



PPS Manual

PTMs Peptide Scanner

Version 1.0

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The CUCKOO Workgroup

The latest version of PPS is available from <http://bioinformatics.lcd-ustc.org/pps>

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Statement

1. **Implementation.** The softwares of the CUCKOO Workgroup are implemented in JAVA (J2SE). Usually, both of online service and local stand-alone packages will be provided.
2. **Availability.** Our softwares are freely available for academic researches. For non-profit users, you can copy, distribute and use the softwares for your scientific studies. Our softwares are not free for commercial usage.
3. **GPS.** Previously, we used the GPS to denote our Group-based Phosphorylation Scoring algorithm. Currently, we are developing an integrated computational platform for post-translational modifications (PTMs) of proteins. We re-denote the GPS as Group-based Prediction Systems. This software is an indispensable part of GPS.
4. **Usage.** Our softwares are designed in an easy-to-use manner. Also, we invite you to read the manual before using the softwares.
5. **Updation.** Our softwares will be updated routinely based on users' suggestions and advices. Thus, your feedback is greatly important for our future updation. Please do not hesitate to contact with us if you have any concerns.
6. **Citation.** Usually, the latest published articles will be shown on the software websites. We wish you could cite the article if the software has been helpful for your work.
7. **Acknowledgements.** The work of CUCKOO Workgroup is supported by grants from Chinese 973 project (2006CBOF0503, and 2006CB933300), Chinese Academy of Sciences (KSCX1-YW-R65, KSCX2-YW-21, and KJCX2-YW-M02), and Chinese Natural Science Foundation (39925018, 30270293, 90508002, 30700138).

Introduction

Recently, an interesting and important question has emerged from feedbacks of experimentalists. Frequently, experimental researchers prefer to study novel PTM sites to elucidate new functions. Thus, it's important to show whether there have been any sites experimentally verified as real PTM sites in given proteins. And such information will be greatly useful for researchers to avoid reduplicate work. Although we and other bioinformaticists developed numerous tools for PTM sites prediction, these softwares could only regard prediction results as potentially real sites. Currently, several public databases, e.g., Phospho.ELM (Diella, et al., 2004; Diella, et al., 2008) and UniProt (Boutet, et al., 2007), have been developed to contain PTM information of proteins. However, these annotations are usually not integrated. Moreover, due to the diversity and heterogeneity of protein names, it's difficult to fully obtain the known PTM information for a given protein, by database searching.

In this work, we developed a novel software of PTMs Peptide Scanner (PPS), to reveal known or highly potential PTM sites in eukaryotic proteins. Five typical PTMs were considered, including phosphorylation, sumoylation, palmitoylation, methylation and acetylation. And the experimentally verified PTM sites were taken from Phospho.ELM 7.0 (Diella, et al., 2004; Diella, et al., 2008) and our previous studies (Chen, et al., 2006; Li, et al., 2006; Ren, et al., 2008; Xue, et al., 2006; Xue, et al., 2006; Zhou, et al., 2006), containing 18 179 known sites. Based on our previous hypothesis of similar peptides with potentially similar functions, we designed a straightforward approach of conserved peptide matching (CPM) algorithm. Given a protein sequence as input, PPS will compare it to the experimentally verified PTMs peptides to find the identical or highly conserved hits. The identical hits might be bona fide modified peptides in experimentally verified proteins or conserved in their highly similar homologs. Thus, PPS could be useful for annotation of covalent modifications information across eukaryotes. As an application, we computationally revealed 71 663 identical hits in six eukaryotic organisms, including *H. sapiens*, *M. musculus*, *D. melanogaster*, *C. elegans*, *S. pombe*, and *S. cerevisiae*. Obviously, most of these results could be highly potential PTM sites and greatly helpful for further experimental verification. Furthermore, highly conserved hits might also point to potentially conserved modifications. In our results, there were 7 911 highly potential PTM hits (with ≤ 3 conservative substitutions) found in the six eukaryotes. In addition, we carried out a proteome-wide study of creation or disruption of covalent modification sites by alternative splicing (AS) in *H. sapiens*. Taken together, we proposed PPS could be a multiple useful tool for PTM sites analyses. Finally, the online service and local packages of PPS 1.0 were implemented in JAVA 1.5.

PTMs

- Post-translational Modification
 - Phosphorylation
 - Methylation
 - Sumoylation
 - Palmitoylation
 - Acetylation

Matching Sites

Position	Code	Peptide	M.Sub	M.Position	M.Peptide	Comments
229	S	QRRSNPPSRKGSQFG	P08034	229	QRRSNPPSRKGSQFG	Phosphorylation: P...
233	S	NPPSRKGSQFGHRLS	P08033	233	NPPSRKGSQFGHRLS	Phosphorylation: P...
233	S	NPPSRKGSQFGHRLS	P08034	233	NPPSRKGSQFGHRLS	Phosphorylation: P...
258	S	LLSEQDGS LKDLIRR	P28230	258	LLSEQDGS LKDLIRR	Phosphorylation
266	S	LKDILRRSPGTGAGL	P28230	266	LKDILRRSPGTGAGL	Phosphorylation
280	C	LAEKSDRCSAC****	P28230	280	LAEKSDRCSAC****	Palmitoylation
283	C	KSDRCSAC*****	P28230	283	KSDRCSAC*****	Palmitoylation

Enter sequence(s) in FASTA format

>Example P08034(Human Connexin-32/Gap junction beta-1 protein)
 MNWVTGLYLLSGVNRHSTAIGRWLVSIFIRIMVLWAESWVGDEKSSFICNTLQPGCNVYDQFFPISHVRLWLSLQLILVSTPALL
 YAMHVAHQGHIEKMLRLEGHGDPLHLEEVKRHKVHISGTLWWTYVISWFRLLFEAVFMYVYLLYPGYAMVRLVKCDVYPCPNTVD
 CFVSRPTEKTVFTVFLAASGICILNVAEWYLIIRACARRAQRRSNPPSRKGSQFGHRLSPEYKQNEINKLSEQDGS LKDLIRRSPG
 TGAGLAEKSDRCSAC

Setting

Up Down Substitution Conserved

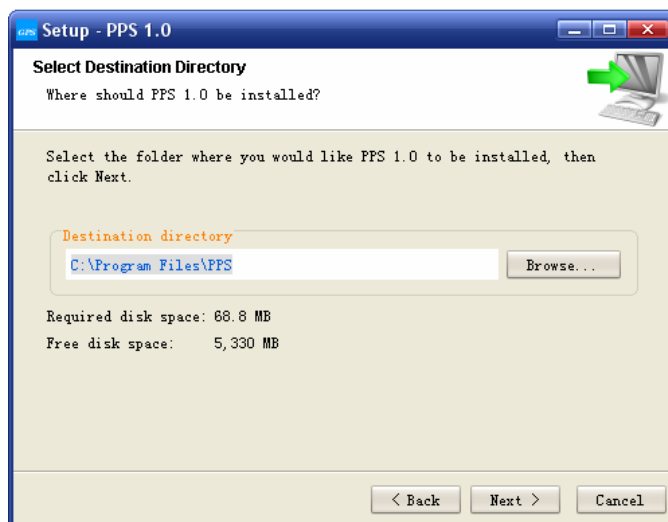
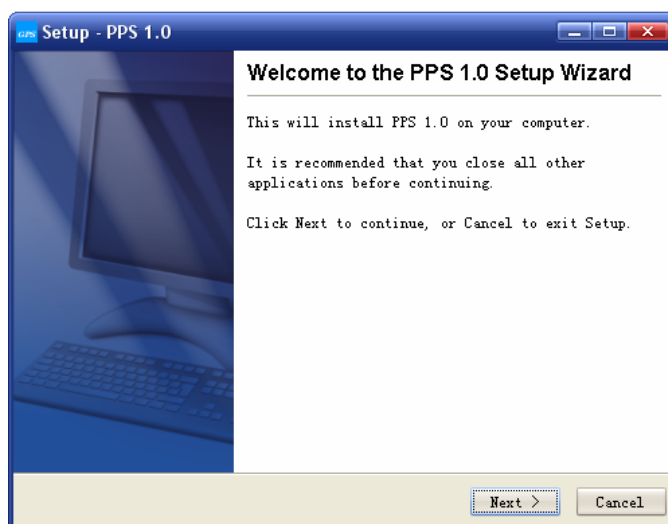
Console

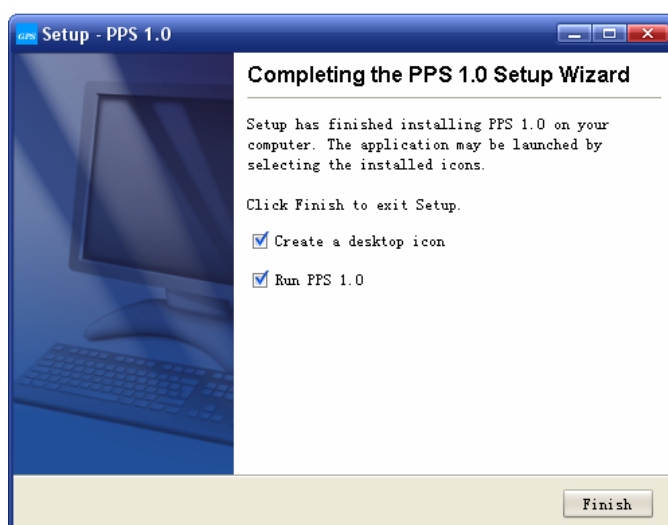
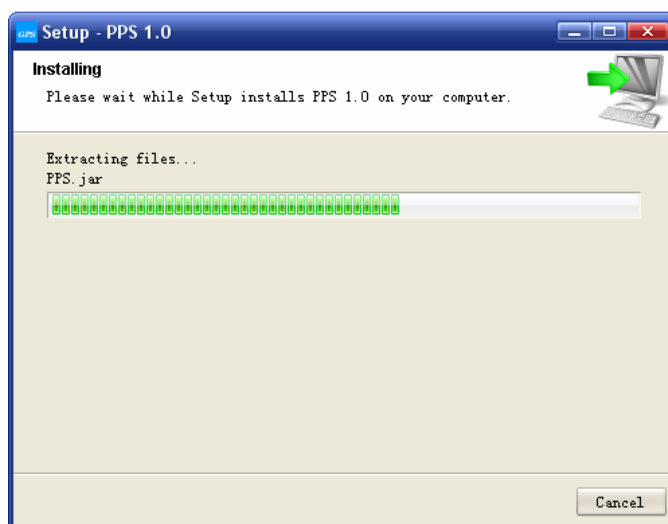
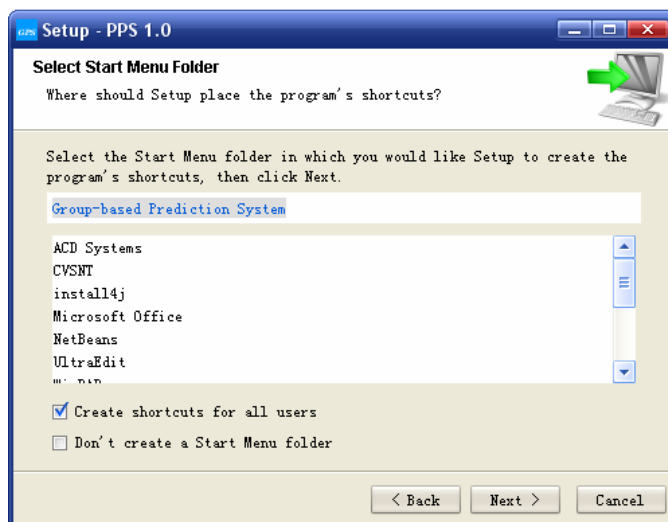
PPS 1.0 User Interface

Download & Installation

The PPS 1.0 was implemented in JAVA (J2SE), and could support three major Operating Systems (OS), including Windows, Linux/Unix or Mac OS X systems. Both of online web service and local stand-alone packages are available from: <http://bioinformatics.lcd-ustc.org/pps/down.php>. We recommend that users could download the latest release.

Please choose the proper package to download. After downloading, please double-click on the software package to begin installation, following the user prompts through the installation. And snapshots of the setup program for windows are shown below:





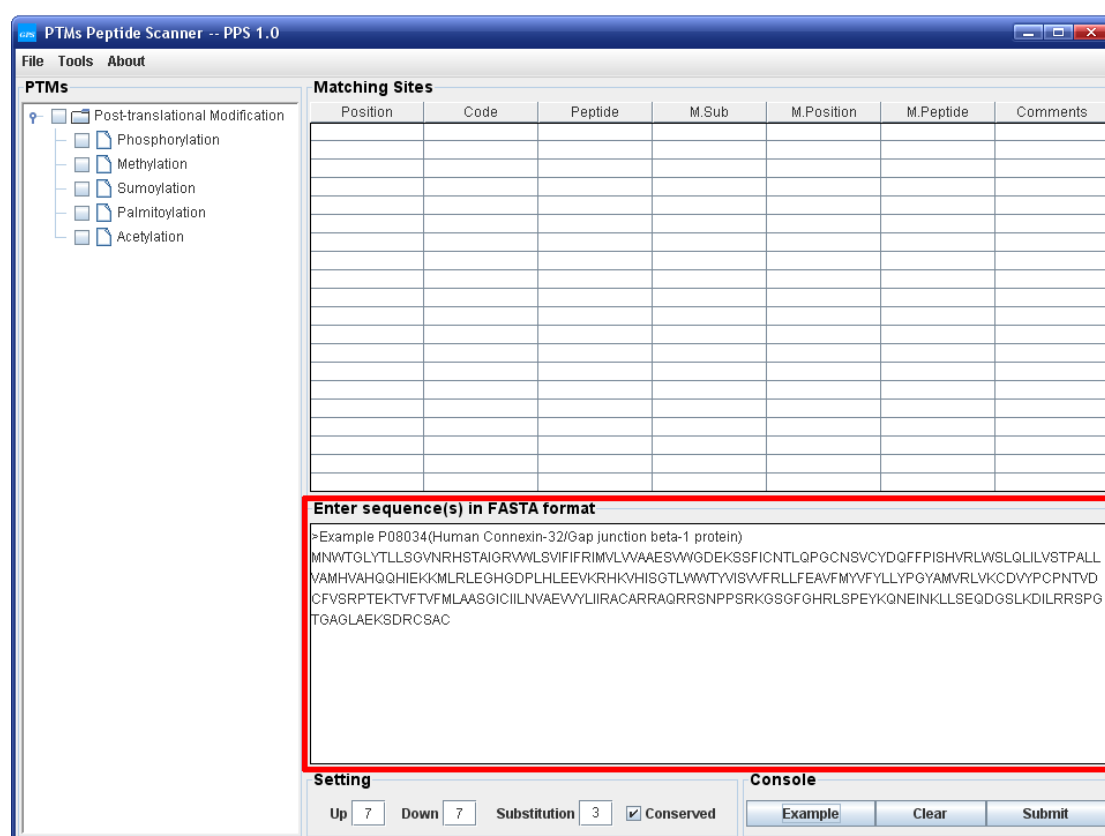
Finally, please click on the **Finish** button to complete the setup program.

Conserved peptide matching

1. A single protein sequence in FASTA format

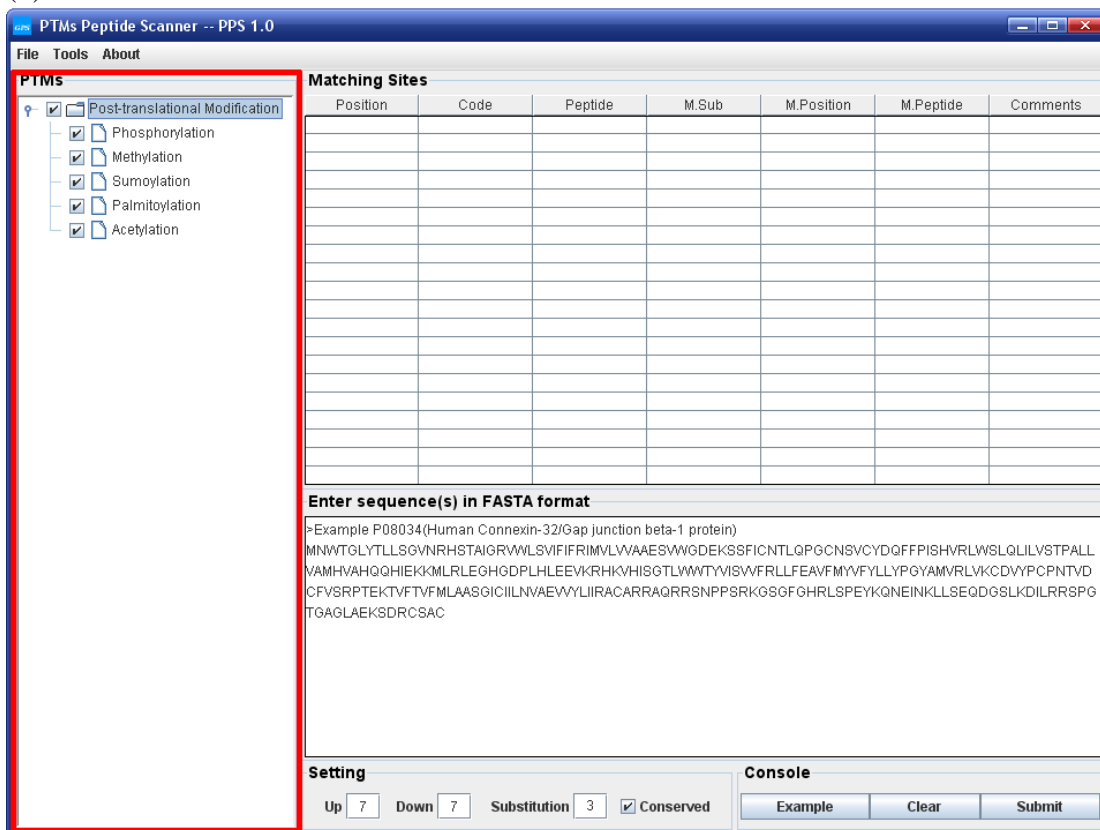
The following steps show you how to use the PPS 1.0 to match the conserved peptides for a single protein sequence in FASTA format.

(1) Firstly, please use “Ctrl+C & Ctrl+V” (Windows & Linux/Unix) or “Command+C & Command+V” (Mac) to copy and paste your sequence into the text form of PPS 1.0

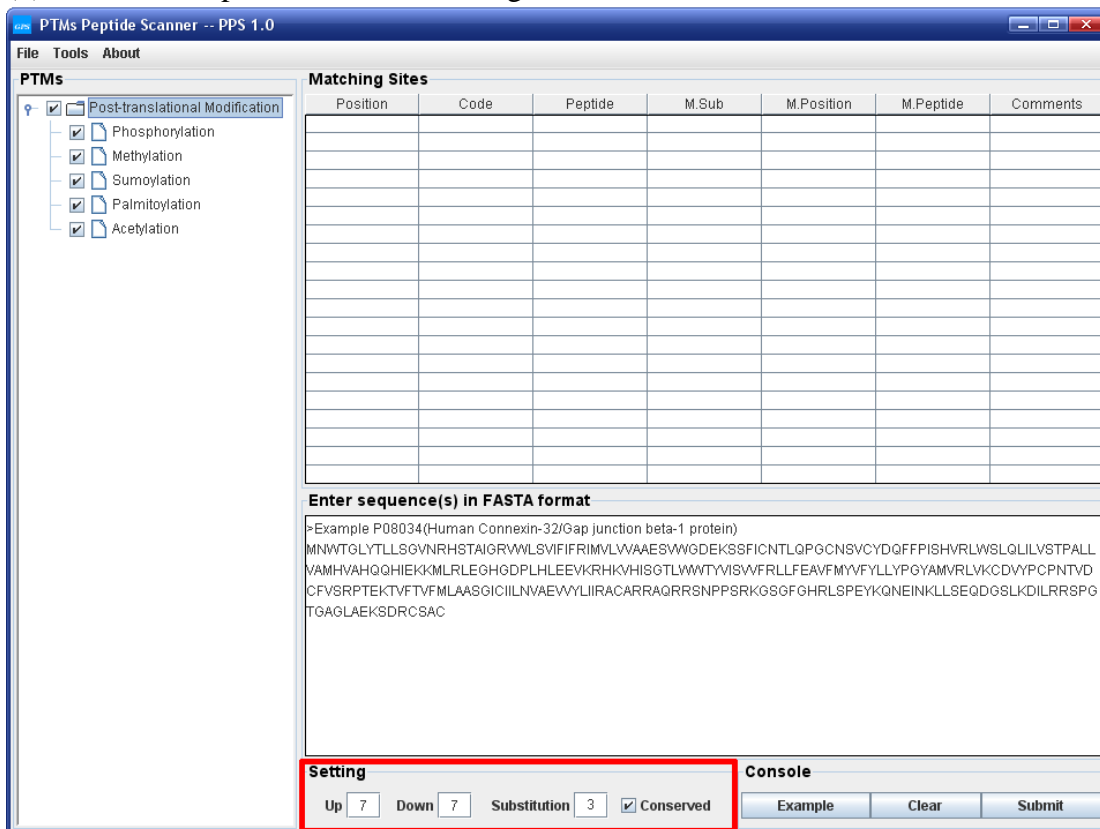


Note: for a single protein, the sequence without a name in raw format is also OK. However, for multiple sequences, the name of each protein should be presented.

(2) Choose one or more kinds of Post-translational Modification from **PTMs Tree**.



(3) Set the basic parameters of matching.



Here we defined a *potentially modified peptide* PMP(m, n) as a modified site flanked by m residues upstream and n residues downstream. Based on a previous article of residues clustering²⁶, we classified twenty common amino acids into five conservative groups, including the residues with large hydrophobic side chains (L, V, I, M), the hydrophobic aromatic residues (F, Y, W), the long-chain positively charged residues (K, R), the alcohol residues (S, T) and the charged/polar residues (E, D, N, Q). At the same position, two different amino acids of the same group will be regarded as a conserved substitution. The parameters of PMP(7, 7) with ≤ 3 conserved substitutions were chosen as default.

(4) Click on the **Submit** button, then the matched conserved sites will be shown.

Position	Code	Peptide	M.Sub	M.Position	M.Peptide	Comments
229	S	QRRSNPPSRKSGSFG	P08034	229	QRRSNPPSRKSGSFG	Phosphorylation: P...
233	S	NPPSRKSGFGHRLS	P08033	233	NPPSRKSGFGHRLS	Phosphorylation: P...
233	S	NPPSRKSGFGHRLS	P08034	233	NPPSRKSGFGHRLS	Phosphorylation: P...
258	S	LLSEQDGS LKDILRR	P28230	258	LLSEQDGS LKDILRR	Phosphorylation
266	S	LKDILRRSPGTGAGL	P28230	266	LKDILRRSPGTGAGL	Phosphorylation
280	C	LAEKSDRC SAC****	P28230	280	LAEKSDRC SAC****	Palmitoylation
283	C	KSDRC SAC*****	P28230	283	KSDRC SAC*****	Palmitoylation

Enter sequence(s) in FASTA format

>Example P08034(Human Connexin-32/Gap junction beta-1 protein)
 MNWTGLYLLSGVNRHSTAIQRVWLSVIFIRIMVLWAAESVWVGDEKSSFCNTLQPGCNVSVYDQFFPISHVRLWLSLQLILVSTPALL
 YAMHVAHQGHIEKMLRLEGHGDPHLLEEVRHKVHISGTLWWTYVISWFRLLFEAVFMVYFLLYPGYAMVRLVKCDVYPCPNTVD
 CFVSRPTEKTVFTVFM LAASGICILNVAEWYLIIRACARRAQRRSNPPSRKSGFGHRLSPEYKQNEINKLLSEQDGS LKDILRRSPG
 TGAGLAEKSDRC SAC

Setting
 Up Down Substitution Conserved

Console

The first three columns contain the site information of peptides in the input protein sequence. The last four columns show the information of matched sites which have been experimentally verified as real PTM sites.

If you want get more information of the matched proteins, you can click on the hyperlinks in the table. The hyperlinks will access the **UniProt** database and show you the detailed information.

Notice: This page will be replaced with www.uniprot.org. Please send us your feedback!

Search for

UniProtKB/Swiss-Prot entry [P08034](#)

[\[Entry info\]](#)
[\[Name and origin\]](#)
[\[References\]](#)
[\[Comments\]](#)
[\[Cross-references\]](#)
[\[Keywords\]](#)
[\[Features\]](#)
[\[Sequence\]](#)
[\[Tools\]](#)

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information	
Entry name	CXB1_HUMAN
Primary accession number	P08034
Secondary accession number	Q5U0S4
Integrated into Swiss-Prot on	August 1, 1988
Sequence was last modified on	August 1, 1988 (Sequence version 1)
Annotations were last modified on	November 25, 2008 (Entry version 105)
Name and origin of the protein	
Protein name	Gap junction beta-1 protein
Synonyms	Connexin-32 Cx32 GAP junction 28 kDa liver protein
Gene name	Name: GJB1 Synonyms: CX32
From	Homo sapiens (Human) [TaxID: 9606]
Taxonomy	Eukaryota, Metazoa, Chordata, Craniata, Vertebrata, Euteleostomi, Mammalia, Eutheria, Euarchontoglires, Primates, Haplorhini, Catarrhini, Homnidae, Homo.
Protein existence	1: Evidence at protein level;
References	
[1]	NUCLEOTIDE SEQUENCE [MRNA]. TISSUE=Liver ; DOI=10.1083/jcb.103.3.767; PubMed=2875078 [NCBI, ExpASY, EBI, Israel, Japan] Kumar N.M., Gilula N.B.; "Cloning and characterization of human and rat liver cDNAs coding for a gap junction protein."; J. Cell Biol. 103:767-776(1986)

(5) Then please click on the **RIGHT** button in the matching form. You can use the **“Select All”** and **“Copy Selected”** to copy the selected results into Clipboard. Then please copy the results into a file, e.g., an EXCEL file for further consideration. Also, you can choose **“Export Result”** to export the results into a tab-delimited text file.

PTMs Peptide Scanner -- PPS 1.0

File Tools About

PTMs

- Post-translational Modification
 - Phosphorylation
 - Methylation
 - Sumoylation
 - Palmitoylation
 - Acetylation

Matching Sites						
Position	Code	Peptide	M.Sub	M.Position	M.Peptide	Comments
229	S	QRRSNPPSRKGSFG	P08034	229	QRRSNPPSRKGSFG	Phosphorylation: P...
233	S	NPPSRKSGFGHRLS	P08033	233	NPPSRKSGFGHRLS	Phosphorylation: P...
233	S	NPPSRKSGFGHRLS	P08034		RKSGFGHRLS	Phosphorylation: P...
258	S	LLSEQDGLKDLIRR	P28230		QDGLKDLIRR	Phosphorylation
266	S	LKDILRRSPGTGAGL	P28230	266	LKDILRRSPGTGAGL	Phosphorylation
280	C	LAEKSDRCAC****	P28230	280	LAEKSDRCAC****	Palmitoylation
283	C	KSDRCAC*****	P28230	283	KSDRCAC*****	Palmitoylation

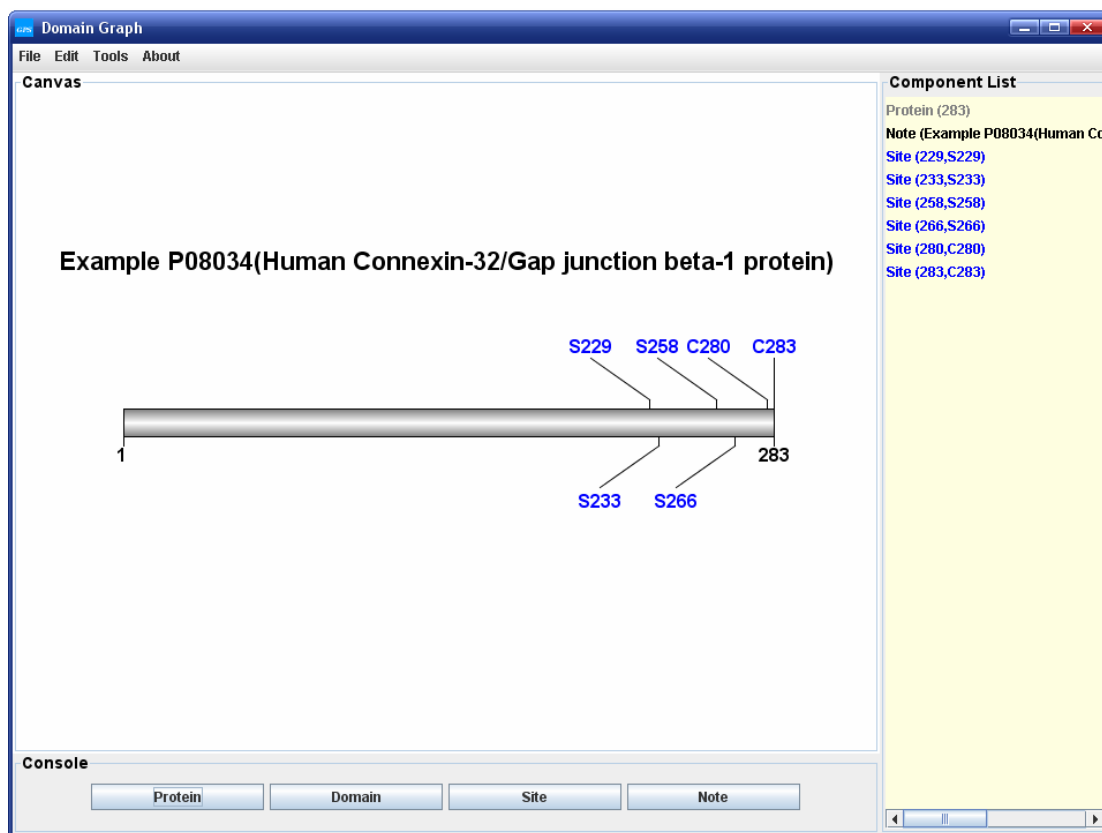
Enter sequence(s) in FASTA format

Example P08034(Human Connexin-32/Gap junction beta-1 protein)
 MNWVTGLYTLISGVNRHSTAIGRWLWLVVIFRIMVLVAAESWVGDEKSSFCNTLQPGCNSVCYDQFFPISHVRLWLSLQLLIVSTPALL
 VAMHVAHQGHIEKMLRLEGHGDLPLHEEVKRHKVHISGLTWWTYVISVFRLLFEAVFMYVYLLYPGYAMVRLVKCDVYPCPNTVD
 CFVSRPTEKTVTFVFLAASGICILNVAEWYLIIRACARRAQRRSNPPSRKSGFGHRLSPEYKQNEINKLLSEGDGSLKDLIRRSFG
 TGAGLAEKSDRCAC

Setting Up Down Substitution Conserved

Console

If you choose the **Visualization** function, the given protein and its matching sites will be visualized with DOG (Domain Graph, Ver 1.0), an illustrator of protein domain structures.



2. Multiple protein sequences in FASTA format

For multiple protein sequences, there are two ways to use the PPS 1.0.

A. Input the sequences into text form directly. (Num. of Seq ≤ 2,000)

If the number of total protein sequences is not greater than 2,000, you can just use “Ctrl+C & Ctrl+V” (Windows & Linux/Unix) or “Command+C & Command+V” (Mac) to copy and paste your sequences into the text form of PPS 1.0 for matching. The name of each protein should be presented.

PTMs Peptide Scanner -- PPS 1.0

File Tools About

PTMs

- Post-translational Modification
 - Phosphorylation
 - Methylation
 - Sumoylation
 - Palmitoylation
 - Acetylation

Matching Sites

Position	Code	Peptide	M.Sub	M.Position	M.Peptide	Comments
>1433B_HUMAN						
47	S	NEERNLLSVAYKNW	Q9CQV8	47	NEERNLLSVAYKNW	Phosphorylation
60	S	WGARRSSWRVSSI	P31946	60	WGARRSSWRVSSI	Phosphorylation: P...
60	S	WGARRSSWRVSSI	Q04917	59	WGARRSSWRVSSI	Phosphorylation: S...
60	S	WGARRSSWRVSSI	P63101	58	WGARRSSWRVSSI	Phosphorylation
60	S	WGARRSSWRVSSI	P63104	58	WGARRSSWRVSSI	Phosphorylation: P...
132	S	GDYFRYLSEVASGDN	P31946	132	GDYFRYLSEVASGDN	Phosphorylation: P...
186	S	FYYEILNSPEKACSL	P63104	184	FYYEILNSPEKACSL	Phosphorylation: M...
186	S	FYYEILNSPEKACSL	P31946	186	FYYEILNSPEKACSL	Phosphorylation: M...
186	S	FYYEILNSPEKACSL	P68250	186	FYYEILNSPEKACSL	Phosphorylation
186	S	FYYEILNSPEKACSL	P29361	184	FYYEILNSPEKACSL	Phosphorylation
143	T	SGDNKQTTVNSGQA	P31946	143	SGDNKQTTVNSGQA	Phosphorylation: P...
>1433E_HUMAN						
217	S	SEESYKDSLIMQLL	Q9UJ20	216	SEESYKDSLIMQLL	Phosphorylation
>1433F_HUMAN						
46	S	NEDRNLLSVAYKNW	Q9CQV8	47	NEERNLLSVAYKNW	Phosphorylation
59	S	WGARRSSWRVSSI	P31946	60	WGARRSSWRVSSI	Phosphorylation: P...
59	S	WGARRSSWRVSSI	Q04917	59	WGARRSSWRVSSI	Phosphorylation: S...
59	S	WGARRSSWRVSSI	P63101	58	WGARRSSWRVSSI	Phosphorylation
59	S	WGARRSSWRVSSI	P63104	58	WGARRSSWRVSSI	Phosphorylation: P...

Enter sequence(s) in FASTA format

```
>1433B_HUMAN
MTMDKSELVQKAKLAQAERYDDMAAMKAVTEQGHELSNEERNLLSVAYKNWVGARRSSWRVISSIEQKTERNEKQKQMGKEY
REKIEAELQDIDNDVLELLDKYLIPNATQPEKVFYLMKMGDYFRYLSEVASGDNKQTTVNSGQAQGEAFEISKEMGPTHPIRLG
LALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTLNEESYKDSLIMQLLRDNLTLWTSENGGDEGDAGEGEN
>1433E_HUMAN
MDDREDLVYQAKLAQAERYDEMVESMKKAVGMDVELTVEERNLLSVAYKNVIGARRASWRIISSIEGKEENKGGEDKLMIREYR
QMVETELKLCDDILDVLDKHLIPAANTGESKVFYKMKGDYHRYLAEFATGNDRKEAENS LVAYKAASDIAMTELPPTHPIRLGLA
LNF SVFYYEILNSPDRACRLAKAAFDAAIAELDTLSEESYKDSLIMQLLRDNLTLWTSDMGQDGEENKEALQDVEDENG
>1433F_HUMAN
MGDREQLLGRARLAQAERYDDMASAMKAVTELNEPLSNEDRNLLSVAYKNWVGARRSSWRVISSIEGKTMADGNEKKEKVKAY
REKIEKELLETVCNDVLSLLDKFLIKNCNDFQYESKVFYLMKMGDYFRYLAEVASGEKKNVWEASEAAYKEAFEISKEQMGPTHPIRL
GLNFCNFCWAFQIAKAEQADLAKAEDDAAIAELDTLNEESYKDSLIMQLLRDNLTLWTSDMGQDGEENKEALQDVEDENG
```

Setting

Up Down Substitution Conserved

Console

B. Use Batch Match tool.

If the number of protein sequences is very large, e.g., yeast or human proteome, please use the **Batch Match Tool**. Please click on the “**Batch Match**” button in the **Tools** menu.

Batch Match

PTMs

- Post-translational Modification
 - Phosphorylation
 - Methylation
 - Sumoylation
 - Palmitoylation
 - Acetylation

Sequence File List

Result File List

Result Export Fold

Setting

Up Down Substitution Conserved

Console

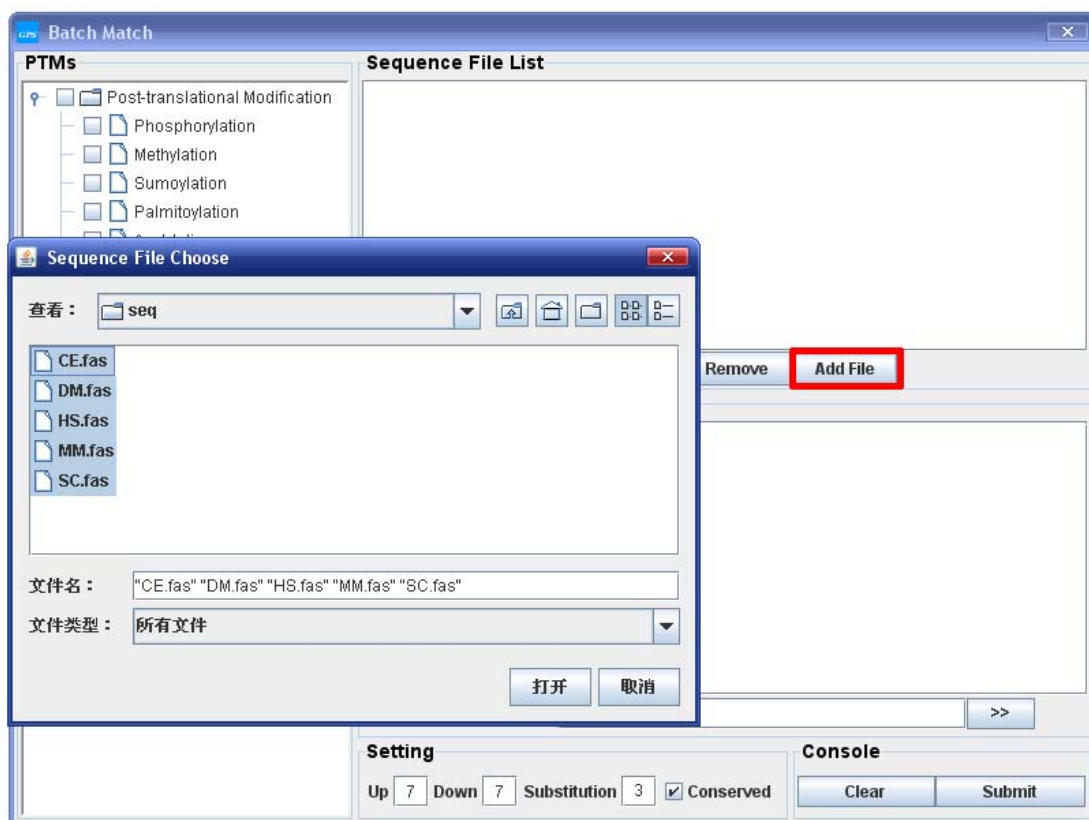
The following steps show you how to use it:

(1) Put protein sequences into one or several files (e.g., SC.fas, CE.fas, and etc) with FATSA format as below:

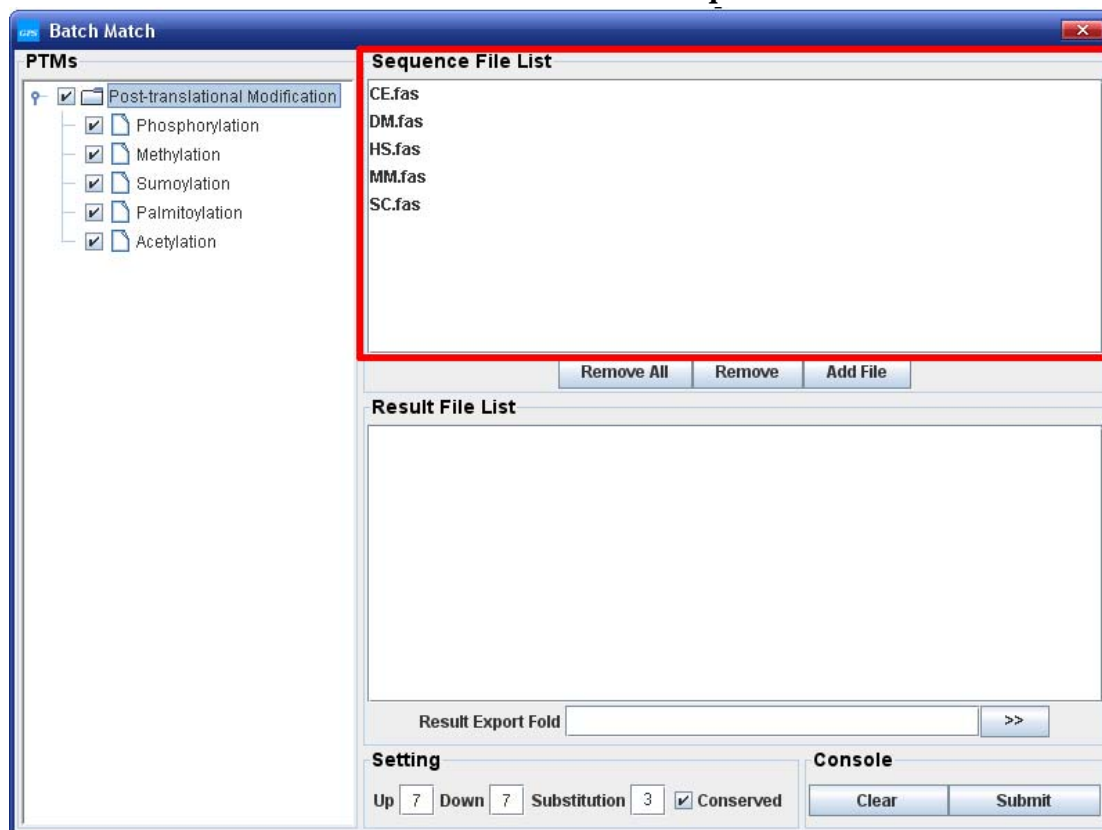
```
>protein1
XXXXXXXXXXXXXXXXX
XXXXXXXXXX
>protein2
XXXXXXXXXXXXXXXXXX...
>protein3
XXXXXXXXXXXXXXXXX
...
```

Most importantly, the name of each protein should be presented.

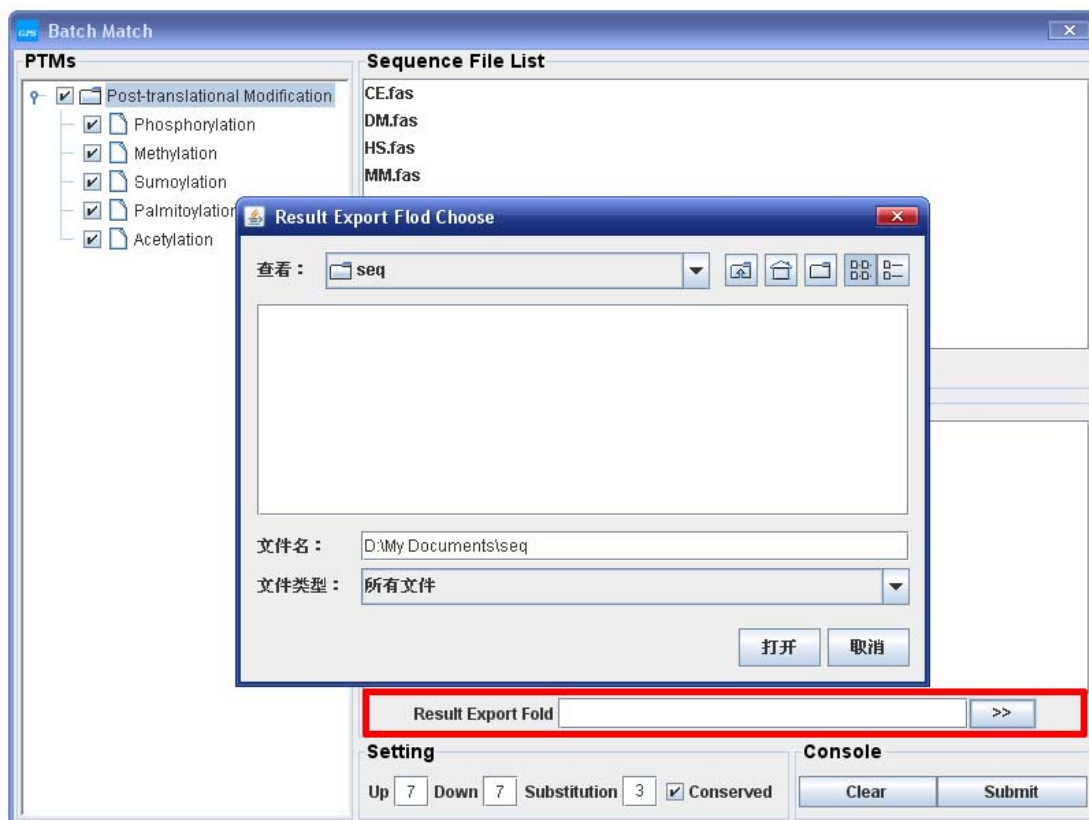
(2) Click on the **Add File** button and add one or more protein sequence files in your hard disk.



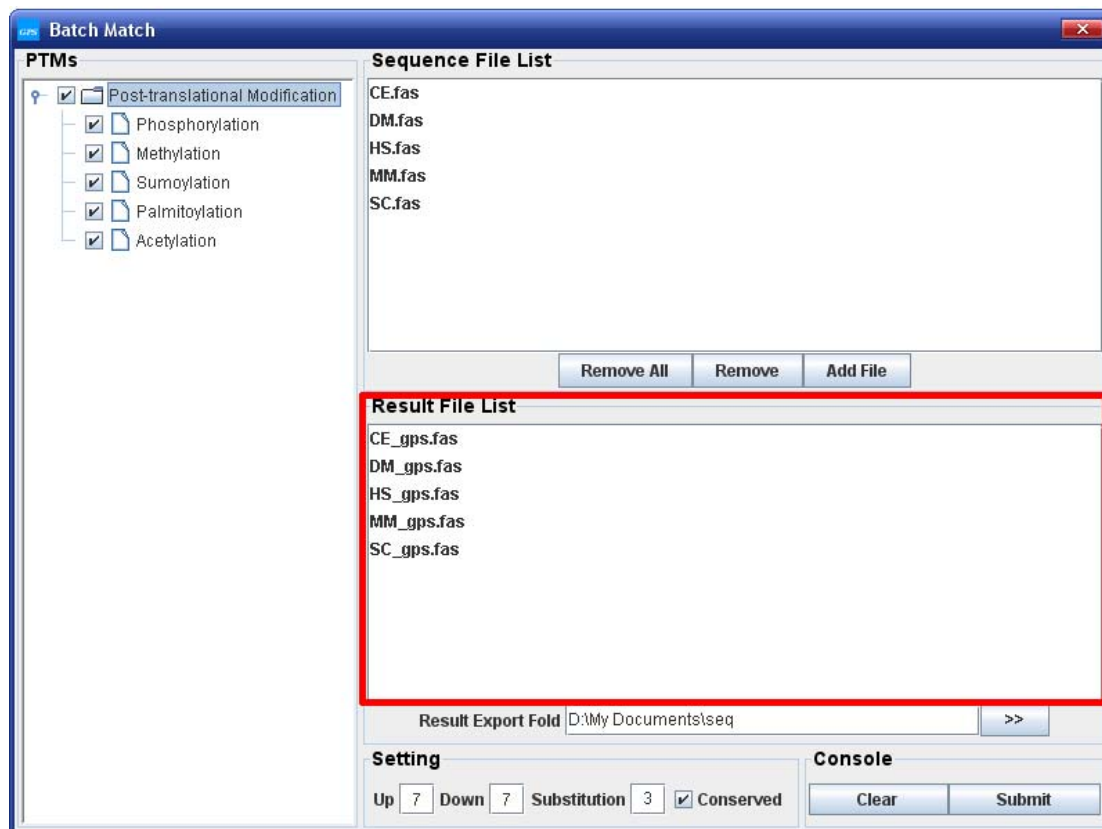
Then the names of added files will be shown in the **Sequence File List**.



(3) The output directory of prediction results should also be defined. Please click on the >> button to specify the export fold.



(4) Please choose one or more kinds of PTMs from **PTMs Tree** before matching. Then click on the **Submit** button and the **Batch Match Tool** begin to process all of the sequence files that have been added to the list. The result of prediction will be export to the **Result Export Fold**, and the name of result files will be shown in the **Result File List**.



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Release Note

1. Dec. 5th, 2008, the online service and the local stand-alone packages of PPS 1.0 were released.