Last updated: 2012-08-20

pLabel (v2.4) User's Manual

by Bioinformatics Group, Institute of Computing Technology, Chinese Academy of Sciences

Tel: 86-10-62601016

Email: zhangkun01@ict.ac.cn, pfind@ict.ac.cn

TABLE OF CONTENTS

1 Overview

The pLabel software tool is developed with two main purposes: (1) to aid users in manually validating peptide identifications via tandem mass spectrometry (MS/MS) through visualization of fragmentation patterns and representation of peptide-spectral match indices, and then to record these indices for the subsequent automatic validation by learning decision-tree model; and (2) to provide an interactive graphical user interface for users to accurately label spectral peaks with corresponding fragment ions once the peptide identification is verified, and then to store detailed fragmentation information for data mining to further deeply and quantitatively investigate the peptide fragmentation mechanisms.

In experiments of protein identification via tandem mass spectrometry, a considerable number of false positives usually exist in peptide identification results. This may be due to chemical contaminants, electrical noises, low mass accuracy, unanticipated post translational modifications (PTMs), or unexpected fragmentation patterns in MS/MS spectra. As a result, manual validation is a vital step in the MS/MS-based proteomics analysis. Meanwhile, more and more identified MS/MS spectra libraries have been constructed and have facilitated the exploratory of peptide fragmentation by data mining methods. The first step toward accomplishing this goal is accurate spectral peak labeling in a high-throughput manner. The remainder of this document shows how to use pLabel step by step.

2 Install Your Own pLabel

pLabel runs on windows operating systems,   you can download the latest setup file here: http://pfind.ict.ac.cn/pLabel_Download.htm.

3 Graphical User Interface

pLabel can be started by clicking the icon pLabel.exe . A friendly interactive graphical user interface (GUI) of pLabel is shown below.
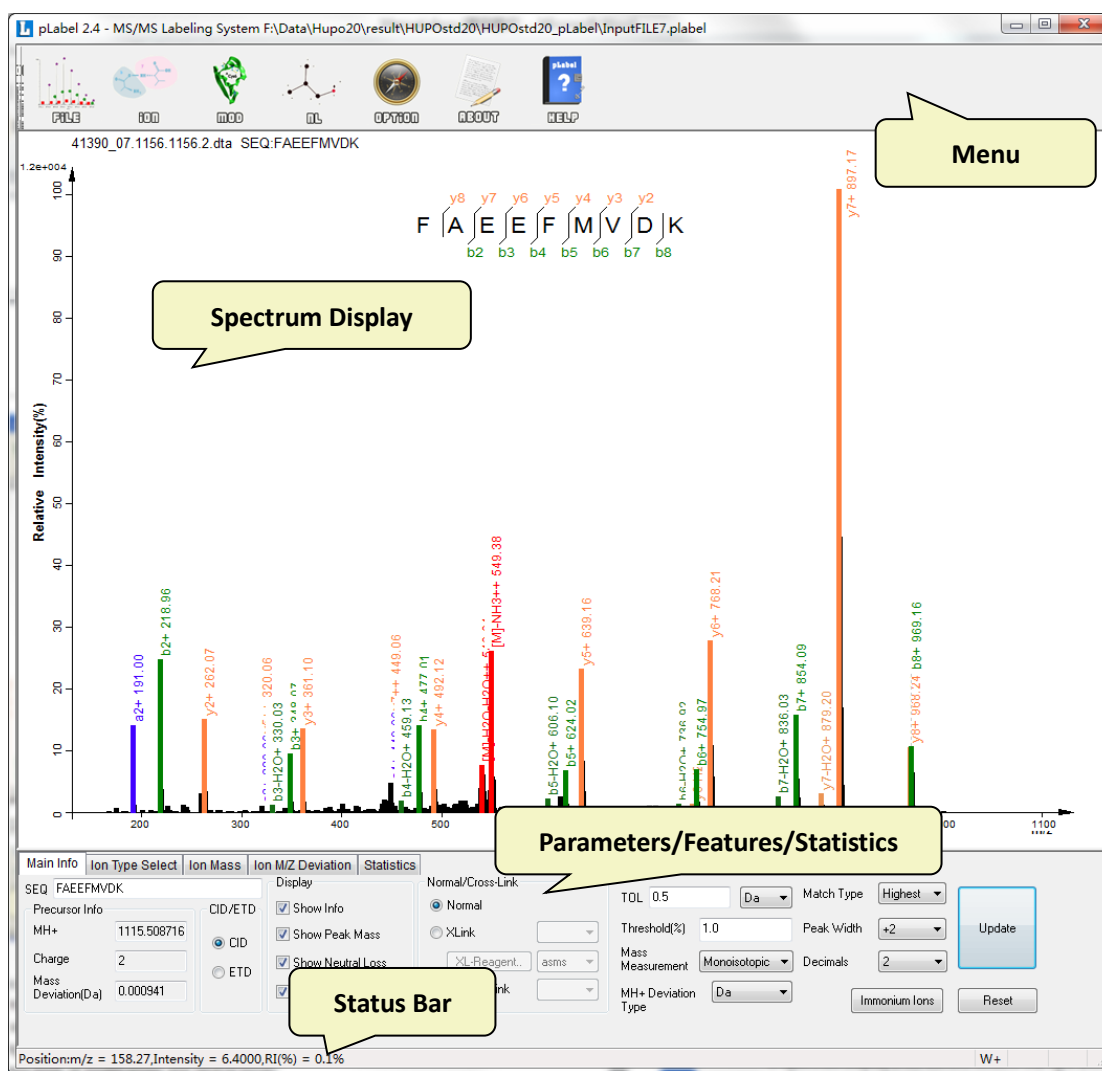
Fig.1 Graphic user interface

The main features of the interface are as follows:

(1) Highlighting matched peaks with different colors in a spectrum with known peptide identification according to a set of user-customized fragment ion types.

(2) Supporting large scale spectra processing.

(3) Supporting many kinds of modifications and neutral losses.

(4) Supporting cross-linking peptide.

4 Highlights of Functionality

4.1 Opening a spectrum file

pLabel 2.4 supports following types of file: DTA file (.dta), MS2 file (.ms2), Mascot Generic Format (.mgf). By clicking **Load MS/MS File** in **File** menu, you can get your file selected. To avoid a huge number of DTA files, clicking **Load DTAs** would be a better choice.
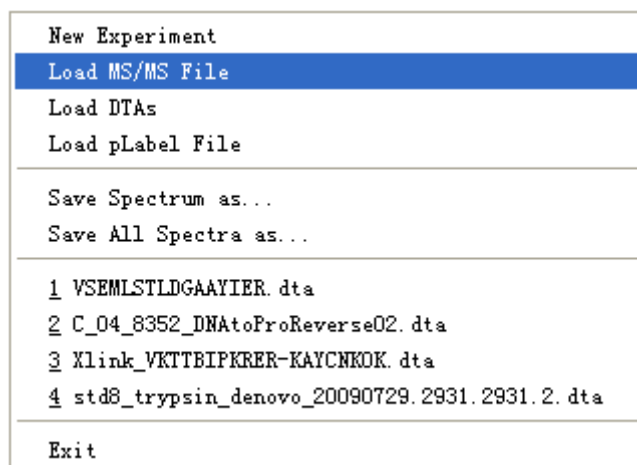
```
New Experiment
Load MS/MS File
Load DTAs
Load pLabel File

Save Spectrum as...
Save All Spectra as...

1 VSEMLSTLDGAAYIER.dta
2 C_04_8352_DNAtoProReverse02.dta
3 Xlink_VKTTBIPKRER-KAYCNKOK.dta
4 std8_trypsin_denovo_20090729.2931.2931.2.dta

Exit
```

Fig.2 Load files

pLabel file (.plabel) is our own file format for labeling spectra with amino acid sequences given by search engines, which is generated by *pBuild*.

A sample plabel file may begin like this (**Fig.3**):

```
[FilePath]
File_Path=G:\testdata\data\n2-090721-etd-ltq-rep1-01.ms2
[Modification]
1=Oxidation_M
2=Carbamidomethyl_C
[Total]
total=5204
[Spectrum1]
name=N2-090721-ETD-LTQ-REP1-01.13.13.2.DTA
pep1=0 EDKAFCK 0.32
[Spectrum2]
```

Fig.3 A sample plabel file

4.2 Open a batch file for multi-spectra processing

In **Spectrum Display** area, buttons listed in the right are used for multi-spectra processing. Getting **All** peptides identified, loading previous (<)/next (>) spectrum (Mouse Wheel), enlarging (+)/narrowing (-) the current spectrum (Alt + Mouse Wheel) do help you out of manual check (**Fig.4**).

Fig.4 Select a spectrum

Spectra are listed in alphabetical order, and formed in groups. Each group is named by its first spectrum, and has 1,000 spectra. To select a spectrum just by clicking the corresponding file name or typing a key word then clicking [Search].

4.3 Labeling a spectrum

4.3.1 Setting parameters

Fig.5 Parameters setting area

SEQ: Your peptide sequence, only characters 'A'- 'Z', a'- 'z', '-' are allowed. For example, a normal peptide sequence will be like this: "VSEMLSTLDGAAYIER", a cross-linking peptide like "VSEMLSTLDK-GAAYIER" is also a valid sequence.

Precursor Info: Precursor charge, precursor mass(MH+) and precursor mass deviation are listed here, they can not be edited. Click "Mass Deviation" to change between Da/ppm mode.

CID/ETD: activation type

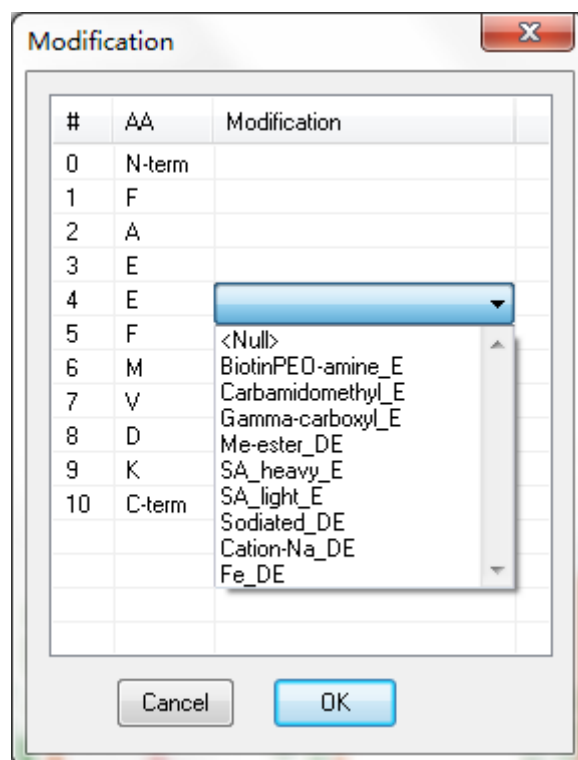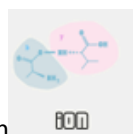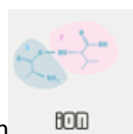**Display:** information on matched peaks

Normal/Cross-link:

Normal	

Cross-link	Xlink	
	Mono	
	Loop	

TOL: The peaks within which will be taken into account.

Threshold: Peaks under this threshold will not be matched.

Mass Measurement: mono/average

Match Type: In the highest mode, the most intensive peak in the tolerance window of a theoretical peak is selected as a matched peak. While in the nearest mode, the peak which is nearest to the theoretical peak is selected.

4.3.2 Set modifications

Setting modifications for an amino acid just by clicking button , then a setting window will be shown (**Fig.6**):

Fig.6 Modification setting dialog

Inserting a new modification, first click the button , then select "Mass Config" in the list (**Fig.7**), finally add your own modification in the dialog, see **Fig.8**.
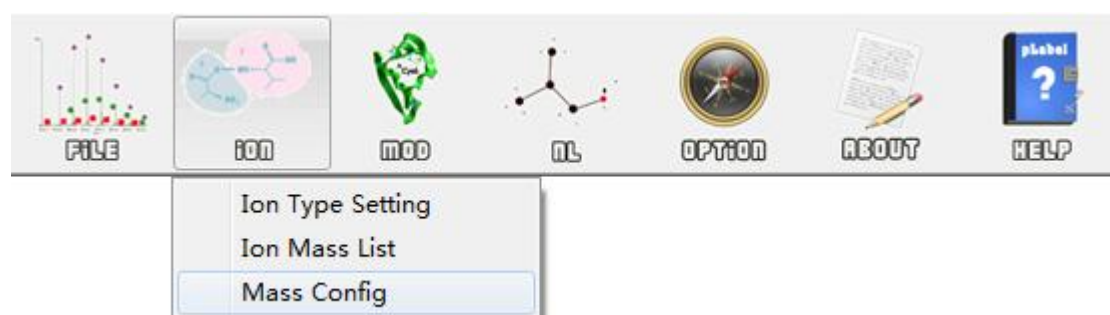
Fig.7 Ion setting
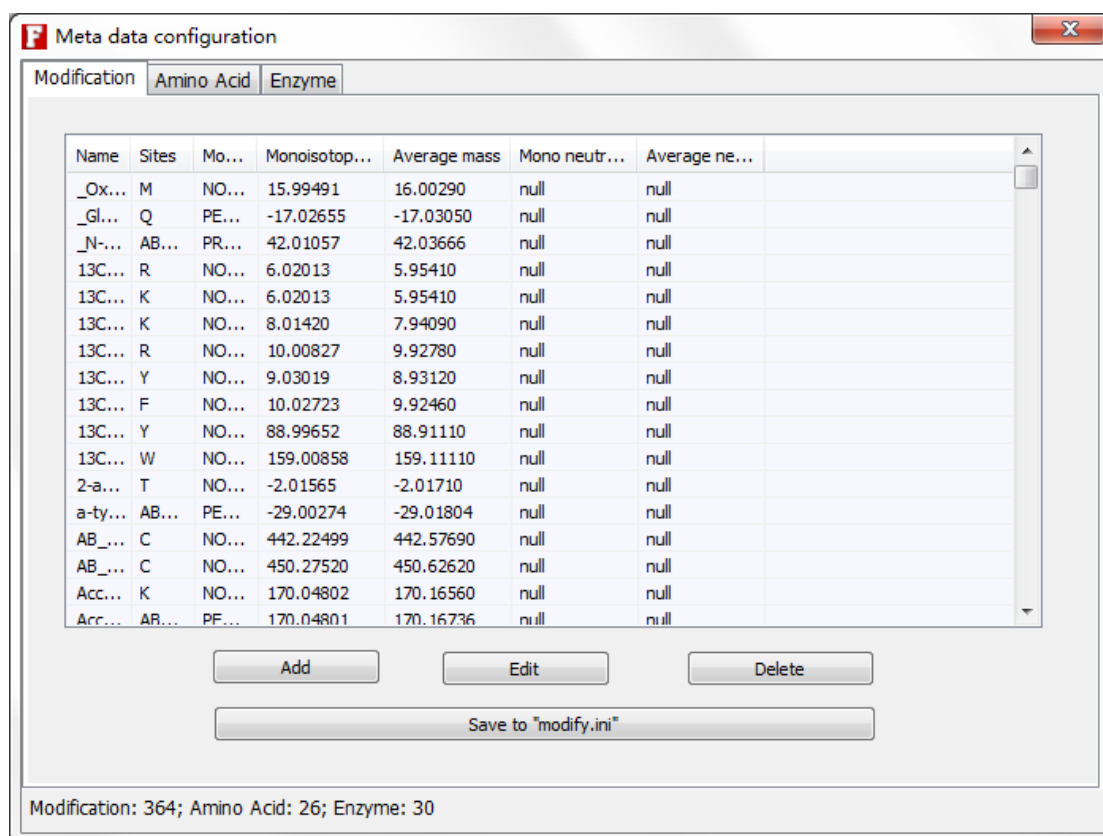
Fig.8 Add new modification

4.3.3 Choosing fragment ion types

Fig.9 Choose ion types

pLabel will use the specified ion types to label peaks in the spectrum display area.

4.3.4 Add new neutral lost

pLabel supports user to define new neutral lost. Try this function by clicking button .

4.4 Statistics

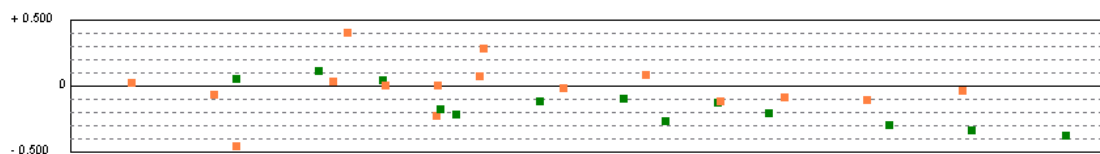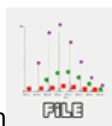"Ion M/Z deviation" area shows all matched peaks' deviations (**Fig. 10**).

Fig.10 Ion m/z deviation for one spectrum

pLabel also allows you to evaluate an experiment, get all ion deviations (**Fig.11**).

Fig.11 Statistic

4.5 Saving pictures

This function lets you to save a picture (.bmp, .jpg, .png, .tiff) for one dta or pictures for an

experiment. Click button , select "Save Spectrum As"/"Save All Spectrum As", then

follow the steps.

5 Contact Us

In case of any problem, please contact us by:

Phone #: 86-10-62601016

E-mail: zhangkun01@ict.ac.cn, pfind@ict.ac.cn

Homepage of pFind studio: http://pfind.ict.ac.cn

Address: Institute of Computing Technology, Chinese Academy of Sciences. No. 6, Kexueyuan South Road, Zhongguancun, Haidian District, Beijing 100190, P.R.China