 5 Tools and Database not available in CoBaltDB

Program	Reference	Analytical method	CoBaltDB features prediction group(s)			
SpLip	[174]	Weight matrix	LIPO		(only *Spirochaetal*)	
PROTEUS2	[175]	Multi-Methods		SEC	αTMB	βBarrel
PRED-TMR2	[176]	NN			αTMB	
PRODIV-TMHMM	[72]	Multi HMM			αTMB	
S_TMHMM	[72]	HMM			αTMB	
TransMem	[69]	NN			αTMB	
BPROMPT	[177]	Bayesian Belief Network			αTMB	
orienTM	[178]	Statistical analysis			αTMB	
APSSP2	[179]	Multi-Methods			Secondary structure	
PRALINE_TM	[180]	Alignment, tools consensus			Secondary structure	
OPM (DB)	[181]	Multi-Methods			Membrane orientation	
MP_Topo (DB)	[182]	Experimental			TMB	
PDBTM (DB)	[183]	TMDET algorithm			TMB	
TMB-HMM	A.Garrow	HMM, SVM				βBarrel
TMBETA-SVM	[86]	SVM				βBarrel
TMBETA-GENOME (DB)	[184]	Multi-Methods				βBarrel
PredictProtein	[185]	Alignment, Multi-Methods	Localization			
EcoProDB (DB)	[186]	Identification on 2D gels	Localization		(only *E.coli*)	
LOCTARGET (DB)	[187]	Multi-Methods	Localization			
DBMLoc (DB)	[188]	_	Localization			

NN: Neural Network, HMM: Hidden Markov Model, SVM: Support Vector Machine

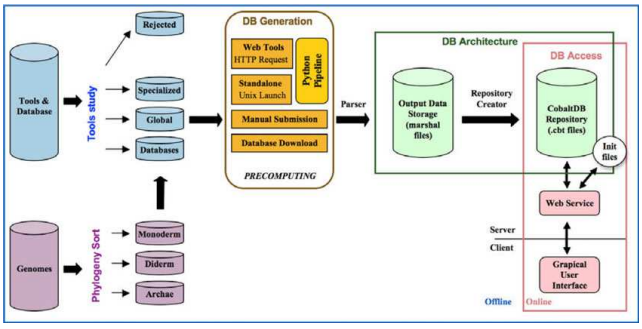


Figure 1 A schematic view of the CoBaltDB workflow. CoBaltDB integrates the results of 43 localization predictors for 784 complete bacterial and archaeal proteomes. Each complete NCBI prokaryotic genome implemented in CoBaltDB was classified as: archaea, or monoderm or diderm bacteria. 101 protein subcellular location predictors were evaluated and few were rejected. Selected tools were classified as: feature localization tools (Specialized), localization meta-tools (Global) or databases. The data recovery process was performed manually or via a Web automat using a python automatic submission workflow for both stand-alone and web-based tools. Databases were downloaded. For each protein, ouptuts collected were parsed and selected items were stored in particular CoBaltDB formatted files (.cbt). The parsing pipeline creates one ".cbt" file per replicon to compose the final CoBaltDB repository. The client CoBaltDB Graphical User Interface communicates with the server-side repository via web services to provide graphical and tabular representations of the results.

initialization web service (that returns the current list of genomes supported); two repository web services that allow querying the database either by specifying a replicon or a list of locus tags; and a raw data web service that retrieves all recorded raw data generated by a given tool for the specified locus tag.

Utility

Running CoBaltDB

Our goal was to build an open-access reference database providing access to protein localization predictions. CoBaltDB was designed to centralize different types of data and to interface them so as to help researchers rapidly analyse and develop hypotheses concerning the subcellular distribution of particular protein(s) or a given proteome. This data management allows comparative evaluation of the output of each tool and database and thus straightforward identification of inaccurate or conflicting predictions.

We developed a user-friendly CoBaltDB GUI as a Java 5 client application using NetBeans 5.5.1 IDE. It presents four tabs that perform specific tasks: the "input" tab (Figure 2) allows selecting the organism whose proteome localizations will be presented, using organism name completion or through an alphabetical list. Alternatively, users may also enter a subset of proteins, specified by their locus tags. The "Specialized tools" tab (Figure 3) supplies a table showing, for each protein identified by its locus tag or protein identifier, some annotation information such as its gene name, description and links to the corresponding NCBI and KEGG web pages. Clicking on a "locus tag" opens a navigator window with the related KEGG link, and clicking on a "protein Id" opens the corresponding NCBI entry web page. The table shows, for each protein and for each feature box (Tat, Sec, Lipo, αTMB, βBarrel), a heat map (white/blue) representing the percentage of tools predicting the truth/presence of the corresponding localization feature in the protein considered. Clicking on the heat map opens a new window that shows the raw data generated by each tool of the considered feature box, thus allowing the investigator to access the tool-specific information they are used to. The predictions of related feature databases are given next to the corresponding heat-map. The proteins which are referred to by the databases implemented in CobaltDB as having an experimentally determined localization appear with a yellow background colour. This representation enables the user to observe graphically the distribution of tools predicting each type of feature. The "meta-tools" tab (Figure 4) provides the predictions given by multi-modular prediction software (meta-tools or global databases) that use various techniques to predict directly three to five subcellular protein localizations in mono- and/or diderm bacteria (Table 4). The descriptions of the localizations were standardised to ease interpretation by the

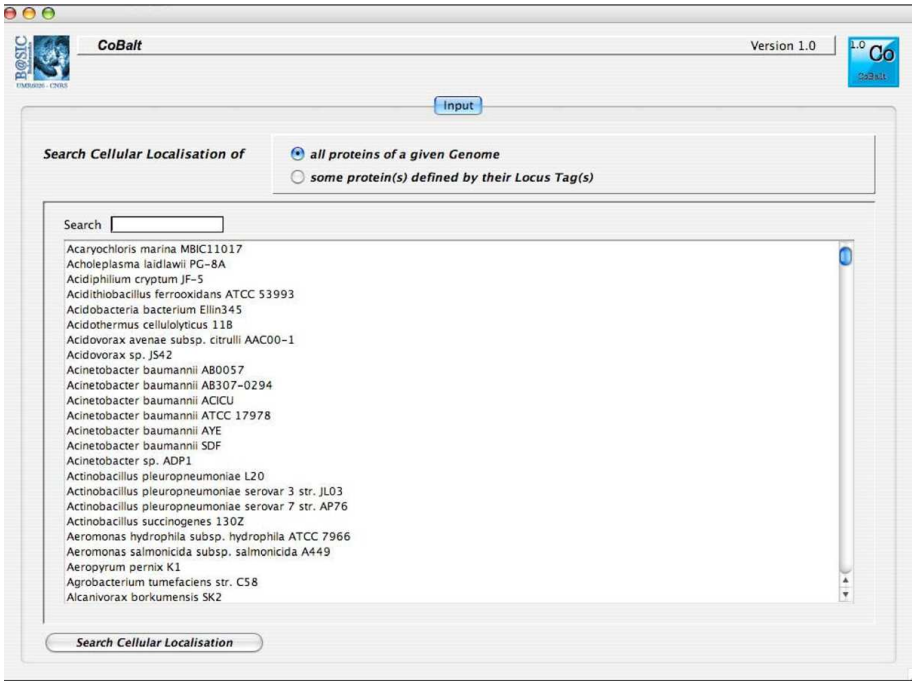


Figure 2 A snapshot of the CoBaltDB input interface. The "input" module allows the selection of organisms, using organism name completion or through an alphabetical list. Users can also enter a subset of proteins, specified by their locus tags.

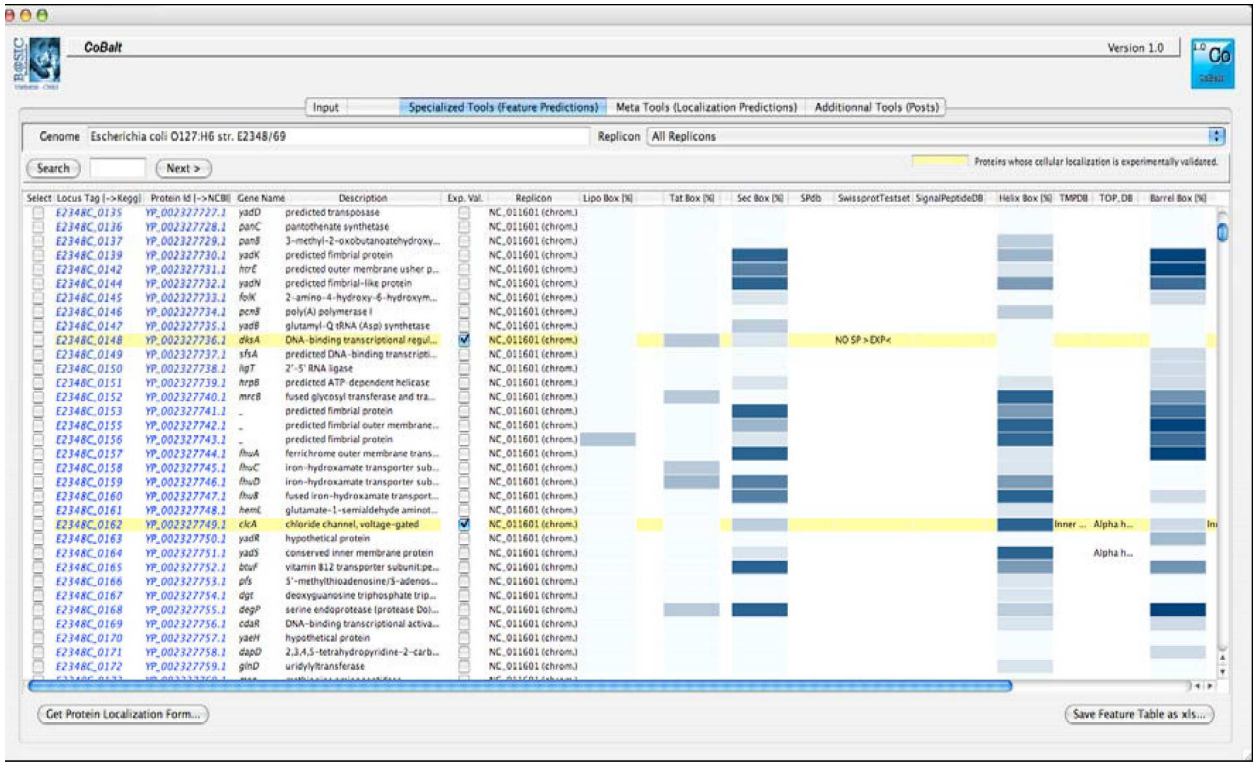


Figure 3 The CoBaltDB Specialized Tools viewer. The "Specialized tools" browser supplies a tabular output for every protein, enriched with the protein's annotation including locus tag, protein identifier, gene name (if available) and product descriptions. Clicking on each "locus tag" opens a navigator window with related KEGG link whereas clicking on every "protein Id" opens the corresponding NCBI entry web page. Clicking on the white/blue heat map reveals the raw results of all tools corresponding to the feature box considered.

investigator. Both tables may be searched for occurrences of any string of characters via the search button, facilitating retrieval of a particular locus tag, protein id, accession number or even a gene name or annotation description. Both tables may be sorted with respect to any column, i.e. in alphanumerical order for the locus tags, protein identifiers, annotation descriptions and localization predictions, or in numerical order for the percentages. This makes it straightforward to identify all proteins with particular combinations of localization features. Both tables may be saved as Excel files. Finally, the CoBaltDB "additional tools" tab (Figure 5) enables queries to be submitted to a set of 50 additional tools by pre-filling the selected forms with the selected protein sequence and Gram information whenever appropriate. For this use, the investigator might have to enter additional parameters.

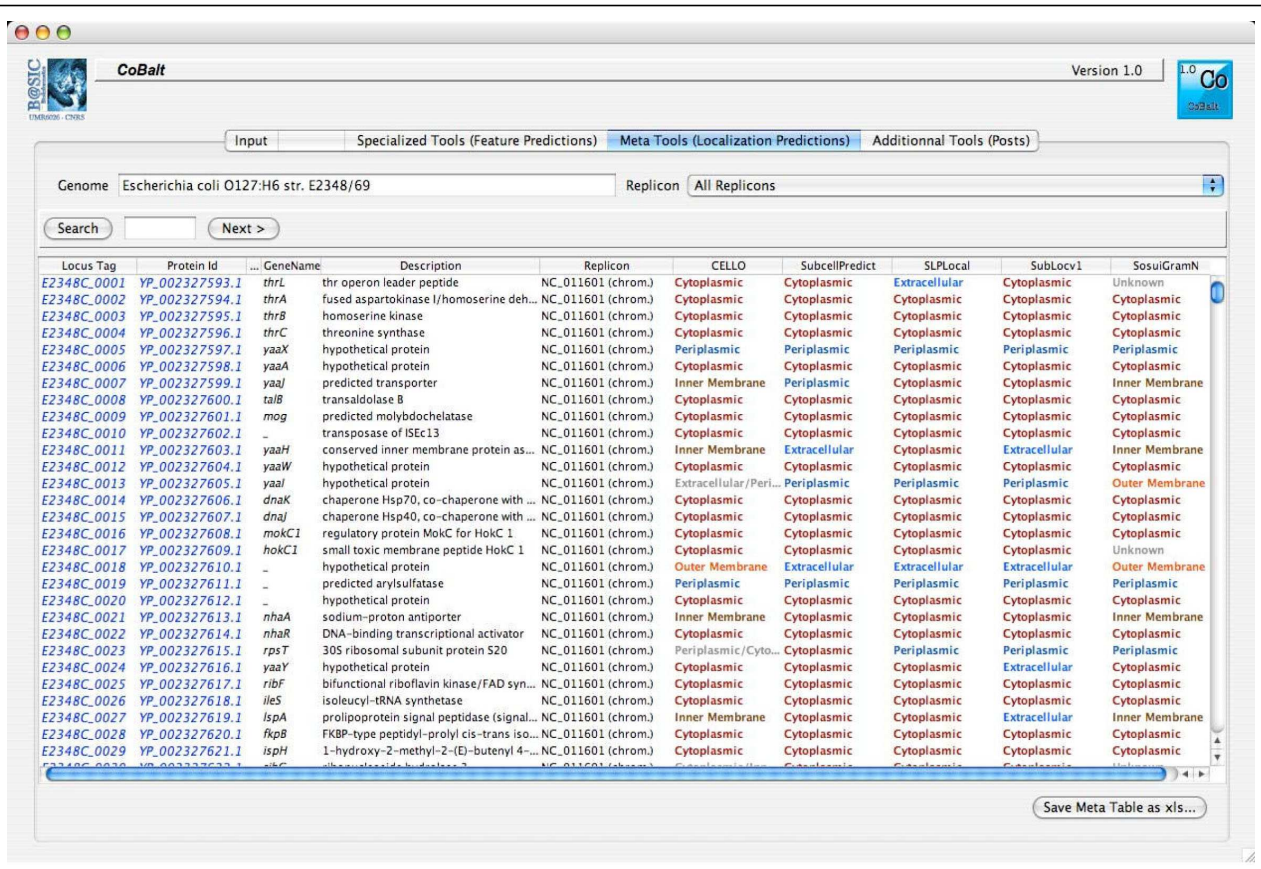
Finally, for each protein, all results were summarized in a synopsis (Figure 6); the synopsis presents the results generated by all the tools in a unified manner, and includes a summary of all predicted cleavage sites and membrane domains. This "standardized" form thus provides all relevant information and lets the investigators establish their own hypotheses and conclusions. This form may be saved as a .pdf file (Figure 6). Examples of using the CoBaltDB synopsis are provided below in the second case study.

Selected CoBaltDB uses

We propose to illustrate briefly some possible uses of CoBaltDB.

1-Using CoBaltDB to compare subcellular prediction tools and databases

The various bioinformatic approaches developed for computational determination of protein subcellular localization exhibit differences in sensitivity and specificity; these differences are mainly the consequences of the types of sequences used as training models (diderms, monoderms, Archaea) and of the methods applied (regular expressions, machine learning or others). By interfacing the results from most of the reliable predictions tools, CoBaltDB provides immediate comparisons and constitutes an accurate and high-performance resource to identify and characterize candidate "non-cytoplasmic" proteins. As an example, using CoBaltDB to analyse the 82 proteins that compose the experimentally confirmed



Locus Tag	Protein Id	...	GeneName	Description	Replicon	CELLO	SubcellPredict	SLPLocal	SubLocv1	SosuiGramN
E2348C_0001	YP_002327593.1		thrL	thr operon leader peptide	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Extracellular	Cytoplasmic	Unknown
E2348C_0002	YP_002327594.1		thrA	fused aspartokinase I/homoserine deh...	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0003	YP_002327595.1		thrB	homoserine kinase	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0004	YP_002327596.1		thrC	threonine synthase	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0005	YP_002327597.1		yaaX	hypothetical protein	NC_011601 (chrom.)	Periplasmic	Periplasmic	Periplasmic	Periplasmic	Periplasmic
E2348C_0006	YP_002327598.1		yaaA	hypothetical protein	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0007	YP_002327599.1		yaaJ	predicted transporter	NC_011601 (chrom.)	Inner Membrane	Periplasmic	Cytoplasmic	Cytoplasmic	Inner Membrane
E2348C_0008	YP_002327600.1		talB	transaldolase B	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0009	YP_002327601.1		mog	predicted molybdochelatase	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0010	YP_002327602.1		_	transposase of ISEc13	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0011	YP_002327603.1		yaaH	conserved inner membrane protein as...	NC_011601 (chrom.)	Inner Membrane	Extracellular	Cytoplasmic	Extracellular	Inner Membrane
E2348C_0012	YP_002327604.1		yaaW	hypothetical protein	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0013	YP_002327605.1		yaaI	hypothetical protein	NC_011601 (chrom.)	Extracellular/Peri...	Periplasmic	Periplasmic	Periplasmic	Outer Membrane
E2348C_0014	YP_002327606.1		dnaK	chaperone Hsp70, co-chaperone with ...	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0015	YP_002327607.1		dnaJ	chaperone Hsp40, co-chaperone with ...	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0016	YP_002327608.1		mokC1	regulatory protein MokC for HokC 1	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0017	YP_002327609.1		hokC1	small toxic membrane peptide HokC 1	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Unknown
E2348C_0018	YP_002327610.1		_	hypothetical protein	NC_011601 (chrom.)	Outer Membrane	Extracellular	Extracellular	Extracellular	Outer Membrane
E2348C_0019	YP_002327611.1		_	predicted arylsulfatase	NC_011601 (chrom.)	Periplasmic	Periplasmic	Periplasmic	Periplasmic	Periplasmic
E2348C_0020	YP_002327612.1		_	hypothetical protein	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0021	YP_002327613.1		nhaA	sodium-proton antiporter	NC_011601 (chrom.)	Inner Membrane	Cytoplasmic	Cytoplasmic	Cytoplasmic	Inner Membrane
E2348C_0022	YP_002327614.1		nhaR	DNA-binding transcriptional activator	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0023	YP_002327615.1		rpsT	30S ribosomal subunit protein S20	NC_011601 (chrom.)	Periplasmic/Cyto...	Cytoplasmic	Periplasmic	Periplasmic	Periplasmic
E2348C_0024	YP_002327616.1		yaaY	hypothetical protein	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Extracellular	Cytoplasmic
E2348C_0025	YP_002327617.1		ribF	bifunctional riboflavin kinase/FAD syn...	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0026	YP_002327618.1		ileS	isoleucyl-tRNA synthetase	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0027	YP_002327619.1		lspA	prolipoprotein signal peptidase (signal...	NC_011601 (chrom.)	Inner Membrane	Cytoplasmic	Cytoplasmic	Extracellular	Inner Membrane
E2348C_0028	YP_002327620.1		fkpB	FKBP-type peptidyl-prolyl cis-trans iso...	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
E2348C_0029	YP_002327621.1		ispH	1-hydroxy-2-methyl-2-(E)-butenyl 4-...	NC_011601 (chrom.)	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic

Figure 4 The CoBaltDB Meta-Tools interface. The "meta-tools" panel presents the CoBaltDB-computed results for multi-modular prediction software that uses various techniques to directly predict 3 to 5 subcellular localizations for proteins in mono- and/or diderm bacteria.

"Lipoproteome" of *E. coli K-12* [97] shows that 72 are correctly predicted by the three precomputed tools (LipoP [59], DOLOP [57] and LIPO [56]), and that the other 10 are only identified by two of the three tools (Additional file 4A). Eight of these lipoproteins were not detected by DOLOP, because the regular expression pattern allowing detection of the lipidation sequence ([LVI] [ASTVI] [GAS] [C] lipobox) is too stringent (Additional file 4B). By comparison, the PROSITE lipobox pattern (PS00013/PDOC00013) is more permissive ([DERK](6)-[LIVMFWSTAG] (2)- [LIVMFYSTAGCQ]- [AGS]-C). This example demonstrates that using a single tool may result in errors and suggests that the best approach is to combine the various "features-based" methods available and compare their findings. This view also applies to meta-tools predictors. *E. coli* K12 lipoproteins can be found anchored to the inner or the outer membrane through attached lipid, but some of them are periplasmic (Additional file 4A). The comparison of *in silico* subcellular localization assignments with experimental findings clearly indicates that all meta-tools require significant improvements in accuracy and precision, that none should be used to the exclusion of the others. It also appears that analysis with specialized tools, organized on a "one feature at a time" basis (Lipo SPs, TAT SPs ...), most reliably gives predictions consistent with experimental data. For this purpose, CoBaltDB is a unique and innovative resource.

2-Using CoBaltDB to analyse protein(s) and a proteome

One valuable property of CoBaldDB is to recapitulate all pre-computed predictions in a unique A4-formated synopsis. This summary is very helpful for assessing computational data such as the variation and frequency in the predictions of signal peptide cleavage sites: such predictions are sometimes significantly consistent, but often are not in agreement with each other (Figure 7A). However, correct identification of signal peptide cleavage sites is essential in many situations, especially for producing secreted recombinant proteins.

The CoBaltDB synopsis could also be used to discriminate between SignalPeptidaseII- and SignalPeptidaseI-cleaved signals and between SPs and N-terminal transmembrane helices. Indeed, most localization predictors have difficulties distinguishing between type I

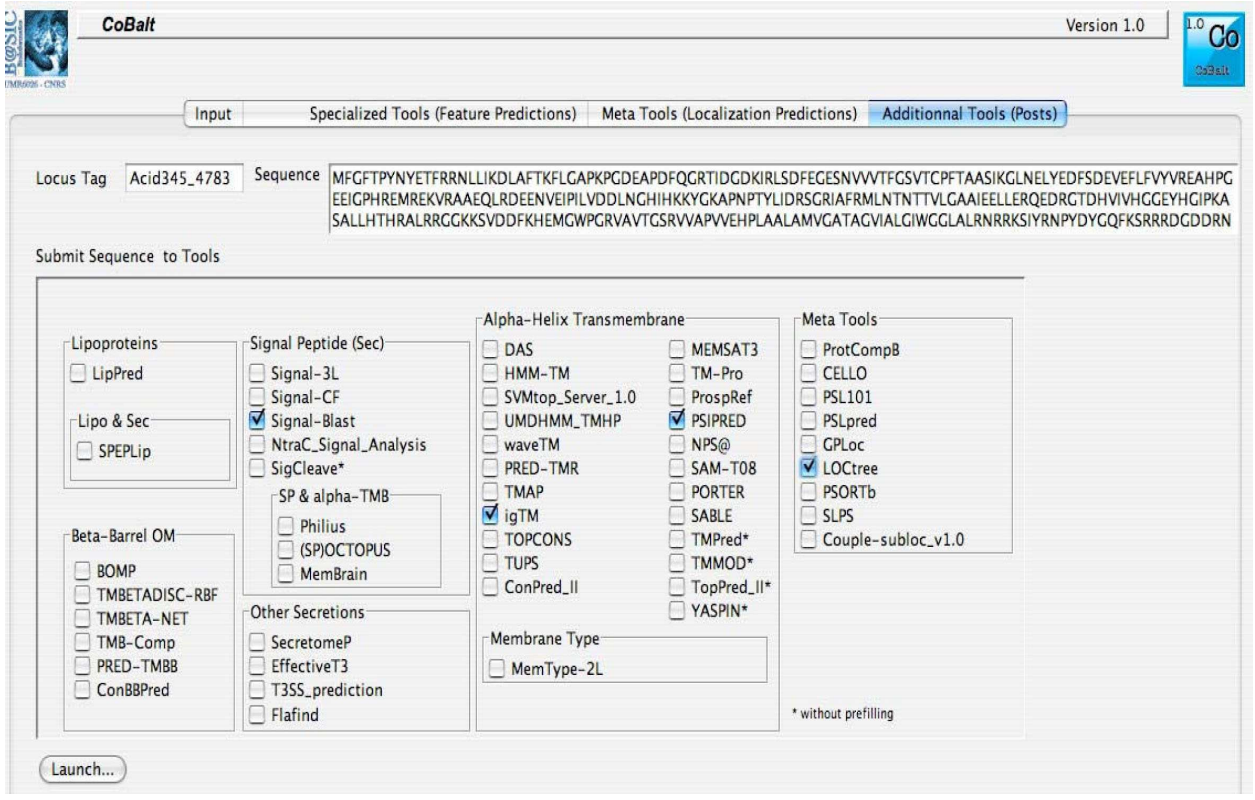


Figure 5 The CoBaltDB Prefilled post window. The "additional tools" panel enables web page submission for a set of 50 additional tools by pre-filling selected forms with selected sequence and Gram information as appropriate.

and type II signal peptidase cleavages. CoBaltDB can be exploited in an interesting way to benchmark this prediction by displaying all cleavage site predictions in a "decreasing sensitivity" arrangement (SpII then Tat-dependant SPI then Sec-SPI). By considering lipoprotein datasets from different organisms, we evidenced two principal profiles (Figure 7B) and found that all experimentally validated lipoproteins score 100% (all tools give the same prediction) or 66% in the CoBaltDB LIPO column (see explanation in the paragraph above). In addition, in almost all of the examined cases, tools dedicated to Twin-arginine SP detection do not identify SpII-dependent SP, whereas the Sec-SP predictors detect both Sec and Tat-type I as well as type II signal-anchor sequences.

These observations allow us to propose, for our data set, thresholds for each box: as previously illustrated, lipoproteins have score > 66% in the LIPO prediction box; Tat-secreted proteins have 0% in the LIPO box and 100% for the two TAT-dedicated tools; Sec-secreted proteins have 33% in the LIPO Box (due to the fact that LipoP detects both SpI and SpII [59]), 0% in the TAT-tools, and > 80% in SEC-specialized tools. Rules of this type can be used to check entire proteomes for evaluation of the different secretomes as illustrated in the following case studies.

3-Using CoBaltDB to compare proteomes

Using CoBaltDB and the thresholds described above, we can compare the predicted lipoproteomes (Figure 8A) of the three completely sequenced substrains of *E. coli* K12: MG1655 and W3110 (both derived from W1485 approximately 40 years ago [98]), and DH10B which was constructed by a series of genetic manipulations [99]. Each of these three substrains encode 89 lipoproteins found in both other substrains (Additional file 4). Four additional lipoproteins are detected in DH10B (BorD, CusC, RlpA and RzoD) and are second copies lipoprotein genes, present in the 113-kb tandemly repeated region of the chromosome (Figure 8B, coordinates 514341 to 627601, [99]), and strain DH10B contains one gene encoding the Rz1 proline-rich lipoprotein from bacteriophage lambda absent from the two other substrains. Lipoprotein YghJ, that shares 64% homology with *V. cholerae* virulence-associated accessory colonization factor AcfD [100], is absent from the DH10B genome annotation. However, comparative genomic analysis

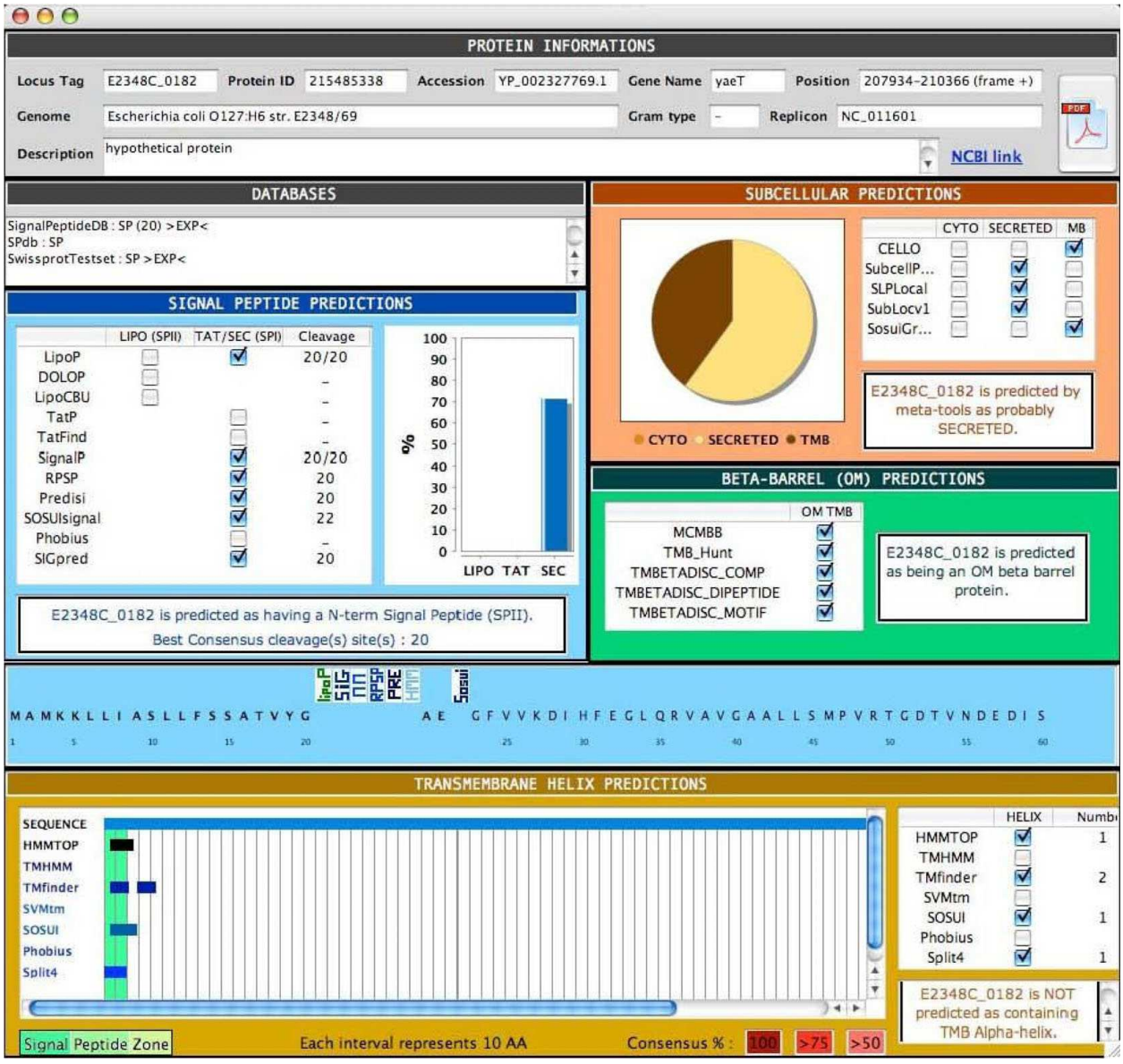


Figure 6 CoBaltDB Synopsis. For any given protein, all results are summarized in a synopsis which presents, in a unified manner, a summary of all predicted cleavage sites and membrane domains. This synopsis can be stored as a .pdf file.

shows that a *yghJ* locus could be annotated in this strain but corresponds to a pseudogene caused by a frameshift event (Figure 8C). YfbK was also overlooked in the DH10B annotation process but in this case, the gene is intact. Finally, differences between lipoprotein prediction results concerning YafY, YfiM and YmbA are due to erroneous N-terminus predictions. YafY in DH10B was predicted to be a lipoprotein due to the N-terminal 17 aa-long type II signal peptide and was published as a new inner membrane lipoprotein [101]. In substrains MG1655 and WS3110, the original annotation fused the *yafY* loci with its upstream pseudogene *ykfK* (137 N-terminal aa longer). The presumed start codons of YfiM and YmbA in MG1655 were recently changed by adding 17 (*lrilfvcslllsgcsh*) and 5 (*mkkwl*) N-terminal amino acids, respectively (PMC1325200). These modifications substantially affect the prediction of their subcellular localization. Inspection of the genomic sequences of the two other substrains leads to equivalent changes such that YfiM and YmbA in all three substrains are now predicted to be lipoproteins. In conclusion, using CoBaltDB to compare lipoproteomes between substrains, we were able to detect genomic events as well as "annotation" errors. After correction, we can conclude

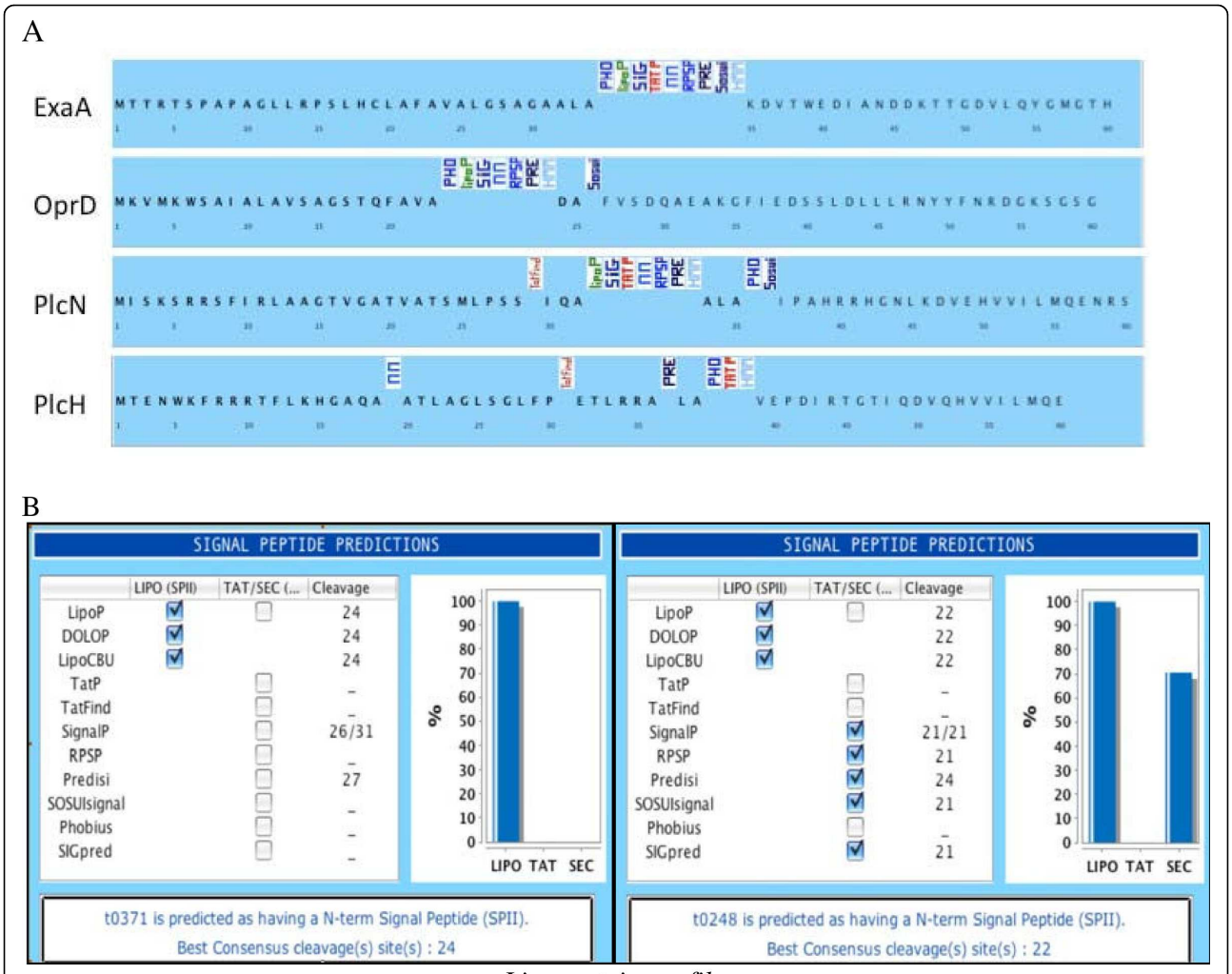


Figure 7 Using CoBaltDB to analyse protein(s) and a proteome. A: Comparative analysis of SP cleavage site predictions (proteinssecreted by *P. aeruginosa*); B: Discriminating between SPI- and SP II cleavage sites.

that the three *E. coli K12* substrains have 93 lipoproteins in common; that one locus **whose** function is related to virulence has been transformed into a pseudogene in DH10B; and that DH10B contains five additional lipoproteins due to duplication events and to the presence of prophages absent from the other two substrains (Figure 8D).

4-Using CoBaltDB to improve the classification of orthologous and paralogous proteins

Protein function is generally related to its subcellular compartment, so orthologous proteins are expected, in most cases, to be in the same subcellular location. Consequently, inconsistencies of location predictions between orthologs potentially indicate distinct functional subclasses. Thus, CobaltDB can be used to help improve the functional annotation of orthologous proteins by adding the subcellular localization dimension. As an example, OxyGene, an anchor-based database of the ROS-RNS (Reactive Oxygen-Nitrogen species) detoxification subsystems for 664 complete bacterial and archaeal genomes, includes 37 detoxicifation enzyme subclasses [102]. Analysis of CoBaltDB subcellular localization information suggested the existence of additional subclasses. For example, 1-cystein peroxiredoxin, PRX_BCPs (bacterioferritin comigratory protein homologs), can be sub-divided into two new subclasses by distinguishing the secreted from the non-secreted forms (Figure 9a). Differences in the location between orthologous proteins are suggestive of functional diversity, and this is important for predictions of phenotype from the genotype.

CoBaltDB is a very useful tool for the comparison of paralogous proteins. For example, quantitative and qualitative analysis of superoxide anion detoxification

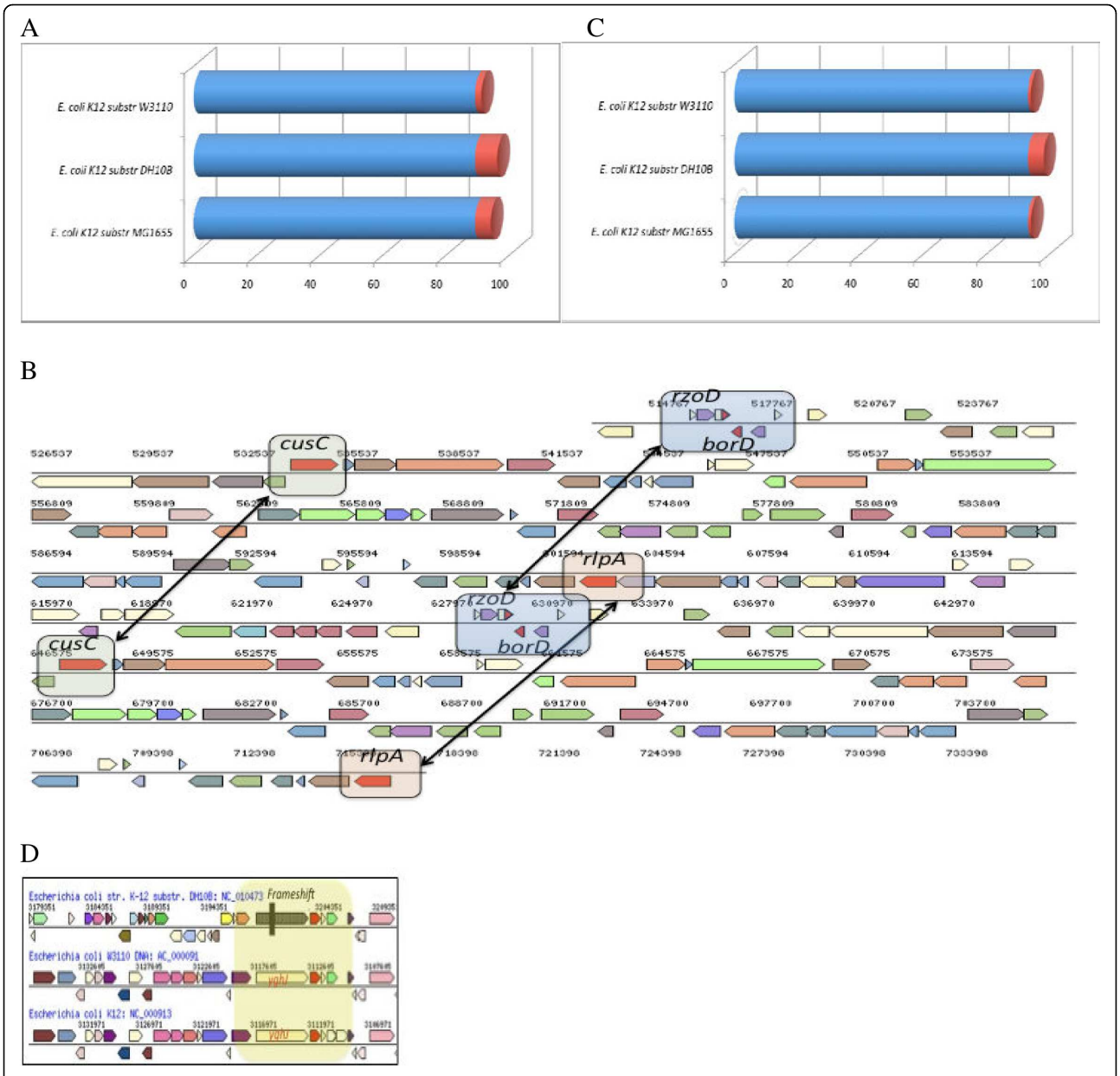


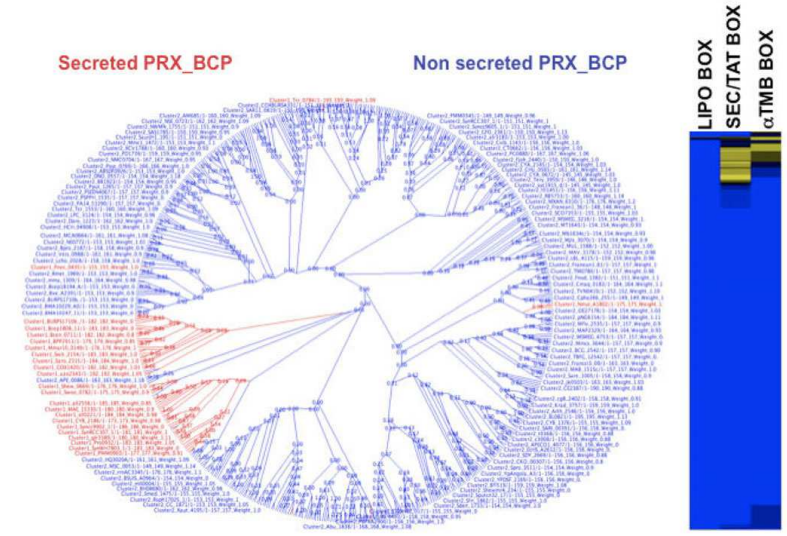
Figure 8 Using CoBaltDB in comparative proteomics. Example of E. coli K12 substrains lipoproteomes.

subsystems using the OxyGene platform identified three iron-manganese Superoxide dismutase (SOD_FMN) in *Agrobacterium tumefaciens* but only one SOD_FMN and one copper-zinc SOD (SOD_CUZ) in *Sinorhizobium meliloti*. The number of paralogs and the class of orthologs thus differ between these two closely related genus. However, adding the subcellular localization dimension reveals that both species have machinery to detoxify superoxide anions in both the periplasm and cytoplasm: both one of the three SOD_FMN of *A. tumefaciens* and the SOD_CUZ of *S. meliloti* are secreted (Figure 9b). CoBaltDB thus helps explain the difference suggested by OxyGene with respect to the ability of the two species to detoxify superoxide.

Discussion

CobaltDB allows biologists to improve their prediction of the subcellular localization of a protein by letting them compare the results of tools based on different methods and bringing complementary information. To facilitate the correct interpretation of the results, biologists have to keep in mind the limitations of the tools especially regarding the methodological strategies employed and the training sets used [93]. For example, most specialized tools

A



B

Genome	Replicon	OxyDB Name	Gene Name	Locus Tag	CoBaltDB
Agrobacterium tumefaciens str. C58					
	NC_003063	SOD_FMN	sodBIII	Atu4726	secreted
	NC_003063	SOD_FMN	sodBII	Atu4583	non secreted
	NC_003062	SOD_FMN	sodBI	Atu0876	non secreted
Sinorhizobium meliloti 1021					
	NC_003047	SOD_FMN	sodB	SMc00043	non secreted
	NC_003047	SOD_CUZ	sodC	SMc02597	secreted

Figure 9 Using CoBalt for the analysis of orthologous and paralogous proteins. A: Phylogenetic tree of 1-cystein peroxiredoxin PRX_BCP proteins and heat map of scores in each box for each PRX_BCP protein. B: OxyGene and CoBalt predictions for SOD in *Agrobacterium tumefacins str. C58* and *Sinorhizobium meliloti 1021*.

tend to detect the presence of N-terminal signal peptides and predict cleavage sites. However the absence of an N-terminal signal peptide does not systematically indicate that the protein is not secreted. Some proteins that are translocated via the Sec system might not necessarily exhibit an N-terminal signal peptide, such as the SodA protein of *M. tuberculosis*, which is dependent on SecA2 for secretion and lacks a classical signal sequence for protein export [103]. Furthermore, there is no systematic cleavage of the N-terminal signal peptide as it can serve as a cytoplasmic membrane anchor [104,105]. Another example: although type II and type V secretion systems generally require the presence of an N-terminal signal peptide in order to utilise the sec pathway for translocation from cytoplasm to periplasm, type I and type III (and usually also type IV) systems can secrete a protein without any such signal [28,106]. Other proteins, such as Yop proteins exported by the *Yersinia* TTS system, have no classical *sec*-dependent signal sequences; however the information required to direct these proteins into the TTS pathway is contained within the N-terminal coding region of each gene [107-109].

Some challenges still need to be addressed in the prediction of the subcellular localization of proteins. For instance, bioinformatics has recently focussed on predicting proteins secreted via other pathways [110,111].

Conclusion

We have developed CoBaltDB, the first friendly interfaced database that compiles a large number of *in silico* subcellular predictions concerning whole bacterial and archaeal proteomes. Currently, CoBaltDB allows fast access to precomputed localizations for 2,548,292 proteins in 784 proteomes. It allows combined management of the predictions of 75 feature tools and 24 global tools and databases. New specialised prediction tools, algorithms and methods are continuously released, so CoBaltDB was designed to have the flexibility to facilitate inclusion of new tools or databases as required.

In general, our analysis indicates that both feature-based and general localization tools and databases have perform diversely in terms of specificity and sensitivity; the diversity arises mainly from the different sets of proteins used during the training process and from the limitations of the mathematical and statistical methodologies applied. In all our analyses with CoBaltDB, it became clear that that the combination and comparative analysis of results of heterogeneous tools improved the computational predictions,

and contributed to identifying the limitations of each tool. Therefore, CoBaltDB can serve as a reference resource to facilitate interpretation of results and to provide a benchmark for accurate and effective *in silico* predictions of the subcellular localization of proteins. We hope that it will make a significant contribution to the exploitation of *in silico* subcellular localization predictions as users can easily create small datasets and determine their own thresholds for each predicted feature (type I or II SPs for example) or proteome. This is very important, as constructing an exhaustive "experimentally validated protein location" dataset is a time-consuming process –including identifying and reading all relevant papers– and as experimental findings about some subcellular locations are very limited.

Availability and requirements

Database name: CoBaltDB

Project home page: http://www.umr6026.univ-rennes1.fr/english/home/research/basic/software/cobalten

Operating system(s): Platform independent

Programming languages: Java, Python and BioPython

CoBaltDB package, requirements and documentations are freely available at http://www.umr6026.univ-rennes1.fr/english/home/research/basic/software/cobalten

Additional file 1: List of precomputed genomes (Excel). A table of all complete procaryotic genomes and corresponding replicons available in CoBaltDB.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2180-10-88-S1.XLS]

Additional file 2: Procaryotic subcellular localisation tools (HTML). This page is an inventory of all tools considered during the construction of CoBaltDB. The tools and databases related to the protein localization in procaryotic genomes are sorted by type of prediction. For each tool, a short description and the corresponding web link are displayed.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2180-10-88-S2.PDF]

Additional file 3: Monoderm and Diderm classification of genomes (PNG). Picture showing the cellular organization type (monoderm or diderm) for phylum in CoBaltDB.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2180-10-88-S3.PNG]

Additional file 4: Using CoBalt in comparative proteomics (PDF). Example of the lipoproteomes *of E. coli K12* substrains, experimentally confirmed by EcoGene. Table1A: Prediction results for the 89 confirmed lipoproteins in the three substrains DH10B, MG1655 et W3110. Table1B: The lipoproteins that are not recognized by DOLOP have a sequence which does not match the DOLOP lipoBox pattern [LVI] [ASTVI] [ASG] [C].
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2180-10-88-S4.PDF]

Abbreviations

(AA): Amino acid; (aTMB): alpha-transmembrane; (CU): chaperone-usher; (ENP): extracellular nucleation-precipitation; (EXP): experimentally valitaded; (FEA): flagella export apparatus; (FPE): fimbrilin-protein exporter; (GUI): Graphical User Interface; (HMM): Hidden Markov Model; (LIPO): lipoprotein; (NN): Neural Network; (PRX_BCPs): bacterioferritin comigratory protein homologs; (PSSM): Position Specific Scoring Matrix; (RE): regular expression; (ROS-RNS): Reactive Oxygen-Nitrogen species; (Sec): Sec apparatus; (SOAP): Simple Object Access Protocol; (SOD_CUZ): one copper-zinc superoxide dismutase; (SOD_FMN): iron-manganese superoxide dismutase; (SP): signal peptide; (SVM): support vector machine; (T1-7SS): Type (1-7) Secretion System; (Tat): Twin-arginine translocation; (TM): transmembrane; (Wss): WXG100 secretion system.

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Authors' contributions

DG designed and implemented the CoBaltDB database and the pre-computing pipeline for automated data retrieval. SA and DG developed the user interface. CLM and FBH tested the database for functionality, and performed bioinformatics analyses leading to valuable suggestions on utility and design. CLM and SA helped coordinate the study. FBH conceived and managed the project. All authors participated in CoBaltDB design, contributed to workflow and interface designs and helped write the manuscript. All authors read and approved the final manuscript.

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References

1. Rost B, Liu J, Nair R, Wrzeszczynski KO, Ofran Y: **Automatic prediction of protein function.** *Cell Mol Life Sci* 2003, **60(12)**:2637-2650.
2. Nagy A, Hegyi H, Farkas K, Tordai H, Kozma E, Banyai L, Patthy L: **Identification and correction of abnormal, incomplete and mispredicted proteins in public databases.** *BMC bioinformatics* 2008, **9**:353.
3. Desvaux M, Hebraud M, Talon R, Henderson IR: **Secretion and subcellular localizations of bacterial proteins: a semantic awareness issue.** *Trends in microbiology* 2009, **17(4)**:139-145.
4. De-la-Pena C, Lei Z, Watson BS, Sumner LW, Vivanco JM: **Root-microbe communication through protein secretion.** *The Journal of biological chemistry* 2008, **283(37)**:25247-25255.
5. Steward O, Pollack A, Rao A: **Evidence that protein constituents of postsynaptic membrane specializations are locally synthesized: time course of appearance of recently synthesized proteins in synaptic junctions.** *Journal of neuroscience research* 1991, **30(4)**:649-660.
6. Russo DM, Williams A, Edwards A, Posadas DM, Finnie C, Dankert M, Downie JA, Zorreguieta A: **Proteins exported via the PrsD-PrsE type I secretion system and the acidic exopolysaccharide are involved in biofilm formation by Rhizobium leguminosarum.** *Journal of bacteriology* 2006, **188(12)**:4474-4486.
7. Zhang L, Zhu Z, Jing H, Zhang J, Xiong Y, Yan M, Gao S, Wu LF, Xu J, Kan B: **Pleiotropic effects of the twin-arginine translocation system on biofilm formation, colonization, and virulence in Vibrio cholerae.** *BMC microbiology* 2009, **9**:114.
8. De Buck E, Anne J, Lammertyn E: **The role of protein secretion systems in the virulence of the intracellular pathogen Legionella pneumophila.** *Microbiology (Reading, England)* 2007, **153(Pt 12)**:3948-3953.
9. Poueymiro M, Genin S: **Secreted proteins from Ralstonia solanacearum: a hundred tricks to kill a plant.** *Current opinion in microbiology* 2009, **12(1)**:44-52.
10. Shrivastava R, Miller JF: **Virulence factor secretion and translocation by Bordetella species.** *Current opinion in microbiology* 2009, **12(1)**:88-93.
11. Natale P, Bruser T, Driessen AJ: **Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane–distinct translocases and mechanisms.** *Biochimica et biophysica acta* 2008, **1778(9)**:1735-1756.
12. Papanikou E, Karamanou S, Economou A: **Bacterial protein secretion through the translocase nanomachine.** *Nature reviews* 2007, **5(11)**:839-851.
13. Muller M: **Twin-arginine-specific protein export in Escherichia coli.** *Research in microbiology* 2005, **156(2)**:131-136.
14. Lee PA, Tullman-Ercek D, Georgiou G: **The bacterial twin-arginine translocation pathway.** *Annual review of microbiology* 2006, **60**:373-395.

15. Albers SV, Szabo Z, Driessen AJ: **Protein secretion in the Archaea: multiple paths towards a unique cell surface.** *Nature reviews* 2006, **4(7)**:537-547.
16. Desvaux M, Parham NJ, Scott-Tucker A, Henderson IR: **The general secretory pathway: a general misnomer?** *Trends in microbiology* 2004, **12(7)**:306-309.
17. Delepelaire P: **Type I secretion in gram-negative bacteria.** *Biochimica et biophysica acta* 2004, **1694(1-3)**:149-161.
18. Holland IB, Schmitt L, Young J: **Type 1 protein secretion in bacteria, the ABC-transporter dependent pathway (review).** *Molecular membrane biology* 2005, **22(1-2)**:29-39.
19. Galan JE, Wolf-Watz H: **Protein delivery into eukaryotic cells by type III secretion machines.** *Nature* 2006, **444(7119)**:567-573.
20. Ghosh P: **Process of protein transport by the type III secretion system.** *Microbiol Mol Biol Rev* 2004, **68(4)**:771-795.
21. Medini D, Covacci A, Donati C: **Protein homology network families reveal step-wise diversification of Type III and Type IV secretion systems.** *PLoS computational biology* 2006, **2(12)**:e173.
22. Pukatzki S, McAuley SB, Miyata ST: **The type VI secretion system: translocation of effectors and effector-domains.** *Current opinion in microbiology* 2009, **12(1)**:11-17.
23. Filloux A, Hachani A, Bleves S: **The bacterial type VI secretion machine: yet another player for protein transport across membranes.** *Microbiology (Reading, England)* 2008, **154(Pt 6)**:1570-1583.
24. Desvaux M, Hebraud M, Henderson IR, Pallen MJ: **Type III secretion: what's in a name?** *Trends in microbiology* 2006, **14(4)**:157-160.
25. Coulthurst SJ, Palmer T: **A new way out: protein localization on the bacterial cell surface via Tat and a novel Type II secretion system.** *Molecular microbiology* 2008, **69(6)**:1331-1335.
26. Cianciotto NP: **Type II secretion: a protein secretion system for all seasons.** *Trends in microbiology* 2005, **13(12)**:581-588.
27. Mueller CA, Broz P, Cornelis GR: **The type III secretion system tip complex and translocon.** *Molecular microbiology* 2008, **68(5)**:1085-1095.
28. Henderson IR, Navarro-Garcia F, Desvaux M, Fernandez RC, Ala'Aldeen D: **Type V protein secretion pathway: the autotransporter story.** *Microbiol Mol Biol Rev* 2004, **68(4)**:692-744.
29. Desvaux M, Parham NJ, Henderson IR: **Type V protein secretion: simplicity gone awry?** *Current issues in molecular biology* 2004, **6(2)**:111-124.
30. Nuccio SP, Baumler AJ: **Evolution of the chaperone/usher assembly pathway: fimbrial classification goes Greek.** *Microbiol Mol Biol Rev* 2007, **71(4)**:551-575.
31. Sauer FG, Remaut H, Hultgren SJ, Waksman G: **Fiber assembly by the chaperone-usher pathway.** *Biochimica et biophysica acta* 2004, **1694(1-3)**:259-267.
32. Kostakioti M, Newman CL, Thanassi DG, Stathopoulos C: **Mechanisms of protein export across the bacterial outer membrane.** *Journal of bacteriology* 2005, **187(13)**:4306-4314.
33. Bitter W, Houben EN, Luirink J, Appelmelk BJ: **Type VII secretion in mycobacteria: classification in line with cell envelope structure.** *Trends in microbiology* 2009, **17(8)**:337-338.
34. Desvaux M, Khan A, Scott-Tucker A, Chaudhuri RR, Pallen MJ, Henderson IR: **Genomic analysis of the protein secretion systems in Clostridium acetobutylicum ATCC 824.** *Biochimica et biophysica acta* 2005, **1745(2)**:223-253.
35. Peabody CR, Chung YJ, Yen MR, Vidal-Ingigliardi D, Pugsley AP, Saier MH Jr: **Type II protein secretion and its relationship to bacterial type IV pili and archaeal flagella.** *Microbiology (Reading, England)* 2003, **149(Pt 11)**:3051-3072.
36. Aldridge P, Hughes KT: **How and when are substrates selected for type III secretion?** *Trends in microbiology* 2001, **9(5)**:209-214.
37. Pallen MJ: **The ESAT-6/WXG100 superfamily – and a new Gram-positive secretion system?** *Trends in microbiology* 2002, **10(5)**:209-212.
38. Desvaux M, Hebraud M, Talon R, Henderson IR: **Outer membrane translocation: numerical protein secretion nomenclature in question in mycobacteria.** *Trends in microbiology* 2009, **17(8)**:338-340.
39. von Heijne G: **Patterns of amino acids near signal-sequence cleavage sites.** *European journal of biochemistry/FEBS* 1983, **133(1)**:17-21.
40. von Heijne G: **A new method for predicting signal sequence cleavage sites.** *Nucleic acids research* 1986, **14(11)**:4683-4690.
41. McGeoch DJ: **On the predictive recognition of signal peptide sequences.** *Virus research* 1985, **3(3)**:271-286.
42. Ladunga I, Czako F, Csabai I, Geszti T: **Improving signal peptide prediction accuracy by simulated neural network.** *Comput Appl Biosci* 1991, **7(4)**:485-487.
43. Schneider G, Rohlk S, Wrede P: **Analysis of cleavage-site patterns in protein precursor sequences with a perceptron-type neural network.** *Biochemical and biophysical research communications* 1993, **194(2)**:951-959.
44. Plewczynski D, Slabinski L, Ginalski K, Rychlewski L: **Prediction of signal peptides in protein sequences by neural networks.** *Acta biochimica Polonica* 2008, **55(2)**:261-267.
45. Nielsen H, Krogh A: **Prediction of signal peptides and signal anchors by a hidden Markov model.** *Proceedings/International Conference on Intelligent Systems for Molecular Biology; ISMB* 1998, **6**:122-130.
46. Bendtsen JD, Nielsen H, von Heijne G, Brunak S: **Improved prediction of signal peptides: SignalP 3.0.** *Journal of molecular biology* 2004, **340(4)**:783-795.
47. Nielsen H, Engelbrecht J, Brunak S, von Heijne G: **Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites.** *Protein Eng* 1997, **10(1)**:1-6.
48. Kall L, Krogh A, Sonnhammer EL: **A combined transmembrane topology and signal peptide prediction method.** *J Mol Biol* 2004, **338(5)**:1027-1036.
49. Kall L, Krogh A, Sonnhammer EL: **Advantages of combined transmembrane topology and signal peptide prediction–the Phobius web server.** *Nucleic Acids Res* 2007, **35 Web Server:** W429-432.
50. Zhang Z, Henzel WJ: **Signal peptide prediction based on analysis of experimentally verified cleavage sites.** *Protein Sci* 2004, **13(10)**:2819-2824.
51. Berks BC: **A common export pathway for proteins binding complex redox cofactors?** *Molecular microbiology* 1996, **22(3)**:393-404.
52. Rose RW, Bruser T, Kissinger JC, Pohlschroder M: **Adaptation of protein secretion to extremely high-salt conditions by extensive use of the twin-arginine translocation pathway.** *Molecular microbiology* 2002, **45(4)**:943-950.
53. Bendtsen JD, Nielsen H, Widdick D, Palmer T, Brunak S: **Prediction of twin-arginine signal peptides.** *BMC Bioinformatics* 2005, **6**:167.
54. von Heijne G: **The structure of signal peptides from bacterial lipoproteins.** *Protein engineering* 1989, **2(7)**:531-534.
55. Sankaran K, Gan K, Rash B, Qi HY, Wu HC, Rick PD: **Roles of histidine-103 and tyrosine-235 in the function of the prolipoprotein diacylglyceryl transferase of Escherichia coli.** *Journal of bacteriology* 1997, **179(9)**:2944-2948.
56. Berven FS, Karlsen OA, Straume AH, Flikka K, Murrell JC, Fjellbirkeland A, Lillehaug JR, Eidhammer I, Jensen HB: **Analysing the outer membrane subproteome of Methylococcus capsulatus (Bath) using proteomics and novel biocomputing tools.** *Archives of microbiology* 2006, **184(6)**:362-377.
57. Babu MM, Priya ML, Selvan AT, Madera M, Gough J, Aravind L, Sankaran K: **A database of bacterial lipoproteins (DOLOP) with functional assignments to predicted lipoproteins.** *Journal of bacteriology* 2006, **188(8)**:2761-2773.
58. Bagos PG, Tsirigos KD, Liakopoulos TD, Hamodrakas SJ: **Prediction of lipoprotein signal peptides in Gram-positive bacteria with a Hidden Markov Model.** *J Proteome Res* 2008, **7(12)**:5082-5093.
59. Juncker AS, Willenbrock H, Von Heijne G, Brunak S, Nielsen H, Krogh A: **Prediction of lipoprotein signal peptides in Gram-negative bacteria.** *Protein Sci* 2003, **12(8)**:1652-1662.
60. Klein P, Kanehisa M, DeLisi C: **The detection and classification of membrane-spanning proteins.** *Biochimica et biophysica acta* 1985, **815(3)**:468-476.
61. Claros MG, von Heijne G: **TopPred II: an improved software for membrane protein structure predictions.** *Comput Appl Biosci* 1994, **10(6)**:685-686.
62. Hirokawa T, Boon-Chieng S, Mitaku S: **SOSUI: classification and secondary structure prediction system for membrane proteins.** *Bioinformatics (Oxford, England)* 1998, **14(4)**:378-379.
63. Jayasinghe S, Hristova K, White SH: **Energetics, stability, and prediction of transmembrane helices.** *Journal of molecular biology* 2001, **312(5)**:927-934.
64. Ganapathiraju M, Jursa CJ, Karimi HA, Klein-Seetharaman J: **TMpro web server and web service: transmembrane helix prediction through amino acid property analysis.** *Bioinformatics* 2007, **23(20)**:2795-2796.
65. Deber CM, Wang C, Liu LP, Prior AS, Agrawal S, Muskat BL, Cuticchia AJ: **TM Finder: a prediction program for transmembrane protein segments using a combination of hydrophobicity and nonpolar phase helicity scales.** *Protein Sci* 2001, **10(1)**:212-219.

66. Jones DT, Taylor WR, Thornton JM: **A model recognition approach to the prediction of all-helical membrane protein structure and topology.** *Biochemistry* 1994, **33(10)**:3038-3049.
67. Persson B, Argos P: **Prediction of membrane protein topology utilizing multiple sequence alignments.** *Journal of protein chemistry* 1997, **16(5)**:453-457.
68. Rost B, Fariselli P, Casadio R: **Topology prediction for helical transmembrane proteins at 86% accuracy.** *Protein Sci* 1996, **5(8)**:1704-1718.
69. Aloy P, Cedano J, Oliva B, Aviles FX, Querol E: **'TransMem': a neural network implemented in Excel spreadsheets for predicting transmembrane domains of proteins.** *Comput Appl Biosci* 1997, **13(3)**:231-234.
70. Krogh A, Larsson B, von Heijne G, Sonnhammer EL: **Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes.** *Journal of molecular biology* 2001, **305(3)**:567-580.
71. Tusnady GE, Simon I: **The HMMTOP transmembrane topology prediction server.** *Bioinformatics* 2001, **17(9)**:849-850.
72. Viklund H, Elofsson A: **Best alpha-helical transmembrane protein topology predictions are achieved using hidden Markov models and evolutionary information.** *Protein Sci* 2004, **13(7)**:1908-1917.
73. Yuan Z, Mattick JS, Teasdale RD: **SVMtm: support vector machines to predict transmembrane segments.** *Journal of computational chemistry* 2004, **25(5)**:632-636.
74. Garrow AG, Agnew A, Westhead DR: **TMB-Hunt: an amino acid composition based method to screen proteomes for beta-barrel transmembrane proteins.** *BMC bioinformatics* 2005, **6**:56.
75. Garrow AG, Westhead DR: **A consensus algorithm to screen genomes for novel families of transmembrane beta barrel proteins.** *Proteins* 2007, **69(1)**:8-18.
76. Bagos PG, Liakopoulos TD, Hamodrakas SJ: **Evaluation of methods for predicting the topology of beta-barrel outer membrane proteins and a consensus prediction method.** *BMC bioinformatics* 2005, **6**:7.
77. Martelli PL, Fariselli P, Krogh A, Casadio R: **A sequence-profile-based HMM for predicting and discriminating beta barrel membrane proteins.** *Bioinformatics (Oxford, England)* 2002, **18(Suppl 1)**:S46-53.
78. Bigelow HR, Petrey DS, Liu J, Przybylski D, Rost B: **Predicting transmembrane beta-barrels in proteomes.** *Nucleic acids research* 2004, **32(8)**:2566-2577.
79. Randall A, Cheng J, Sweredoski M, Baldi P: **TMBpro: secondary structure, beta-contact and tertiary structure prediction of transmembrane beta-barrel proteins.** *Bioinformatics (Oxford, England)* 2008, **24(4)**:513-520.
80. Bigelow H, Rost B: **PROFtmb: a web server for predicting bacterial transmembrane beta barrel proteins.** *Nucleic acids research* 2006, , **34 Web Server:** W186-188.
81. Hu J, Yan C: **A method for discovering transmembrane beta-barrel proteins in Gram-negative bacterial proteomes.** *Computational biology and chemistry* 2008, **32(4)**:298-301.
82. Waldispuhl J, Berger B, Clote P, Steyaert JM: **transFold: a web server for predicting the structure and residue contacts of transmembrane beta-barrels.** *Nucleic acids research* 2006, , **34 Web Server:** W189-193.
83. Zhai Y, Saier MH Jr: **The beta-barrel finder (BBF) program, allowing identification of outer membrane beta-barrel proteins encoded within prokaryotic genomes.** *Protein Sci* 2002, **11(9)**:2196-2207.
84. Berven FS, Flikka K, Jensen HB, Eidhammer I: **BOMP: a program to predict integral beta-barrel outer membrane proteins encoded within genomes of Gram-negative bacteria.** *Nucleic Acids Res* 2004, , **32 Web Server:** W394-399.
85. Bagos PG, Liakopoulos TD, Spyropoulos IC, Hamodrakas SJ: **PRED-TMBB: a web server for predicting the topology of beta-barrel outer membrane proteins.** *Nucleic Acids Res* 2004, , **32 Web Server:** W400-404.
86. Park KJ, Gromiha MM, Horton P, Suwa M: **Discrimination of outer membrane proteins using support vector machines.** *Bioinformatics* 2005, **21(23)**:4223-4229.
87. Ou YY, Gromiha MM, Chen SA, Suwa M: **TMBETADISC-RBF: Discrimination of beta-barrel membrane proteins using RBF networks and PSSM profiles.** *Computational biology and chemistry* 2008, **32(3)**:227-231.
88. Billion A, Ghai R, Chakraborty T, Hain T: **Augur–a computational pipeline for whole genome microbial surface protein prediction and classification.** *Bioinformatics* 2006, **22(22)**:2819-2820.
89. Zhou M, Boekhorst J, Francke C, Siezen RJ: **LocateP: genome-scale subcellular-location predictor for bacterial proteins.** *BMC bioinformatics* 2008, **9**:173.
90. Choo KH, Tan TW, Ranganathan S: **SPdb–a signal peptide database.** *BMC bioinformatics* 2005, **6**:249.
91. Rey S, Acab M, Gardy JL, Laird MR, deFays K, Lambert C, Brinkman FS: **PSORTdb: a protein subcellular localization database for bacteria.** *Nucleic Acids Res* 2005, , **33 Database:** D164-168.
92. Park S, Yang JS, Jang SK, Kim S: **Construction of Functional Interaction Networks through Consensus Localization Predictions of the Human Proteome.** *J Proteome Res* 2009, **8(7)**:3367-3376.
93. Restrepo-Montoya D, Vizcaino C, Nino LF, Ocampo M, Patarroyo ME, Patarroyo MA: **Validating subcellular localization prediction tools with mycobacterial proteins.** *BMC Bioinformatics* 2009, **10**:134.
94. Shen YQ, Burger G: **'Unite and conquer': enhanced prediction of protein subcellular localization by integrating multiple specialized tools.** *BMC Bioinformatics* 2007, **8**:420.
95. Gupta RS: **The natural evolutionary relationships among prokaryotes.** *Critical reviews in microbiology* 2000, **26(2)**:111-131.
96. Rachel R, Wyschkony I, Riehl S, Huber H: **The ultrastructure of Ignicoccus: evidence for a novel outer membrane and for intracellular vesicle budding in an archaeon.** *Archaea (Vancouver, BC)* 2002, **1(1)**:9-18.
97. Rudd KE: **EcoGene: a genome sequence database for Escherichia coli K-12.** *Nucleic Acids Res* 2000, **28(1)**:60-64.
98. Itoh T, Okayama T, Hashimoto H, Takeda J, Davis RW, Mori H, Gojobori T: **A low rate of nucleotide changes in Escherichia coli K-12 estimated from a comparison of the genome sequences between two different substrains.** *FEBS letters* 1999, **450(1-2)**:72-76.
99. Durfee T, Nelson R, Baldwin S, Plunkett G, Burland V, Mau B, Petrosino JF, Qin X, Muzny DM, Ayele M, *et al*: **The complete genome sequence of Escherichia coli DH10B: insights into the biology of a laboratory workhorse.** *J Bacteriol* 2008, **190(7)**:2597-2606.
100. Peterson KM, Mekalanos JJ: **Characterization of the Vibrio cholerae ToxR regulon: identification of novel genes involved in intestinal colonization.** *Infection and immunity* 1988, **56(11)**:2822-2829.
101. Miyadai H, Tanaka-Masuda K, Matsuyama S, Tokuda H: **Effects of lipoprotein overproduction on the induction of DegP (HtrA) involved in quality control in the Escherichia coli periplasm.** *The Journal of biological chemistry* 2004, **279(38)**:39807-39813.
102. Thybert D, Avner S, Lucchetti-Miganeh C, Cheron A, Barloy-Hubler F: **OxyGene: an innovative platform for investigating oxidative-response genes in whole prokaryotic genomes.** *BMC genomics* 2008, **9**:637.
103. Braunstein M, Espinosa BJ, Chan J, Belisle JT, Jacobs WR Jr: **SecA2 functions in the secretion of superoxide dismutase A and in the virulence of Mycobacterium tuberculosis.** *Molecular microbiology* 2003, **48(2)**:453-464.
104. Goder V, Spiess M: **Topogenesis of membrane proteins: determinants and dynamics.** *FEBS letters* 2001, **504(3)**:87-93.
105. Martoglio B, Dobberstein B: **Signal sequences: more than just greasy peptides.** *Trends in cell biology* 1998, **8(10)**:410-415.
106. Bingle LE, Bailey CM, Pallen MJ: **Type VI secretion: a beginner's guide.** *Current opinion in microbiology* 2008, **11(1)**:3-8.
107. Anderson DM, Schneewind O: **A mRNA signal for the type III secretion of Yop proteins by Yersinia enterocolitica.** *Science (New York, NY)* 1997, **278(5340)**:1140-1143.
108. Anderson DM, Schneewind O: **Yersinia enterocolitica type III secretion: an mRNA signal that couples translation and secretion of YopQ.** *Molecular microbiology* 1999, **31(4)**:1139-1148.
109. Michiels T, Wattiau P, Brasseur R, Ruysschaert JM, Cornelis G: **Secretion of Yop proteins by Yersiniae.** *Infection and immunity* 1990, **58(9)**:2840-2849.
110. Lower M, Schneider G: **Prediction of type III secretion signals in genomes of gram-negative bacteria.** *PLoS One* 2009, **4(6)**:e5917.
111. Arnold R, Brandmaier S, Kleine F, Tischler P, Heinz E, Behrens S, Niinikoski A, Mewes HW, Horn M, Rattei T: **Sequence-based prediction of type III secreted proteins.** *PLoS pathogens* 2009, **5(4)**:e1000376.
112. Hiller K, Grote A, Scheer M, Munch R, Jahn D: **PrediSi: prediction of signal peptides and their cleavage positions.** *Nucleic Acids Res* 2004, , **32 Web Server:** W375-379.
113. Gomi M, Sonoyama M, Mitaku S: **High performance system for signal peptide prediction: SOSUIsignal.** *Chem-Bio Informatics Journal* 2004, **4(4)**:142-147.

114. Mitaku S, Hirokawa T, Tsuji T: **Amphiphilicity index of polar amino acids as an aid in the characterization of amino acid preference at membrane-water interfaces.** *Bioinformatics* 2002, **18(4)**:608-616.
115. Juretic D, Zoranic L, Zucic D: **Basic charge clusters and predictions of membrane protein topology.** *J Chem Inf Comput Sci* 2002, **42(3)**:620-632.
116. Bagos PG, Liakopoulos TD, Hamodrakas SJ: **Finding beta-barrel outer membrane proteins with a Markov Chain Model.** *WSEAS Transactions on Biology and Biomedecine* 2004, **1(2)**:186-189.
117. Gromiha MM, Ahmad S, Suwa M: **TMBETA-NET: discrimination and prediction of membrane spanning beta-strands in outer membrane proteins.** *Nucleic Acids Res* 2005, , **33 Web Server:** W164-167.
118. Garrow AG, Agnew A, Westhead DR: **TMB-Hunt: a web server to screen sequence sets for transmembrane beta-barrel proteins.** *Nucleic Acids Res* 2005, , **33 Web Server:** W188-192.
119. Lu Z, Szafron D, Greiner R, Lu P, Wishart DS, Poulin B, Anvik J, Macdonell C, Eisner R: **Predicting subcellular localization of proteins using machine-learned classifiers.** *Bioinformatics* 2004, **20(4)**:547-556.
120. Matsuda S, Vert JP, Saigo H, Ueda N, Toh H, Akutsu T: **A novel representation of protein sequences for prediction of subcellular location using support vector machines.** *Protein Sci* 2005, **14(11)**:2804-2813.
121. Hua S, Sun Z: **Support vector machine approach for protein subcellular localization prediction.** *Bioinformatics* 2001, **17(8)**:721-728.
122. Niu B, Jin YH, Feng KY, Lu WC, Cai YD, Li GZ: **Using AdaBoost for the prediction of subcellular location of prokaryotic and eukaryotic proteins.** *Molecular diversity* 2008, **12(1)**:41-45.
123. Imai K, Asakawa N, Tsuji T, Akazawa F, Ino A, Sonoyama M, Mitaku S: **SOSUI-GramN: high performance prediction for sub-cellular localization of proteins in Gram-negative bacteria.** *Bioinformation* 2008, **2(9)**:417-421.
124. Horler RS, Butcher A, Papangelopoulos N, Ashton PD, Thomas GH: **EchoLOCATION: an in silico analysis of the subcellular locations of Escherichia coli proteins and comparison with experimentally derived locations.** *Bioinformatics* 2009, **25(2)**:163-166.
125. Fernando SA, Selvarani P, Das S, Kumar Ch K, Mondal S, Ramakumar S, Sekar K: **THGS: a web-based database of Transmembrane Helices in Genome Sequences.** *Nucleic Acids Res* 2004, , **32 Database:** D125-128.
126. Litou ZI, Bagos PG, Tsirigos KD, Liakopoulos TD, Hamodrakas SJ: **Prediction of cell wall sorting signals in gram-positive bacteria with a hidden markov model: application to complete genomes.** *Journal of bioinformatics and computational biology* 2008, **6(2)**:387-401.
127. Remmert M, Linke D, Lupas AN, Soding J: **HHomp–prediction and classification of outer membrane proteins.** *Nucleic Acids Res* 2009, , **37 Web Server:** W446-451.
128. Saleh MT, Fillon M, Brennan PJ, Belisle JT: **Identification of putative exported/secreted proteins in prokaryotic proteomes.** *Gene* 2001, **269(1-2)**:195-204.
129. Bagos PG, Tsirigos KD, Plessas SK, Liakopoulos TD, Hamodrakas SJ: **Prediction of signal peptides in archaea.** *Protein Eng Des Sel* 2009, **22(1)**:27-35.
130. Ikeda M, Arai M, Okuno T, Shimizu T: **TMPDB: a database of experimentally-characterized transmembrane topologies.** *Nucleic Acids Res* 2003, **31(1)**:406-409.
131. Tusnady GE, Kalmar L, Simon I: **TOPDB: topology data bank of transmembrane proteins.** *Nucleic Acids Res* 2008, , **36 Database:** D234-239.
132. Menne KM, Hermjakob H, Apweiler R: **A comparison of signal sequence prediction methods using a test set of signal peptides.** *Bioinformatics* 2000, **16(8)**:741-742.
133. Taylor PD, Toseland CP, Attwood TK, Flower DR: **LIPPRED: A web server for accurate prediction of lipoprotein signal sequences and cleavage sites.** *Bioinformation* 2006, **1(5)**:176-179.
134. Fariselli P, Finocchiaro G, Casadio R: **SPEPlip: the detection of signal peptide and lipoprotein cleavage sites.** *Bioinformatics* 2003, **19(18)**:2498-2499.
135. Bendtsen JD, Kiemer L, Fausboll A, Brunak S: **Non-classical protein secretion in bacteria.** *BMC Microbiol* 2005, **5**:58.
136. Shen HB, Chou KC: **Signal-3L: A 3-layer approach for predicting signal peptides.** *Biochem Biophys Res Commun* 2007, **363(2)**:297-303.
137. Chou KC, Shen HB: **Signal-CF: a subsite-coupled and window-fusing approach for predicting signal peptides.** *Biochem Biophys Res Commun* 2007, **357(3)**:633-640.
138. Frank K, Sippl MJ: **High-performance signal peptide prediction based on sequence alignment techniques.** *Bioinformatics* 2008, **24(19)**:2172-2176.
139. Szabo Z, Stahl AO, Albers SV, Kissinger JC, Driessen AJ, Pohlschroder M: **Identification of diverse archaeal proteins with class III signal peptides cleaved by distinct archaeal prepilin peptidases.** *J Bacteriol* 2007, **189(3)**:772-778.
140. Hiss JA, Resch E, Schreiner A, Meissner M, Starzinski-Powitz A, Schneider G: **Domain organization of long signal peptides of single-pass integral membrane proteins reveals multiple functional capacity.** *PLoS One* 2008, **3(7)**:e2767.
141. Reynolds SM, Kall L, Riffle ME, Bilmes JA, Noble WS: **Transmembrane topology and signal peptide prediction using dynamic bayesian networks.** *PLoS Comput Biol* 2008, **4(11)**:e1000213.
142. Viklund H, Elofsson A: **OCTOPUS: improving topology prediction by two-track ANN-based preference scores and an extended topological grammar.** *Bioinformatics* 2008, **24(15)**:1662-1668.
143. Viklund H, Bernsel A, Skwark M, Elofsson A: **SPOCTOPUS: a combined predictor of signal peptides and membrane protein topology.** *Bioinformatics* 2008, **24(24)**:2928-2929.
144. Shen H, Chou JJ: **MemBrain: improving the accuracy of predicting transmembrane helices.** *PLoS One* 2008, **3(6)**:e2399.
145. Cserzo M, Wallin E, Simon I, von Heijne G, Elofsson A: **Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method.** *Protein Eng* 1997, **10(6)**:673-676.
146. Bagos PG, Liakopoulos TD, Hamodrakas SJ: **Algorithms for incorporating prior topological information in HMMs: application to transmembrane proteins.** *BMC Bioinformatics* 2006, **7**:189.
147. Lo A, Chiu HS, Sung TY, Lyu PC, Hsu WL: **Enhanced membrane protein topology prediction using a hierarchical classification method and a new scoring function.** *J Proteome Res* 2008, **7(2)**:487-496.
148. Zhou H, Zhou Y: **Predicting the topology of transmembrane helical proteins using mean burial propensity and a hidden-Markov-model-based method.** *Protein Sci* 2003, **12(7)**:1547-1555.
149. Pashou EE, Litou ZI, Liakopoulos TD, Hamodrakas SJ: **waveTM: wavelet-based transmembrane segment prediction.** *Silico Biol* 2004, **4(2)**:127-131.
150. Pasquier C, Promponas VJ, Palaios GA, Hamodrakas JS, Hamodrakas SJ: **A novel method for predicting transmembrane segments in proteins based on a statistical analysis of the SwissProt database: the PRED-TMR algorithm.** *Protein Eng* 1999, **12(5)**:381-385.
151. Peris P, Lopez D, Campos M: **IgTM: an algorithm to predict transmembrane domains and topology in proteins.** *BMC Bioinformatics* 2008, **9**:367.
152. Bernsel A, Viklund H, Hennerdal A, Elofsson A: **TOPCONS: consensus prediction of membrane protein topology.** *Nucleic Acids Res* 2009, , **37 Web Server:** W465-468.
153. Zhou H, Zhang C, Liu S, Zhou Y: **Web-based toolkits for topology prediction of transmembrane helical proteins, fold recognition, structure and binding scoring, folding-kinetics analysis and comparative analysis of domain combinations.** *Nucleic Acids Res* 2005, , **33 Web Server:** W193-197.
154. Arai M, Mitsuke H, Ikeda M, Xia JX, Kikuchi T, Satake M, Shimizu T: **ConPred II: a consensus prediction method for obtaining transmembrane topology models with high reliability.** *Nucleic Acids Res* 2004, , **32 Web Server:** W390-393.
155. Jones DT: **Improving the accuracy of transmembrane protein topology prediction using evolutionary information.** *Bioinformatics* 2007, **23(5)**:538-544.
156. Adamczak R, Porollo A, Meller J: **Combining prediction of secondary structure and solvent accessibility in proteins.** *Proteins* 2005, **59(3)**:467-475.
157. Ganapathiraju M, Balakrishnan N, Reddy R, Klein-Seetharaman J: **Transmembrane helix prediction using amino acid property features and latent semantic analysis.** *BMC Bioinformatics* 2008, **9(Suppl 1)**:S4.
158. Jones DT: **Protein secondary structure prediction based on position-specific scoring matrices.** *J Mol Biol* 1999, **292(2)**:195-202.
159. Bryson K, McGuffin LJ, Marsden RL, Ward JJ, Sodhi JS, Jones DT: **Protein structure prediction servers at University College London.** *Nucleic Acids Res* 2005, , **33 Web Server:** W36-38.
160. Combet C, Blanchet C, Geourjon C, Deleage G: **NPS@: network protein sequence analysis.** *Trends Biochem Sci* 2000, **25(3)**:147-150.

161. Karplus K: **SAM-T08, HMM-based protein structure prediction.** *Nucleic Acids Res* 2009, , **37 Web Server:** W492-497.
162. Pollastri G, McLysaght A: **Porter: a new, accurate server for protein secondary structure prediction.** *Bioinformatics* 2005, **21(8)**:1719-1720.
163. Kahsay RY, Gao G, Liao L: **An improved hidden Markov model for transmembrane protein detection and topology prediction and its applications to complete genomes.** *Bioinformatics* 2005, **21(9)**:1853-1858.
164. Lin K, Simossis VA, Taylor WR, Heringa J: **A simple and fast secondary structure prediction method using hidden neural networks.** *Bioinformatics* 2005, **21(2)**:152-159.
165. Chou KC, Shen HB: **MemType-2L: a web server for predicting membrane proteins and their types by incorporating evolution information through Pse-PSSM.** *Biochem Biophys Res Commun* 2007, **360(2)**:339-345.
166. Yu CS, Chen YC, Lu CH, Hwang JK: **Prediction of protein subcellular localization.** *Proteins* 2006, **64(3)**:643-651.
167. Su EC, Chiu HS, Lo A, Hwang JK, Sung TY, Hsu WL: **Protein subcellular localization prediction based on compartment-specific features and structure conservation.** *BMC Bioinformatics* 2007, **8**:330.
168. Bhasin M, Garg A, Raghava GP: **PSLpred: prediction of subcellular localization of bacterial proteins.** *Bioinformatics* 2005, **21(10)**:2522-2524.
169. Chou KC, Shen HB: **Large-scale predictions of gram-negative bacterial protein subcellular locations.** *J Proteome Res* 2006, **5(12)**:3420-3428.
170. Shen HB, Chou KC: **Gpos-PLoc: an ensemble classifier for predicting subcellular localization of Gram-positive bacterial proteins.** *Protein Eng Des Sel* 2007, **20(1)**:39-46.
171. Nair R, Rost B: **Mimicking cellular sorting improves prediction of subcellular localization.** *J Mol Biol* 2005, **348(1)**:85-100.
172. Jia P, Qian Z, Zeng Z, Cai Y, Li Y: **Prediction of subcellular protein localization based on functional domain composition.** *Biochem Biophys Res Commun* 2007, **357(2)**:366-370.
173. Rashid M, Saha S, Raghava GP: **Support Vector Machine-based method for predicting subcellular localization of mycobacterial proteins using evolutionary information and motifs.** *BMC Bioinformatics* 2007, **8**:337.
174. Setubal JC, Reis M, Matsunaga J, Haake DA: **Lipoprotein computational prediction in spirochaetal genomes.** *Microbiology* 2006, **152(Pt 1)**:113-121.
175. Montgomerie S, Cruz JA, Shrivastava S, Arndt D, Berjanskii M, Wishart DS: **PROTEUS2: a web server for comprehensive protein structure prediction and structure-based annotation.** *Nucleic Acids Res* 2008, , **36 Web Server:** W202-209.
176. Pasquier C, Hamodrakas SJ: **An hierarchical artificial neural network system for the classification of transmembrane proteins.** *Protein Eng* 1999, **12(8)**:631-634.
177. Taylor PD, Attwood TK, Flower DR: **BPROMPT: A consensus server for membrane protein prediction.** *Nucleic Acids Res* 2003, **31(13)**:3698-3700.
178. Liakopoulos TD, Pasquier C, Hamodrakas SJ: **A novel tool for the prediction of transmembrane protein topology based on a statistical analysis of the SwissProt database: the OrienTM algorithm.** *Protein Eng* 2001, **14(6)**:387-390.
179. Raghava GP: **APSSP2: A combination method for protein secondary structure prediction based on neural network and example based learning.** *CASP5* 2002, **A-132.**
180. Simossis VA, Heringa J: **PRALINE: a multiple sequence alignment toolbox that integrates homology-extended and secondary structure information.** *Nucleic Acids Res* 2005, , **33 Web Server:** W289-294.
181. Lomize MA, Lomize AL, Pogozheva ID, Mosberg HI: **OPM: orientations of proteins in membranes database.** *Bioinformatics* 2006, **22(5)**:623-625.
182. Jayasinghe S, Hristova K, White SH: **MPtopo: A database of membrane protein topology.** *Protein Sci* 2001, **10(2)**:455-458.
183. Tusnady GE, Dosztanyi Z, Simon I: **PDB_TM: selection and membrane localization of transmembrane proteins in the protein data bank.** *Nucleic Acids Res* 2005, , **33 Database:** D275-278.
184. Gromiha MM, Yabuki Y, Kundu S, Suharnan S, Suwa M: **TMBETA-GENOME: database for annotated beta-barrel membrane proteins in genomic sequences.** *Nucleic Acids Res* 2007, , **35 Database:** D314-316.
185. Rost B, Yachdav G, Liu J: **The PredictProtein server.** *Nucleic Acids Res* 2004, , **32 Web Server:** W321-326.
186. Yun H, Lee JW, Jeong J, Chung J, Park JM, Myoung HN, Lee SY: **EcoProDB: the Escherichia coli protein database.** *Bioinformatics* 2007, **23(18)**:2501-2503.
187. Nair R, Rost B: **LOCnet and LOCtarget: sub-cellular localization for structural genomics targets.** *Nucleic Acids Res* 2004, , **32 Web Server:** W517-521.
188. Zhang S, Xia X, Shen J, Zhou Y, Sun Z: **DBMLoc: a Database of proteins with multiple subcellular localizations.** *BMC Bioinformatics* 2008, **9**:127.

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