



GPS-Lipid Manual

Prediction of Protein Lipid Modification Sites

Version 1.0

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Author: Jian Ren & Yu Xue

Contact: Dr. Jian Ren, renjian.sysu@gmail.com; Dr. Yu Xue, xueyu@hust.edu.cn

The software is only free for academic research.

The latest version of GPS-Lipid software is available from <http://lipid.biocuckoo.org>

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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 the National Basic Research Program (973 project) [2013CB933902, 2012CB911201, 2012CB910101]; National Natural Science Foundation of China [31171263, 81272578]; the Guangdong Natural Science Funds for Distinguished Young Scholar [S20120011335]; Zhujiang Nova Program of Guangzhou [2011J2200042]; Program of International S&T Cooperation [2014DFB30020].

Introduction

Most proteins in eucaryote cells are post-transcriptionally modified by a wide range of chemical groups. Among these modifications, the attachment of lipid groups to certain amino acids is a key modification that orchestrates the cellular protein's trafficking (1,2), signaling (3,4) and membrane association (5). With the rapid development of testing techniques, several lipid modifications, such as palmitoylation, prenylation and N-myristoylation, are now extensively studied.

Palmitoylation is a reversible post-translational modification that involves the addition of a 16-carbon chain palmitic acid on cysteine (6). As the palmityl group can effectively increase the hydrophobicity, the palmitoylation process can dynamically regulate the membrane targeting of cellular proteins (1,2). Particularly, the palmitoylation of small GTPase N-Ras and H-Ras has been extensively illustrated (7), which indicates a crucial role in modulating the signal transduction pathway (8,9). Another important type of lipid modification is prenylation. This process involves the addition of a 15-carbon farnesyl group or a 20-carbon geranylgeranyl group to a C-terminal cysteine that conform to a consensus **CAAX** motif (10). Typically, the farnesylation is catalyzed by protein farnesyltransferase (FTase) (11), whereas the geranylgeranylation is performed by protein geranylgeranyltransferase type I (GGTase-I) (12,13). However, in case of Rab proteins, the geranylgeranyltransferase type II (GGTase-II) which recognized a C-terminal **CC/CXC** motif is found to catalyze the geranylgeranylation process (10,14). In addition to the above complex mechanism, prenylation also tightly controls the signaling activities in cell by modifying small GPTase Ras (15,16), Rho (17,18) and Rab (18,19). As for the last type of lipid modification, Protein N-myristoylation is catalyzed by the N-myristoyl transferase (NMT), which recognizes a **MGXXXS/T** signature at N-terminus (20,21). It is reported that N-myristoylation can promote weak protein-protein and protein-membrane interaction (22). Moreover, with an essential role in regulating the relocation of signaling proteins, N-myristoylation functions widely in a variety of signal transduction pathways (23).

In view of the above essential physiological functions, the irregular lipid modification may lead to all sorts of diseases. As reported in published literatures (24,25), the abnormal lipid modification level is highly correlated with oncogenesis. Besides, the elevated levels of lipid modification may also linked with other severe diseases. For example, the overexpression of palmitoyl acyltransferases (PATs) may implicate in schizophrenia (26) and Huntington's disease (27). Also, the N-myristoylation is observed to mediate the viral Infectivity and eukaryotic infections (28). Taken together, the research on lipid modification will be particularly important for the diagnosis and treatment of diseases. However, due to the limitations of

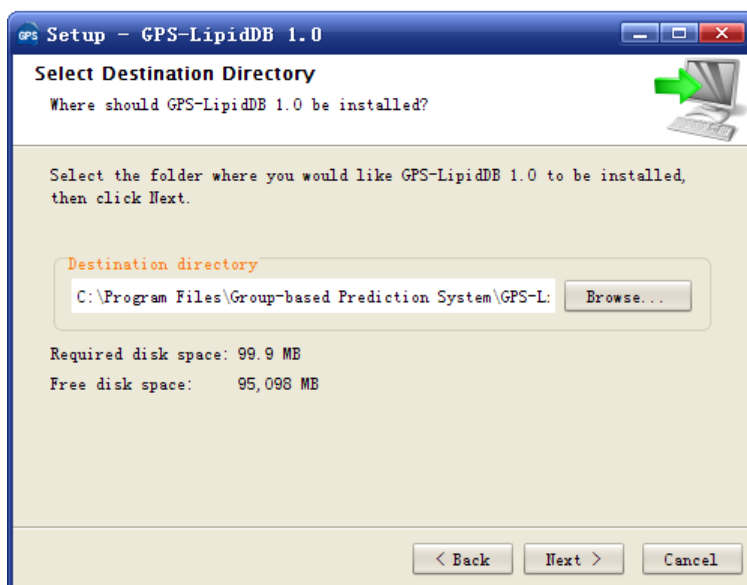
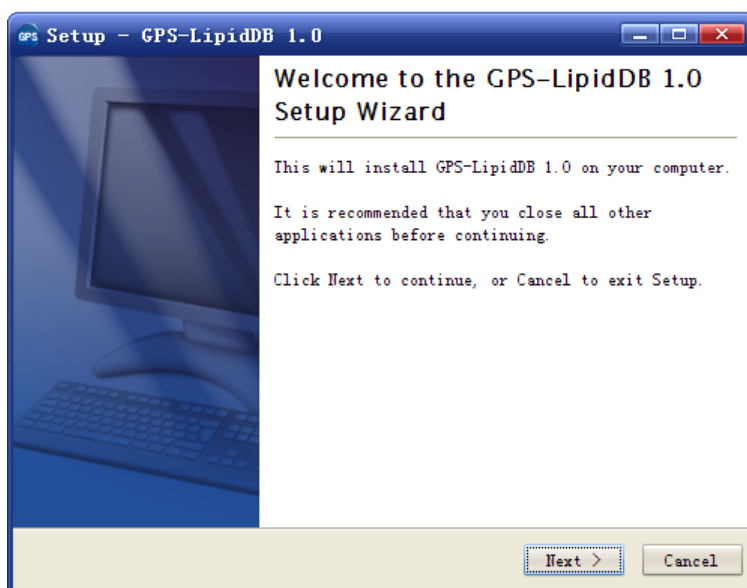
[illegible]

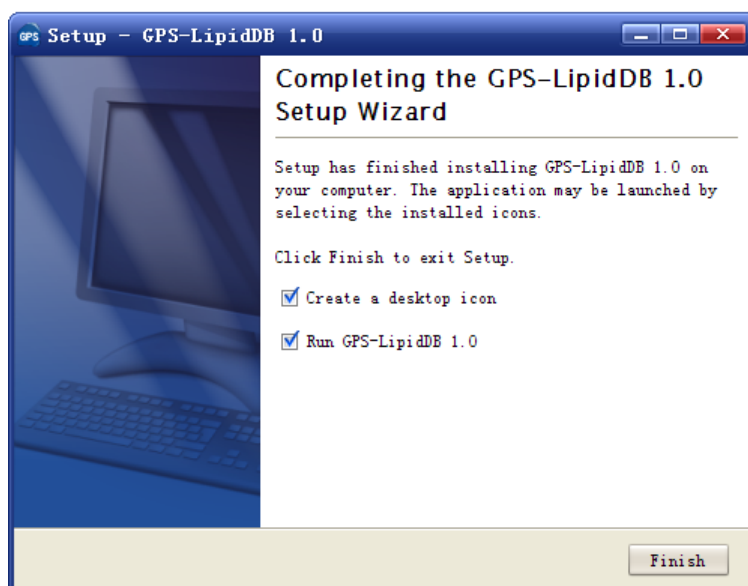
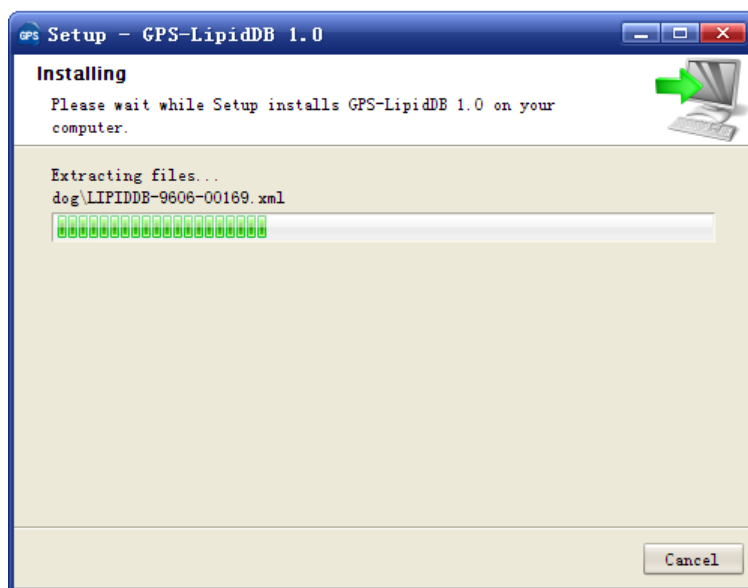
5

Download & Installation

The local packages of GPS-Lipid 1.0 database were implemented in JAVA, and could be installed on Windows, Mac OS X or Unix/Linux systems. The latest distributions of GPS-Lipid could be found at <http://lipid.biocuckoo.org/download.php>. We recommend that users could download the latest release.

After downloading, please double-click on the install package to begin installation. Follow the user prompts through the installation. And snapshots of the setup program are shown below:





Click on the **Finish** button to complete the setup program.

GPS-Lipid 1.0 - Prediction of Protein Lipid Modification Sites

File Tools Help

| ID | Position | Peptide | Score | Cutoff | Cluster |
|-------|----------|-----------------|--------|--------|--------------------------------------|
| RAP1B | 181 | KARKKSSCQLL**** | 13.303 | 4.003 | S-Farnesylation: Non-consensus |
| RAP1B | 181 | KARKKSSCQLL**** | 6.189 | 1.617 | S-Geranylgeranylation: Non-consensus |

Enter sequence(s) in FASTA format

```
>RAP1B
MREYKLVVLGSGGVGKSALTQVFVQGIFVEKYDPTIEDSYRKQVEVDAQQCMLIEDTAGTEQFTAMRDLYMKNGQGALVYSITAGSTFNDLQDLREQILRVKDTDDVPMILVGNKCDLEDER
```

PTM type

☒ Palmitoylation ☒ N-Myristoylation ☒ Farnesylation ☒ Geranylgeranylation

Threshold

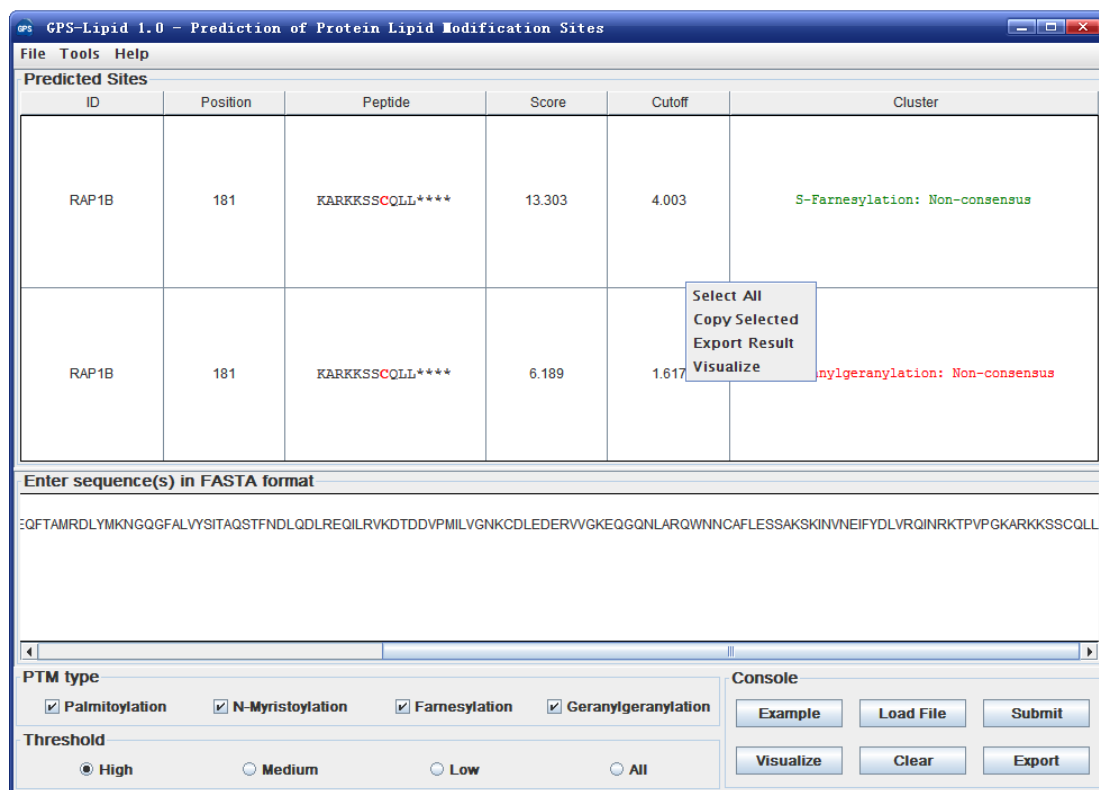
☒ High ☐ Medium ☐ Low ☐ All

Console

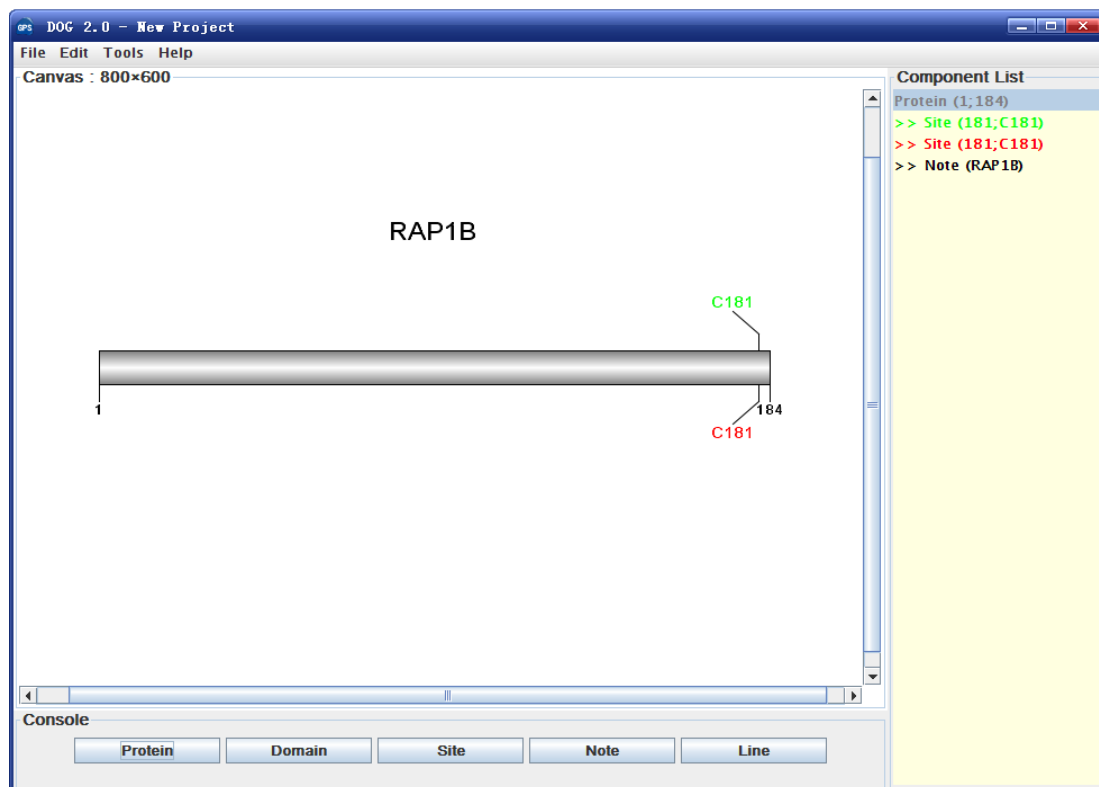
Example Load File Submit

Visualize Clear Export

(5) Then please click on the **RIGHT** button in the prediction 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, eg., an EXCEL file for further consideration. Also, you can choose “**Export Prediction**” to export the prediction results into a tab-delimited text file.



To visualize the predicted site please click on the “**Visualize**” button. Again, different types of modification sites will be marked in different color.



Notably, you can also click the “**Export Prediction**” in **File** menu to export the results.

GPS-Lipid 1.0 - Prediction of Protein Lipid Modification Sites

File Tools Help

Load Sequence File Ctrl-L

Export Result Ctrl-E

Exit Alt-F4

| Position | Peptide | Score | Cutoff | Cluster |
|-----------|-----------------|--------|--------|--------------------------------------|
| RAP1B 181 | KARKKSSCQLL**** | 13.303 | 4.003 | S-Farnesylation: Non-consensus |
| RAP1B 181 | KARKKSSCQLL**** | 6.189 | 1.617 | S-Geranylgeranylation: Non-consensus |

Enter sequence(s) in FASTA format

EQFTAMRDLYMKNQGQGFALVYSITAQSTFNDLQDLREQILRVKDDTDDVPMILVGNKCDLEDERVWGKEQGQNLARQWNNCAFLESSAKSKINVNEIFYDLVRQINRKTPVPGKARKKSSCQLL

PTM type

☒ Palmitoylation ☒ N-Myristoylation ☒ Farnesylation ☒ Geranylgeranylation

Threshold

☒ High ☐ Medium ☐ Low ☐ All

Console

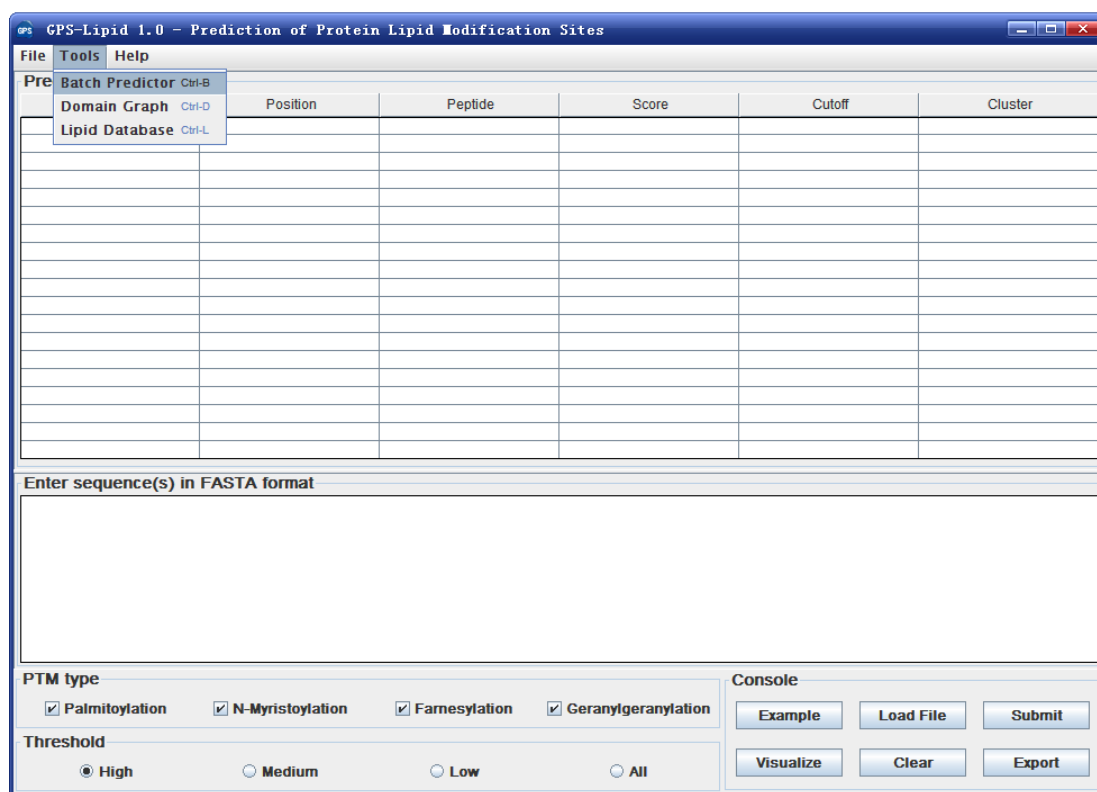
Example Load File Submit

Visualize Clear Export

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

[illegible]

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



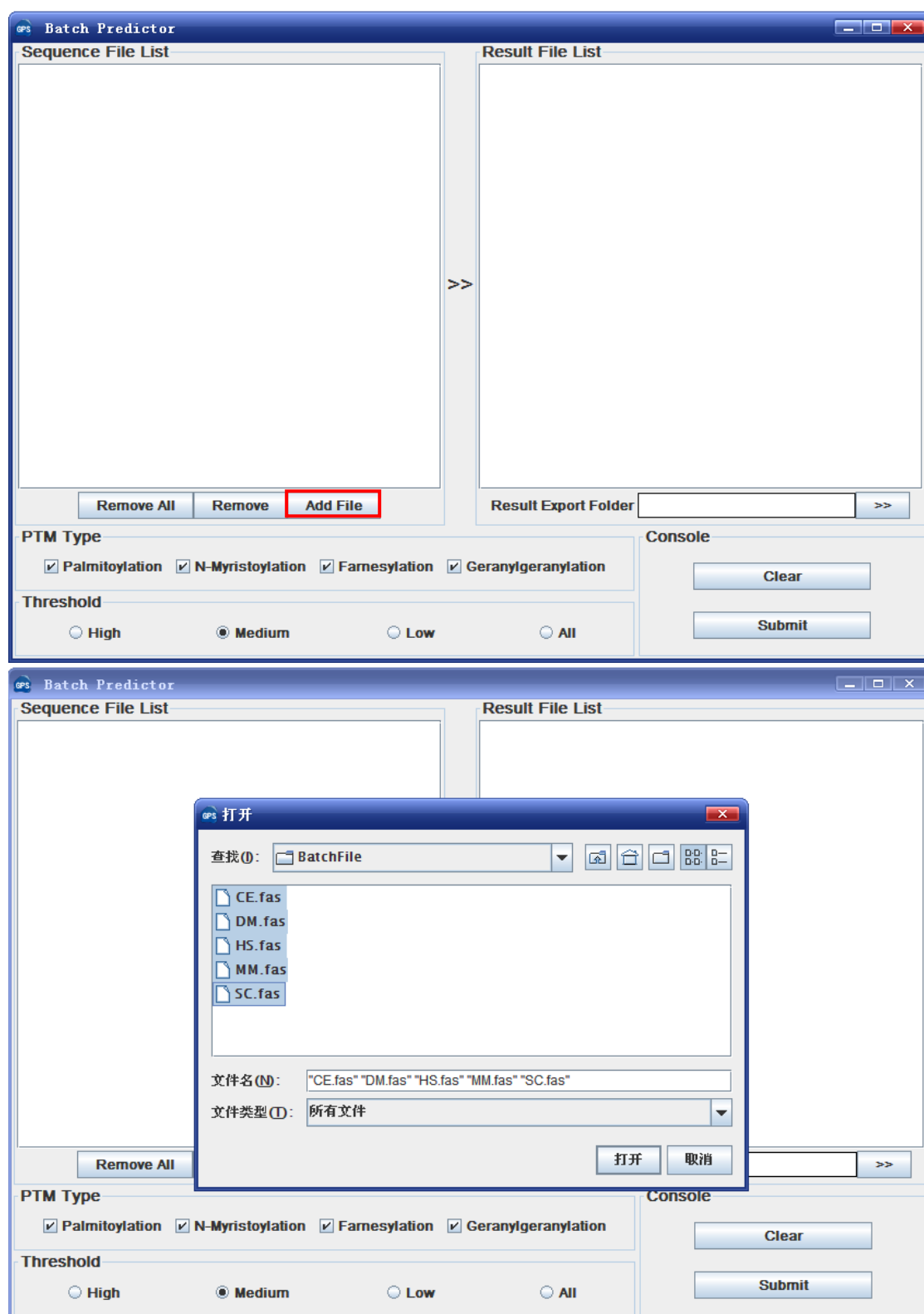
The following steps show you how to use it:

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

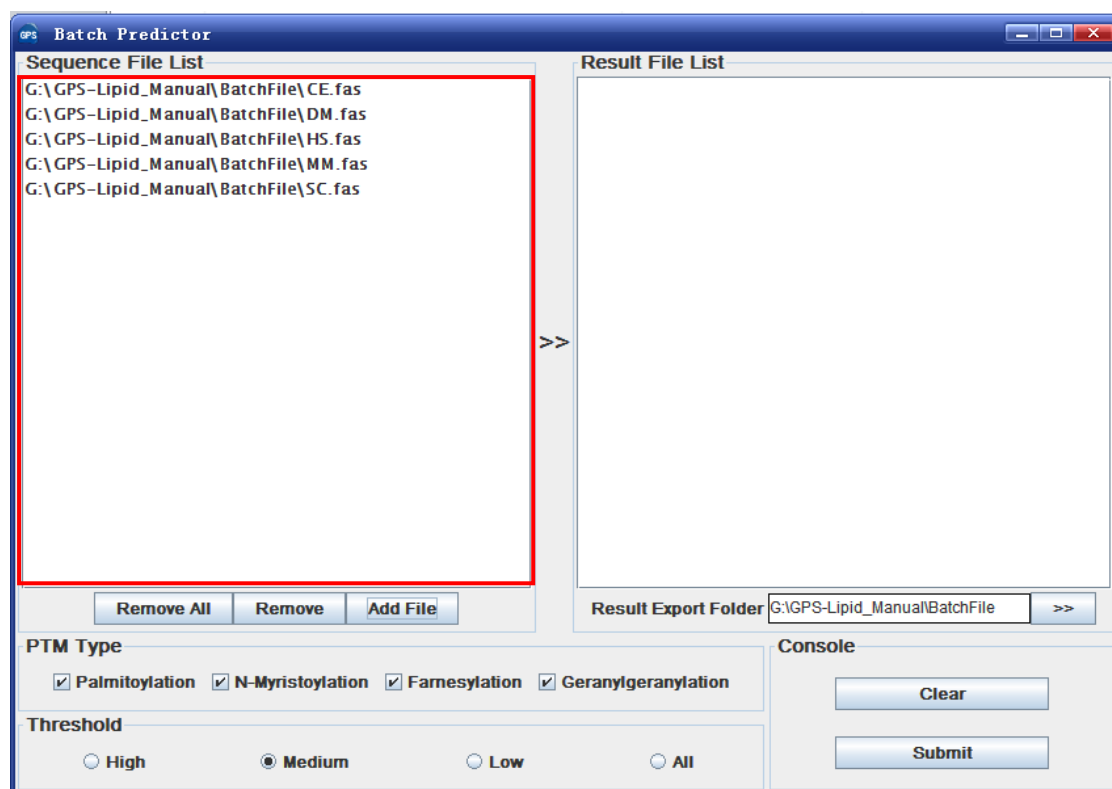
```
>protein1
XXXXXXXXXXXXXXXXXX
XXXXXXXXXX
>protein2
XXXXXXXXXXXXXXXXXXXX...
>protein3
XXXXXXXXXXXXXXXXXX
...
```

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

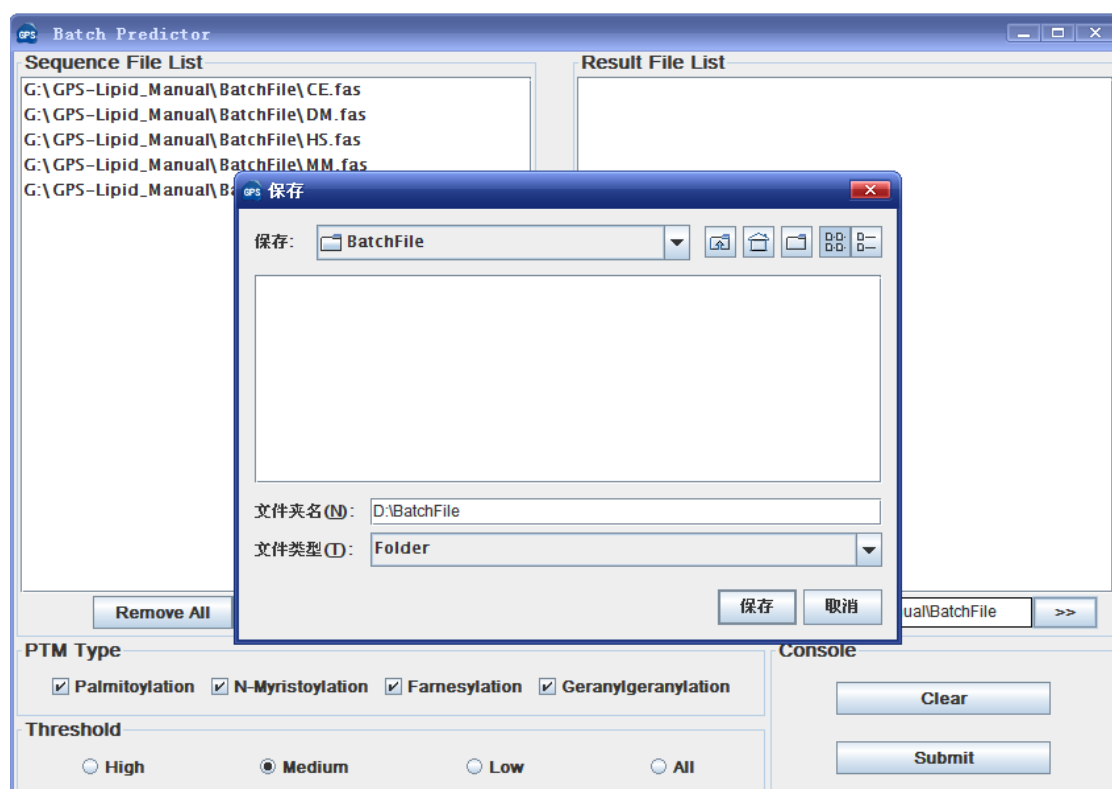
(2) Click on the **Batch Predictor** button and then click on the **Add File** button and add one or more protein sequence files in your hard disk.



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

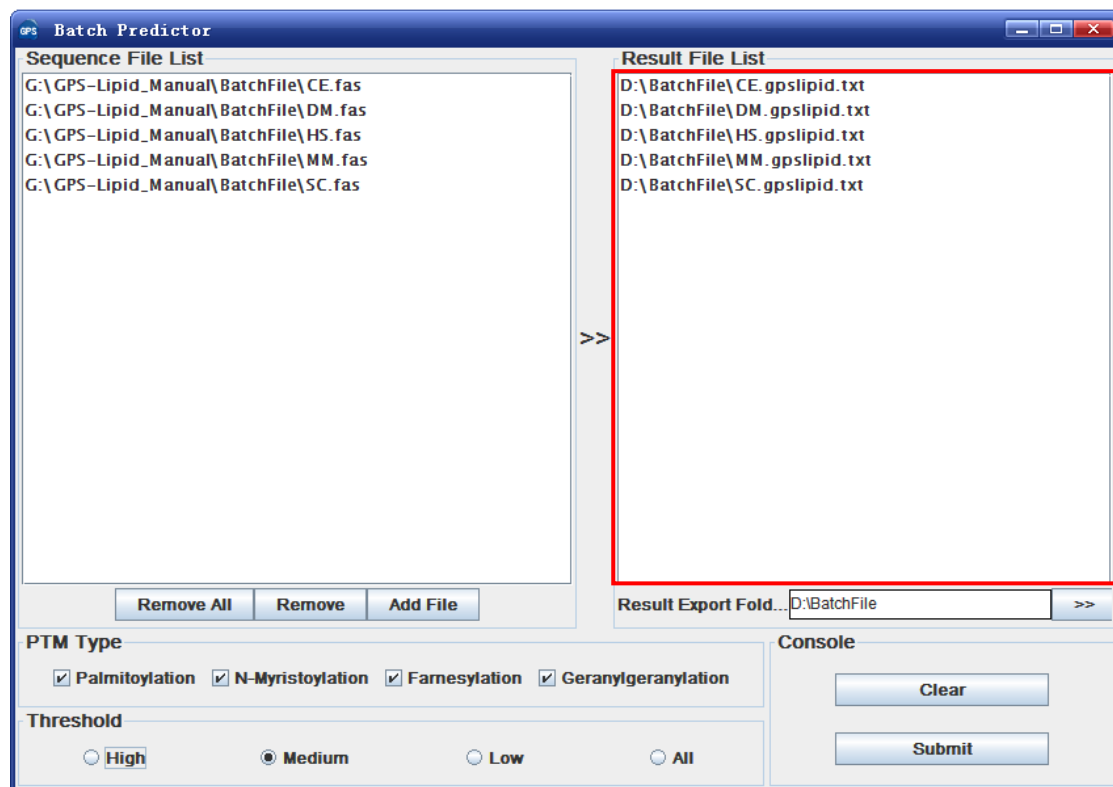


(3) The output directory of prediction results should also be defined. Please click on the >> button to specify the export fold.



(4) Please choose a proper modification type and threshold before prediction. Then

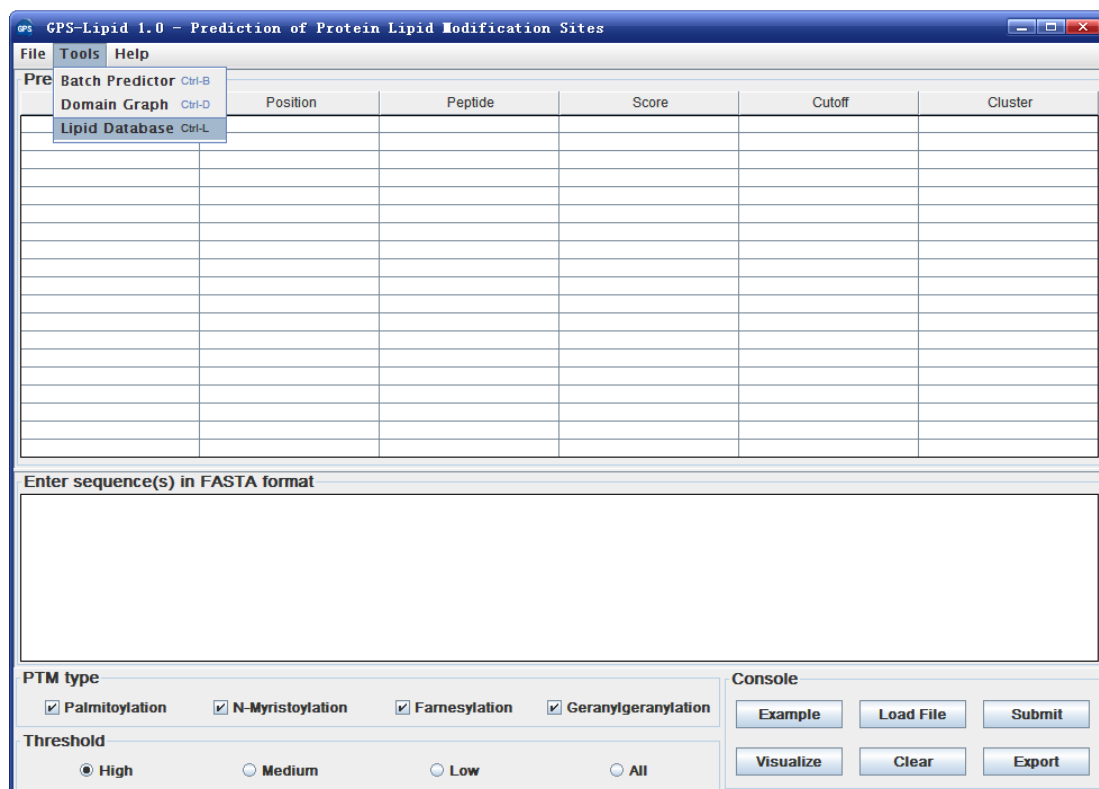
please click on the **Submit** button, after that, the **Batch Predictor** begin to process all of the sequence files that have been added to the list. The result of prediction will be export to the **Result Export Fold**, and the name of result files will be shown in the **Result File List**.



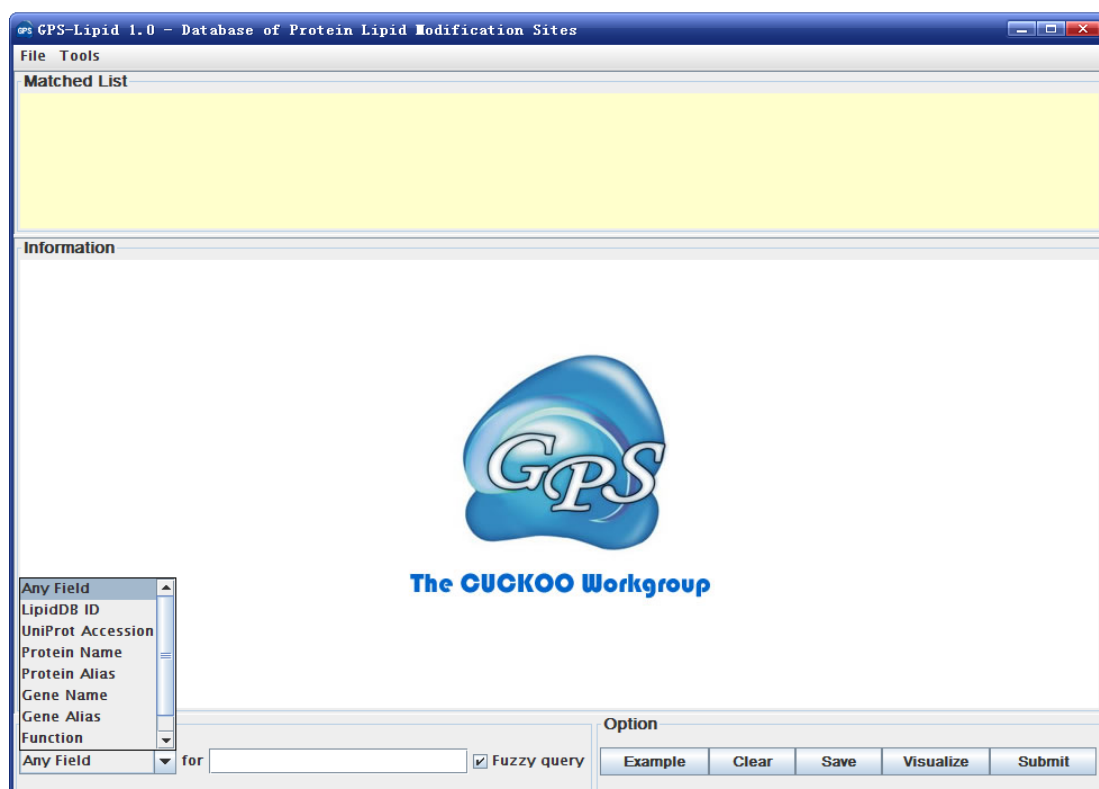
The usage of Lipid database

Search

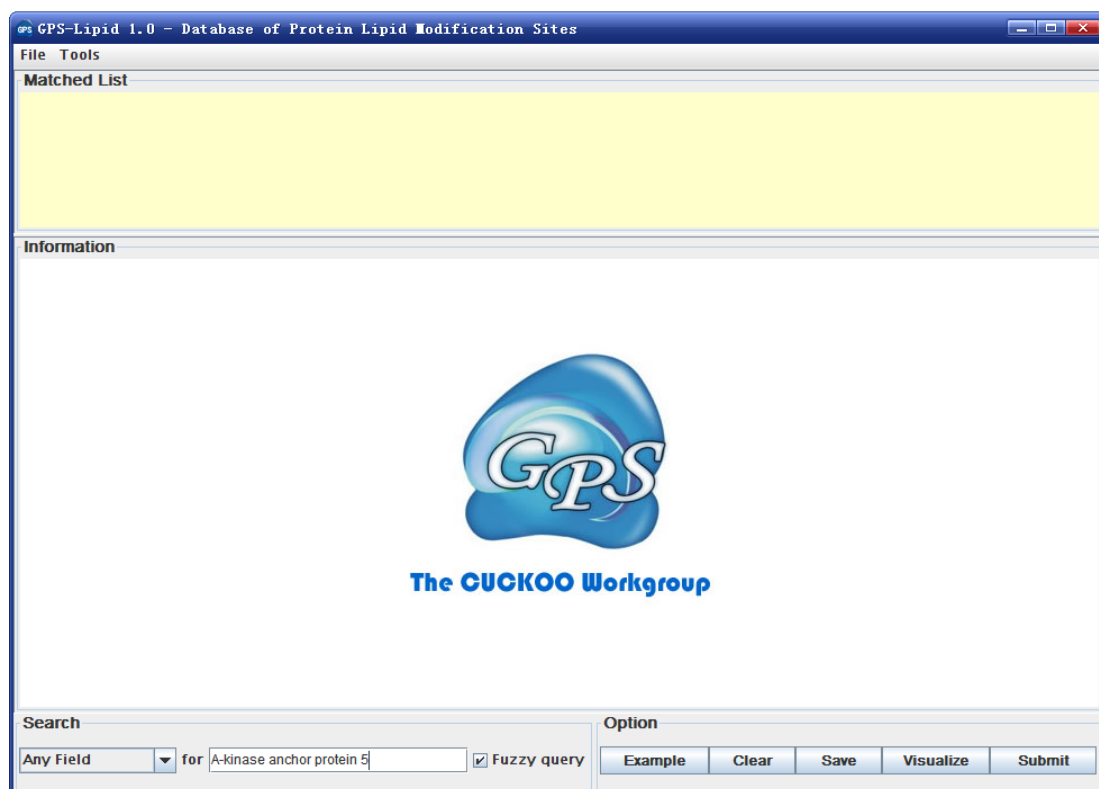
The GPS-Lipid was also provide a database for lipid modifications. Click on the **Tools -> Lipid Database** menu to open the Lipid database.



For simple search, users could input a LipidDB ID with LipidDB-XXXX-XXXXXX, a UniProt ID (Q07065), protein/gene names/aliases (eg., cytoskeleton-associated protein 4) or functions. Users could click the “Example” button one or several times to view the instances.



For example, users could input a protein/gene name/alias, e.g., A-kinase anchor protein 5, specify the “**Any Field**”, and then click on the “**Submit**” button to search the related information for this protein.



Then the information for human kinase anchor protein 5 will be shown in the “Information” form.

The screenshot shows the GPS-Lipid 1.0 software window. The top menu bar includes 'File' and 'Tools'. Below it is a 'Matched List' showing several lipid modification entries. The main section is titled 'Information' and contains a table with two columns: 'Tag' and 'Content'. The table lists various identifiers for the protein, including LipidDB ID, Entry Name, UniProt Accession, Theoretical PI, Molecular Weight, Genbank Protein ID, Genbank Nucleotide ID, Protein Name, Protein, Synonyms/Alias, Gene Name, and Gene. At the bottom, there is a 'Search' section with a dropdown menu set to 'Any Field', a text input field containing 'A-kinase anchor protein 5', and a checked 'Fuzzy query' checkbox. To the right of the search section is an 'Option' section with buttons for 'Example', 'Clear', 'Save', 'Visualize', and 'Submit'.

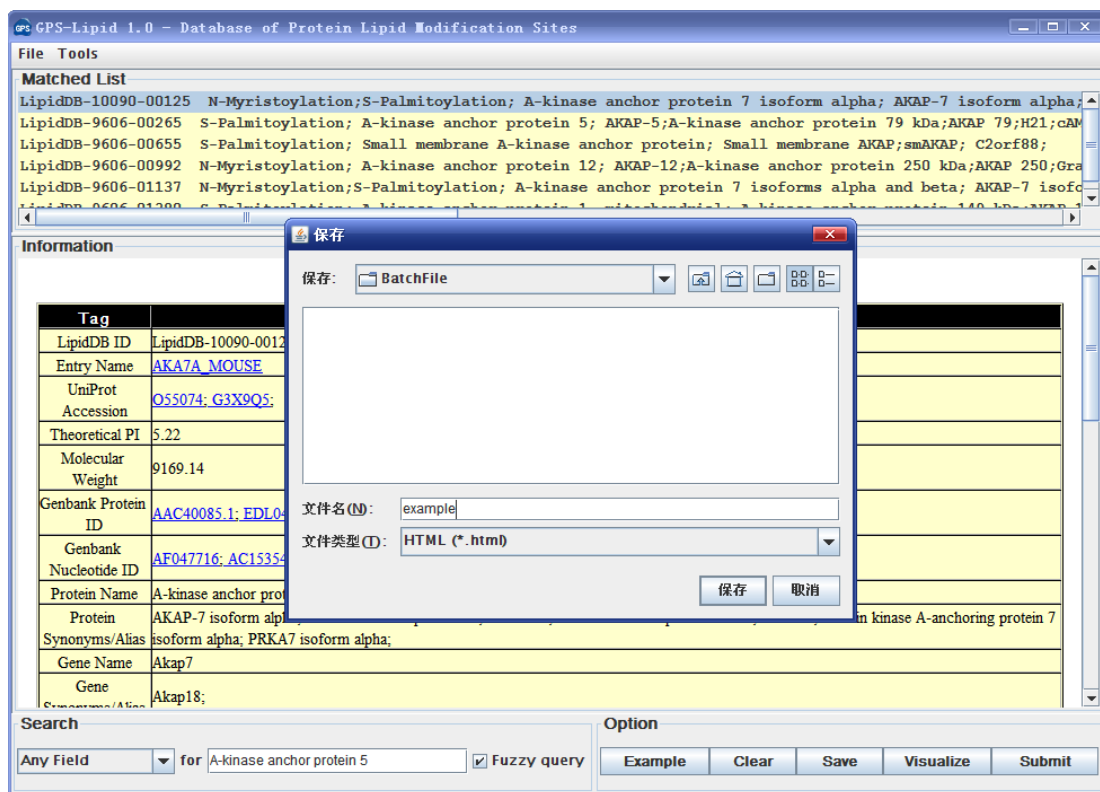
| Tag | Content |
|-----------------------|--|
| LipidDB ID | LipidDB-10090-00125 |
| Entry Name | AKA7A_MOUSE |
| UniProt Accession | Q55074 ; G3X9Q5 |
| Theoretical PI | 5.22 |
| Molecular Weight | 9169.14 |
| Genbank Protein ID | AAC40085.1 ; EDL04795.1 |
| Genbank Nucleotide ID | AF047716 ; AC153549 ; AC153550 ; CH466540 |
| Protein Name | A-kinase anchor protein 7 isoform alpha |
| Protein | AKAP-7 isoform alpha; A-kinase anchor protein 18; AKAP-18; A-kinase anchor protein 9 kDa; AKAP 9; Protein kinase A-anchoring protein 7 |
| Synonyms/Alias | isoform alpha; PRKA7 isoform alpha; |
| Gene Name | Akap7 |
| Gene | Akap18; |

Search: Any Field for A-kinase anchor protein 5 ☒ Fuzzy query

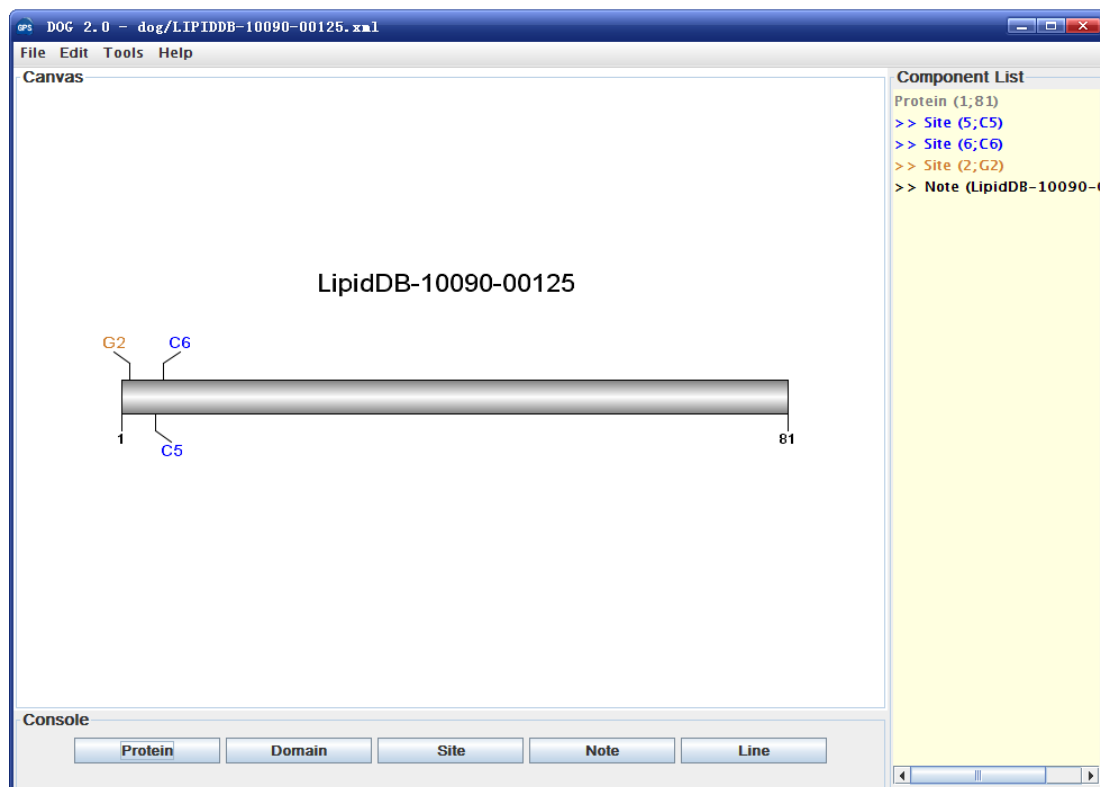
Option: Example Clear Save Visualize Submit

The protein and nucleotide sequences for this protein could be shown by clicking on the related NCBI or Uniprot identifiers:

The searched results could be saved in an HTML format.

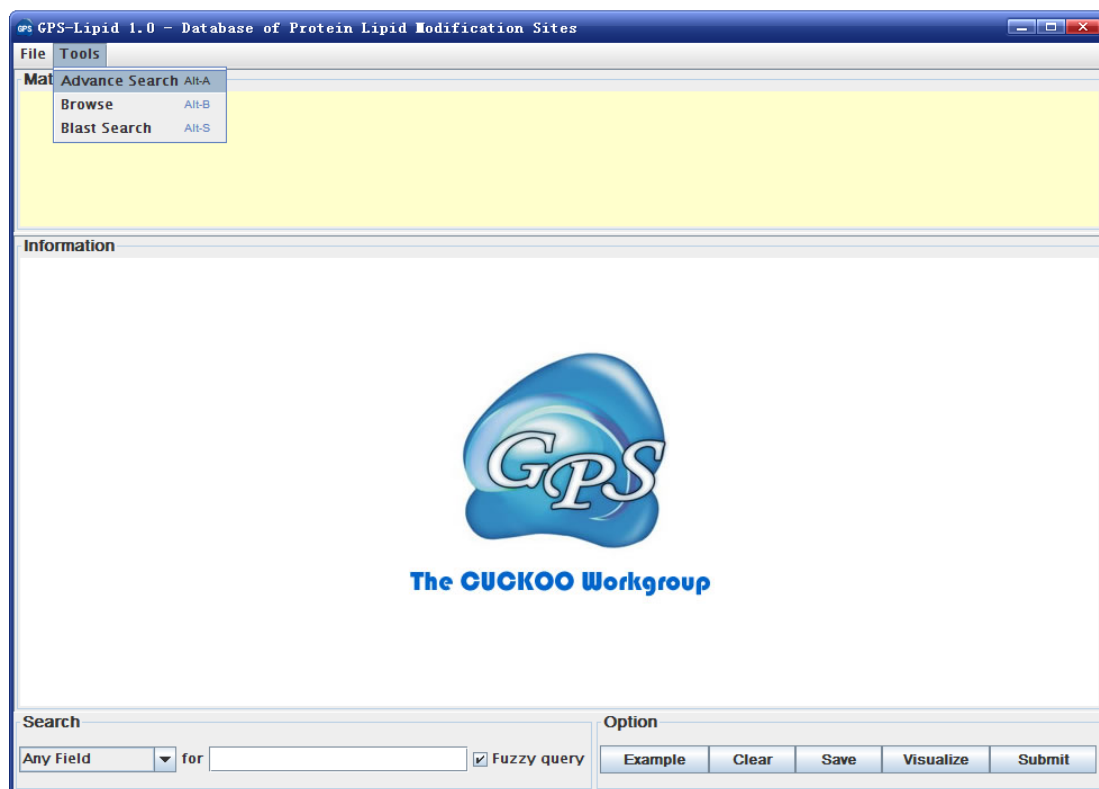


Also, when clicking on the **Visualize** button, you can view the schematic diagram for your search result.



Advance Search

The GPS-Lipid 1.0 database supports three advance options, including advance search, browse, BLAST search. The Advance search option allows you to input up to three terms to find the information more specifically. The querying fields can be empty if fewer terms are needed.



First, users could click on the “**Tools**” button then click on the “**Advance Search**” button to open this option.

GPS-LipidDB - Advance Search

Matched List

Information

Search

☐ Exclude Any Field ☒ and ☐ or

☐ Exclude Any Field ☒ and ☐ or

☐ Exclude Any Field ☒ and ☐ or

Option

Example

Clear

Save

Visualize

Submit

By clicking the “**Example**” button, you can try an instance for usage. You can input Phosphatidylcholine (Protein Name), S-Palmitoylation (Modification type), and 9606 (LipidDB ID) for querying.

GPS-LipidDB - Advance Search

Matched List

Information

Search

☐ Exclude Function ☒ and ☐ or signal transduction

☐ Exclude Modification Type ☒ and ☐ or S-Palmitoylation

☐ Exclude LipidDB ID ☒ and ☐ or 9606

Option

Example

Clear

Save

Visualize

Submit

Then the human's sphingomyelin synthase 2 will be shown.

GPS-LipidDB - Advance Search

Matched List

LipidDB-9606-00114 S-Palmitoylation; Transforming protein RhoA; Rho cDNA clone 12;h12; RHOA; ARH12;ARHA;RHO12

LipidDB-9606-00121 S-Palmitoylation; cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A; 3.1.4.17;3.1.4.17

LipidDB-9606-00151 S-Palmitoylation; CD81 antigen; 26 kDa cell surface protein TAPA-1;Target of the antiproliferative

LipidDB-9606-00238 S-Palmitoylation; Phosphatidylcholine:ceramide cholinephosphotransferase 2; 2.7.8.27;Sphingomyelin

LipidDB-9606-00399 S-Palmitoylation; Regulator of G-protein signaling 7; RGS7; RGS7;

LipidDB-9606-00423 S-Palmitoylation; Phospholipase D1; PLD1;hPLD1;2.1.4.4;Choline phosphatase 1;Phosphatidylcholine

Information

Lipid Modification Database

| Tag | Content |
|------------------------|---|
| LipidDB ID | LipidDB-9606-00114 |
| Entry Name | RHOA_HUMAN |
| UniProt Accession | P61586 ; P06749 ; Q53HM4 ; Q5U024 ; Q9UDJ0 ; Q9UEJ4 ; |
| Theoretical PI | 5.83 |
| Molecular Weight | 21768.13 |
| Genbank Protein ID | CAA28690.1 ; AAC33178.1 ; AAM21117.1 ; AAV38673.1 ; BAD96276.1 ; CAE46190.1 ; AAH01360.1 ; AAH05976.1 ; AAA50612.1 ; AAA67539.1 ; |
| Genbank Nucleotide ID | X05026 ; L25080 ; AF498970 ; BT019870 ; AK222556 ; BX647063 ; AC104452 ; AC121247 ; AC137114 ; BC001360 ; BC005976 ; L09159 ; |
| Protein Name | Transforming protein RhoA |
| Protein Synonyms/Alias | Rho cDNA clone 12; h12; |

Search

☐ Exclude **Function** ☒ and ☐ or

☐ Exclude **Modification Type** ☒ and ☐ or

☐ Exclude **LipidDB ID**

Option

Notably, by clicking the “**Visualize**” button, you can open a schematic diagram of the human's sphingomyelin synthase 2 with a palmitoylation site marked in blue.

DOG 2.0 - dog/LIPIDDB-9606-00114.xml

File Edit Tools Help

Canvas

LipidDB-9606-00114

Component List

Protein (1;193)

>> Site (159;C159)

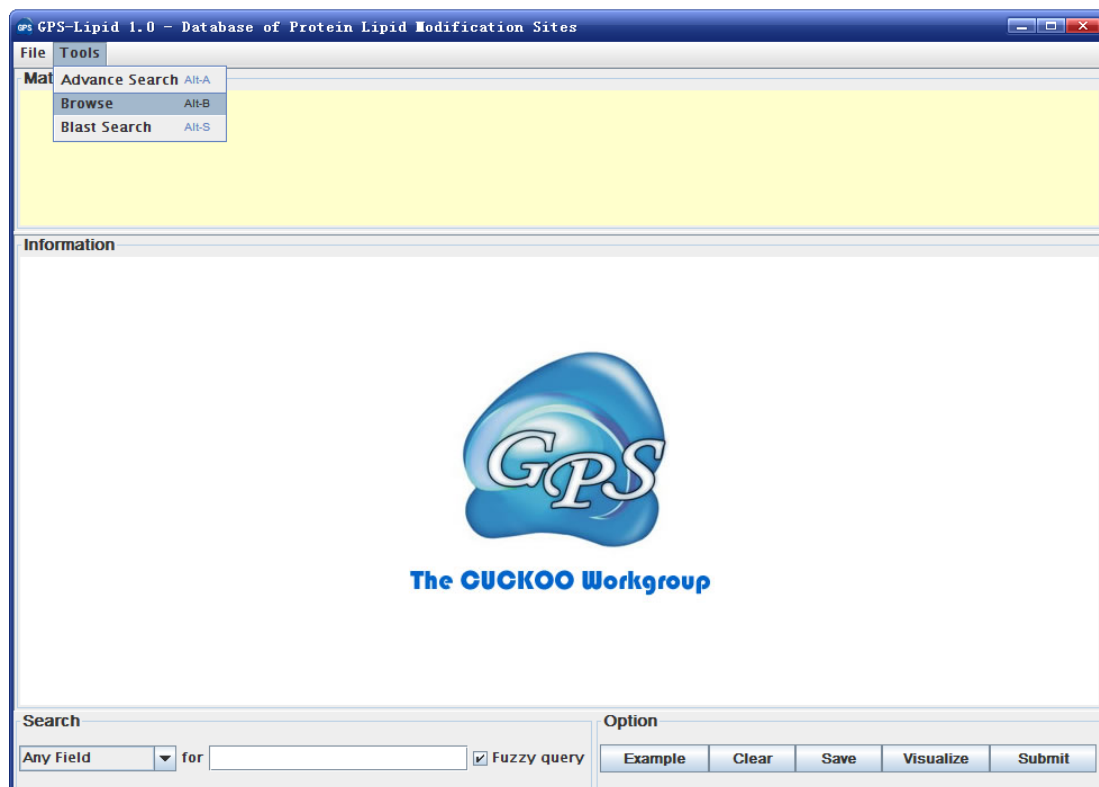
>> Site (190;C190)

>> Note (LipidDB-9606-00114)

Console

Browse

The GPS-Lipid 1.0 database supports the browse function. The Browse search allows users to view all entries in GPS-Lipid 1.0 database.



First, users could click on the “**Tools**” button then click on the “**Browse**” button to visualize all GPS-Lipid proteins. Users could visualize any protein by click on the entries listed in the “LipidDB ID” form.

THE RESULTS WILL BE SHOWN IN SEVERAL COLUMNS OF THE REPORT IN A SEPARATE COLUMN.

[illegible]

GPS-Lipid 1.0 - Database of Protein Lipid Modification Sites

File Tools

Mat

- Advance Search Alt-A
- Browse Alt-B
- Blast Search Alt-S

Information

Search

Any Field for ☒ Fuzzy query

Option

Example Clear Save Visualize Submit

GPS-Lipid - Blast Search

Matched List

Information

Enter a sequence in FASTA format

Option

E-Value < 0.01

Species All

Types All

Example

Clear

Save

Visualize

Submit

Then users could either click on the “**Example**” button in the Option form or directly input a protein sequence in FASTA or RAW format. Please note that only one protein is permitted a time. Then please click on the “Submit” button to search identical or homologous entries. The E-value cut-off could be user-defined in the Option form.

GPS-Lipid - Blast Search

Matched List

Information

Enter a sequence in FASTA format

```
>sp|P01889|I1B07_HUMAN HLA class I histocompatibility antigen, B-7 alpha chain OS=Homo sapiens
GN=HLA-B PE=1 SV=3
MLVMAPIRTVLLLSAALALLETWAGSHSMRYFYTSVSRPGRGEPRFISVGYVDDTQFVRF
DSDAASPREEPRAPWIEQEGPEYWDNRNTQIYKAAQTDRESLRNLRGYYNQSEAGSHILQ
SMYGCUGVPGDGRLLRGHDQYAYDGKDYIALNEDLRSWTAADTAAQITORKWEAAREAEQR
RAYLEGECEVWLRRLRYLENGKDKLERADPKPTHVTHHPISDHEATLRCAWLGFPYPAEITLT
WORDGEDQTDQDELVEVTRPAGDRITFKWAAVVVSSEEGQRYTCHVOHEGLPKPLTLRWEP
SSQSTVPIVGIVAGLAVLVVVIGAVVAVMCRKRKSSGGKGGSSQAACSDSAQGSQSDVSL
TA
```

Option

E-Value < 0.01

Species All

Types All

Example

Clear

Save

Visualize

Submit

GPS-Lipid - Blast Search

Matched List

| LipidDB ID | Modification type | Identities | E-Value | Score (bits) |
|--------------------|-----------------------|------------|---------|--------------|
| LipidDB-9606-00435 | type=S-Palmitoylation | 100.00% | 0.0 | 748 |
| LipidDB-9606-00457 | type=S-Palmitoylation | 31.27% | 7e-037 | 135 |

Information

Lipid Modification Database

| Tag | Content |
|-----------------------|---|
| LipidDB ID | LipidDB-9606-00435 |
| Entry Name | I1B07_HUMAN |
| UniProt Accession | P01889; Q29638; Q29681; Q29854; Q29861; Q31613; Q5SRJ2; Q9GIX1; Q9TP95; |
| Theoretical PI | 5.57 |
| Molecular Weight | 40460.08 |
| Genbank Protein ID | AAA36230.1; AAA59622.1; AAA91229.1; CAA45785.1; AAA87398.1; AAA65639.1; AAA92563.1; AAA92564.1; CAA62864.1; AAF01052.1; CAC35468.1; CAC33440.1; CAI18148.1; BAG36634.1; CAC10402.1; |
| Genbank Nucleotide ID | M32317; M16102; U29057; X64454; U04245; L33922; U21052; U21053; X91749; AF189017; AJ309047; AJ292075; AL671883; |

Enter a sequence in FASTA format

```
>sp|P01889|I1B07_HUMAN HLA class I histocompatibility antigen, B-7 alpha chain OS=Homo sapiens
GN=HLA-B PE=1 SV=3
MLVMAPIRTVLLLSAALALLETWAGSHSMRYFYTSVSRPGRGEPRFISVGYVDDTQFVRF
DSDAASPREEPRAPWIEQEGPEYWDNRNTQIYKAAQTDRESLRNLRGYYNQSEAGSHILQ
SMYGCUGVPGDGRLLRGHDQYAYDGKDYIALNEDLRSWTAADTAAQITORKWEAAREAEQR
RAYLEGECEVWLRRLRYLENGKDKLERADPKPTHVTHHPISDHEATLRCAWLGFPYPAEITLT
WORDGEDQTDQDELVEVTRPAGDRITFKWAAVVVSSEEGQRYTCHVOHEGLPKPLTLRWEP
SSQSTVPIVGIVAGLAVLVVVIGAVVAVMCRKRKSSGGKGGSSQAACSDSAQGSQSDVSL
TA
```

Option

E-Value < 0.01

Species All

Types All

Example

Clear

Save

Visualize

Submit

Again, users could visualize any GPS-LipidDB proteins by clicking on the entries listed in the “Matched list” form. And the results could be saved by clicking on the “Save” button in the Option form.

GPS-Lipid - Blast Search

Matched List

| LipidDB ID | Modification | type | Identities | E-Value | Score (bits) |
|------------|------------------|------|------------|---------|--------------|
| 9606-00435 | S-Palmitoylation | | 100.00% | 0.0 | 748 |
| 9606-00457 | S-Palmitoylation | | 31.27% | 7e-037 | 135 |

Information

| Tag | |
|--------------------|--------------------|
| LipidDB ID | LipidDB-9606-00435 |
| Entry Name | 1B07_HUMAN |
| UniProt Accession | P01889; Q29638 |
| Theoretical PI | 5.57 |
| Molecular Weight | 40460.08 |
| Genbank Protein ID | AAA36230.1; AA |
| Genbank | AAF01052.1; CA |
| Genbank | M32317; M16102 |
| Genbank | AK212011; AT40 |

Enter a sequence in FASTA format

```
>sp|P01889|1B07_HUMAN HLA class II histocompatibility antigen, DR alpha chain
GN=HLA-B PE=1 SV=3
MLVMAPIRTVLLLSAALALTETWAGSHSMRY
DSDAASPREEPAPWIEQEGPEYWRDNTQIYKAAQTDRESLRNLRGYYNQSEAGSHTLQ
SMYGCQVGPGRLLRGHDQYAYDGKDYIALNEDLRSWTAADTAQTORKWEAAREAEQR
RAYLEGECEVWELRRYLENGKKLERADPKTRHVTTHRPISDHEATLRWALGFYPAEITLT
WORDGEDQTDTELVETRPAGDRIFQKWAIVVPSGEEQRYTCHVQHEGLPKPLTLRWEP
SSQSTVPVIGIVAGLAVLAVVVIGAVVAVMCRKSSGGKGGSYQAACSDSAQGSQSDVSL
TA
```

保存

保存: BatchFile

文件名(N): example

文件类型(T): HTML (*.html)

保存 取消

Option

E-Value < 0.01

Species All

Types All

Example

Clear

Save

Visualize

Submit

References

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Release Note

1. June 22th, 2014, the GPS-Lipid 1.0 was released. The online service was developed with PHP + MySQL + JavaScript, while the local packages were developed in JAVA 1.6 (J2SE 6.0). Several redundant entries were removed, with 738 lipid-modified proteins in GPS-Lipid 1.0.