

pLabel User Guide

Version 2.4.0.5

Released time: 2017.12.22

pFind Group

2017.12

Contents

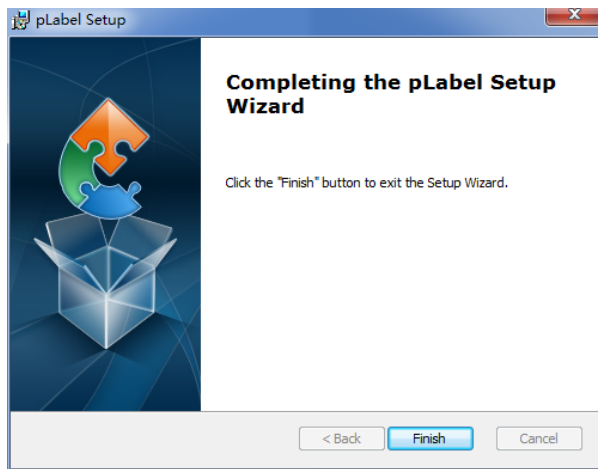
- ❖ **Software installation**
- ❖ **Opening a spectrum file**
- ❖ **Labeling a spectrum**
- ❖ **Saving pictures**

Software installation

- ❖ **pLabel is written by C++.**
- ❖ **Microsoft Visual C++ environment is required.**
- ❖ **pLabel runs on windows operating systems.**
- ❖ **Latest setup file download link:**
 - **<http://pfind.ict.ac.cn/software/pLabel/index.html>**

Installation completed

❖ Click “pLabel.exe” to run pLabel.



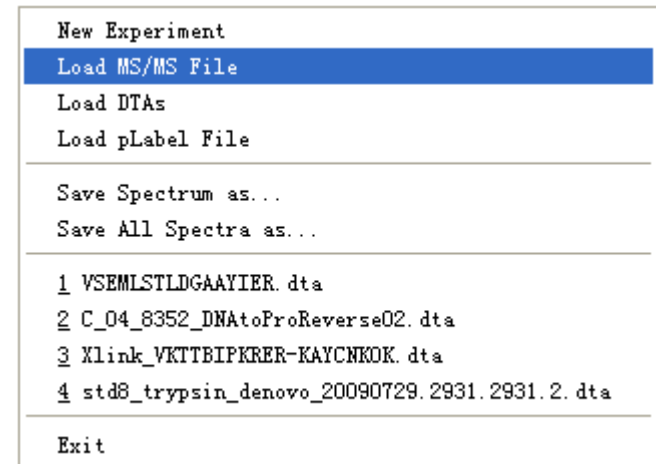
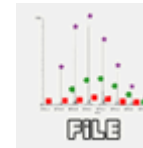
System (C:) > Program Files (x86) > pFind > pLabel > bin

共享 ▾ 刻录 兼容性文件 新建文件夹

名称	修改日期	类型	大小
aa.ini	2010/4/1 17:38	配置设置	2 KB
AniGIF.ocx	1998/12/5 7:18	ActiveX 控件	168 KB
AniGIF2.lic	2010/7/8 15:55	License	1 KB
enzyme.ini	2010/4/1 17:38	配置设置	1 KB
MassConfig.exe	2010/4/1 17:38	应用程序	112 KB
MFC71.dll	2003/3/19 13:20	应用程序扩展	1,036 KB
mfc110.dll	2013/6/9 19:53	应用程序扩展	4,318 KB
modification.ini	2016/7/25 17:30	配置设置	153 KB
modify.ini	2013/11/29 14:36	配置设置	145 KB
msvc71.dll	2003/3/19 12:14	应用程序扩展	488 KB
msvc7110.dll	2012/11/6 2:20	应用程序扩展	523 KB
msvcr71.dll	2003/2/21 20:42	应用程序扩展	340 KB
msvcr110.dll	2012/11/6 2:20	应用程序扩展	855 KB
NL.ini	2010/4/1 17:38	配置设置	1 KB
pLabel.exe	2017/12/22 10:53	应用程序	2,496 KB
regsvr32.exe	2008/6/9 7:00	应用程序	14 KB
ResultParser.dll	2010/4/17 14:47	应用程序扩展	80 KB
scheme.ini	2012/8/20 21:15	配置设置	1 KB
sumo.ini	2017/12/19 20:32	配置设置	1 KB
xlink.ini	2017/12/22 11:20	配置设置	2 KB

Opening a spectrum file

- ❖ Clicking “Load MS/MS File” in File menu.
- ❖ pLabel 2.4 supports DTA file (.dta), MS2 file (.ms2), Mascot Generic Format (.mgf).
- ❖ To avoid a huge number of DTA files, clicking Load DTAs would be a better choice.



pLabel file format

- ❖ 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:

```
[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]
```


Graphic user interface

The screenshot displays the pLabel 2.4 software interface. At the top, a menu bar contains icons for File, Edit, View, Tools, Help, and a question mark icon labeled "Menu". The main window shows a mass spectrum plot with "Relative Intensity (%)" on the y-axis (0 to 100) and "m/z" on the x-axis (0 to 1600). The base peak is at m/z 589.3. A peptide sequence is shown above the spectrum: $K|V|P|Q|V|S|T|P|T|L|V|E|V|S|R$. The sequence is annotated with b-ions (b1 to b10) and y-ions (y1 to y10). A callout box labeled "Spectrum Display" points to the plot. Below the spectrum is a control panel with sections for "Main Info", "Ion Type Select", "Ion Mass", "Ion M/Z Deviation", "Statistics", "Display", "Normal/Cross-Link", "TOL", "Match Type", "Threshold", "Peak Width", "Mass Measurement", "Decimals", "MH+ Deviation Type", "Immunium Ions", and "Update/Reset" buttons. A callout box labeled "Parameters/Features/Statistics" points to this panel. At the bottom, a status bar shows "Position: m/z = 589.328, Intensity = 302837.3000, RI(%) = 97.77%", "y5+", "Matched Intensity = 48.77%", and "SCRL". A callout box labeled "Status Bar" points to this information.

Labeling a spectrum

❖ Setting Parameters-1:

The screenshot shows the 'Setting Parameters-1' interface. The 'Main Info' section is highlighted with a green border and contains the following fields:

- SEQ: KVPQVSTPTLVEVSR
- Precursor Info: MH+ 1639.933569, Charge 3, Mass Deviation(Da) -0.004152
- CID/ETD: CID (selected)
- Display: Show Info, Show Peak Mass, Show Neutral Loss, Show AA Sequence (all checked)

The other sections include:

- Ion Type Select: Normal (selected)
- Ion Mass: SUMO (selected)
- Ion M/Z Deviation: A6 (selected)
- Statistics: TOL 20 ppm, Match Type Highest, Threshold(%) 1.0, Peak Width +2, Mass Measurement Monoisotopic, Decimals 1, MH+ Deviation Type Da

Buttons: Update, Reset, Immonium Ions.

SEQ: Peptide sequence, only characters ‘A’- ‘Z’, ‘a’- ‘z’, ‘-’ are allowed.

Precursor Info: Precursor charge, precursor mass(MH+) and precursor mass deviation are listed here, they can not be edited.

CID/ETD: Activation type.

Display: Spectrum number, AA Sequence and other information.

Labeling a spectrum

❖ Setting Parameters-2:

Main Info	Ion Type Select	Ion Mass	Ion M/Z Deviation	Statistics
SEQ KVPQVSTPTLVEVSR				
Precursor Info			Display	
MH+ 1639.933569	CID/ETD		<input checked="" type="checkbox"/> Show Info	
Charge 3	<input checked="" type="radio"/> CID		<input checked="" type="checkbox"/> Show Peak Mass	
Mass Deviation(Da) -0.004152	<input type="radio"/> ETD		<input checked="" type="checkbox"/> Show Neutral Loss	
			<input checked="" type="checkbox"/> Show AA Sequence	
Normal/Cross-Link				
<input checked="" type="radio"/> Normal				
<input type="radio"/> XLink				
XL-Reagent SUMO				
Possible link A6				
TOL 20 ppm	Match Type Highest	Update		
Threshold(%) 1.0	Peak Width +2			
Mass Measurement Monoisotopic	Decimals 1			
MH+ Deviation Type Da	Immonium Ions	Reset		

Normal/Cross-Link:

Normal		$ \begin{array}{cccccc} & y5 & y4 & y3 & y2 & y1 \\ E & \int L & \int C & \int D & \int I & \int R \\ & b1 & b2 & b3 & b4 & b5 \end{array} $
Cross-link	Xlink	$ \begin{array}{cccccccccc} & y10 & y9 & y8 & y7 & y6 & y5 & y4 & y3 & y2 \\ V & \int K & \int T & \int T & \int B & \int I & \int P & \int K & \int R & \int E & \int R \\ & b1 & b2 & b3 & b4 & b5 & b6 & b7 & b8 & b9 & b10 \\ & & & & & & & & & & \\ & & & & & & & & & & \\ K & \int A & \int Y & \int C & \int N & \int K & O & K \\ & b1 & b2 & b3 & b4 & b5 \end{array} $
	Mono	$ \begin{array}{cccccc} & y7 & y6 & y5 & y4 & y3 \\ K & \int A & \int Y & \int C & \int N & \int K & O & K \\ & b1 & b2 & b3 & b4 & b5 \end{array} $
	Loop	$ \begin{array}{cccccccc} & y10 & & & & & y3 & y2 \\ V & \int K & T & T & B & I & P & K & \int R & \int E & \int R \\ & b1 & & & & & & & b8 & b9 & b10 \end{array} $

Labeling a spectrum

❖ Setting Parameters-3:

The screenshot shows a software interface with several tabs: 'Main Info', 'Ion Type Select', 'Ion Mass', 'Ion M/Z Deviation', and 'Statistics'. The 'Main Info' tab is active, displaying sequence information (SEQ: KVPQVSTPTLVEVSR) and precursor data (MH+, Charge, Mass Deviation). The 'Ion M/Z Deviation' tab is also visible, showing 'Display' options (Show Info, Show Peak Mass, Show Neutral Loss, Show AA Sequence) and 'Normal/Cross-Link' settings (Normal, XLink, XL-Reagent, Possible link). The 'Update' button is highlighted with a green rounded rectangle.

TOL: The peaks within which will be taken into account. Click the button on the right to change between Da/ppm mode.

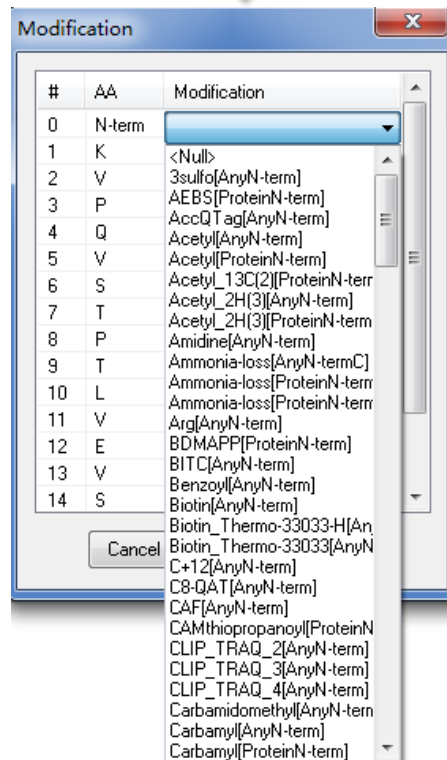
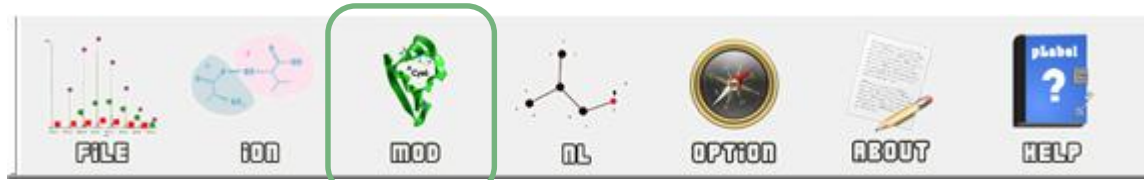
Threshold: Peaks under this threshold will not be matched.

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.

Labeling a spectrum

❖ Choosing Modifications:



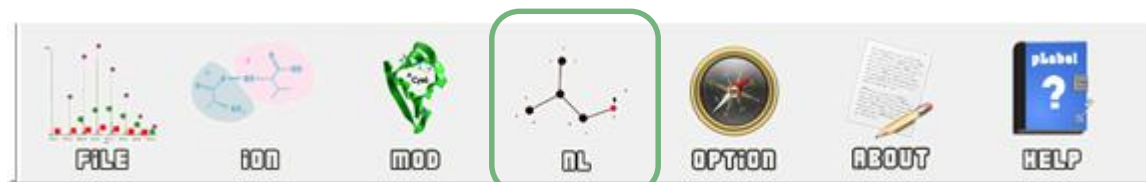
Labeling a spectrum

- ❖ Choosing fragment ion types:
- ❖ pLabel will use the specified ion types to label peaks in the spectrum display area.

	Ion Type Select			Ion Mass			Ion M/Z Deviation			Statistics								
	a	a-H2O	a-NH3	b	b-H2O	b-NH3	c	c-H2O	c-NH3	x	x-H2O	x-NH3	y	y-H2O	y-NH3	z	z-H2O	z-NH3
+	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
++	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
+++	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Labeling a spectrum

❖ Adding new neutral lost:



Neutral Loss Setting

Ion type		Charge	Lost molecular group	
#	Ion typ	Charge	#	Molecular
1	a	1	1	H2O
2	b	2	2	H2O+H2O
3	c	3	3	NH3
4	x	4	4	NH3+NH3
5	y	5	5	H2O+NH3
6	z	6	6	others

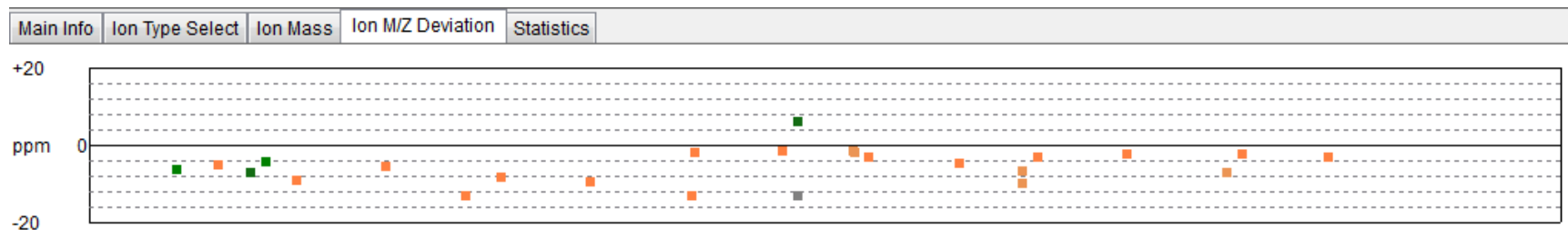
Lost type

Lost type	
b-H2O+	
b-NH3+	
y-H2O+	
y-NH3+	
[M]-H2O+	
O+C8H14NO5+	

Add
Delete
Clear
Save
OK
Cancel

Viewing deviations

❖ All matched peaks' deviations:

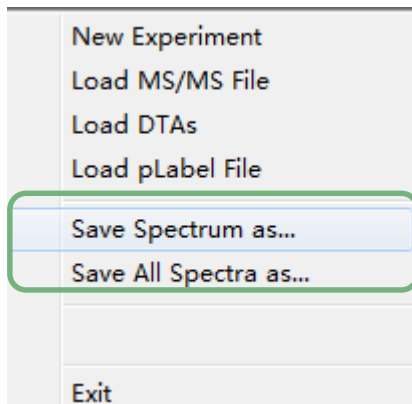
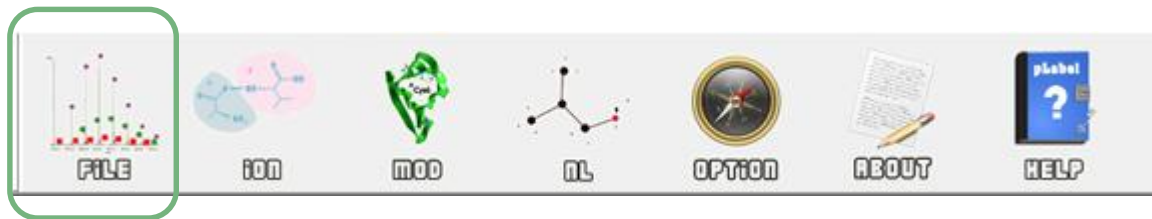


❖ Get all ion deviations:

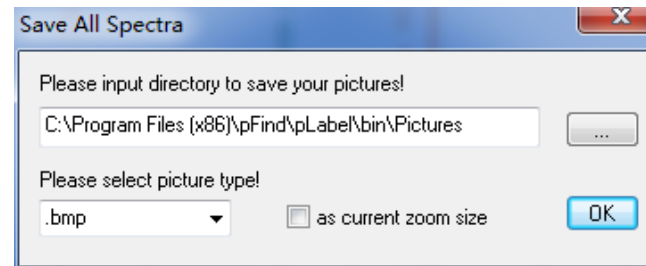
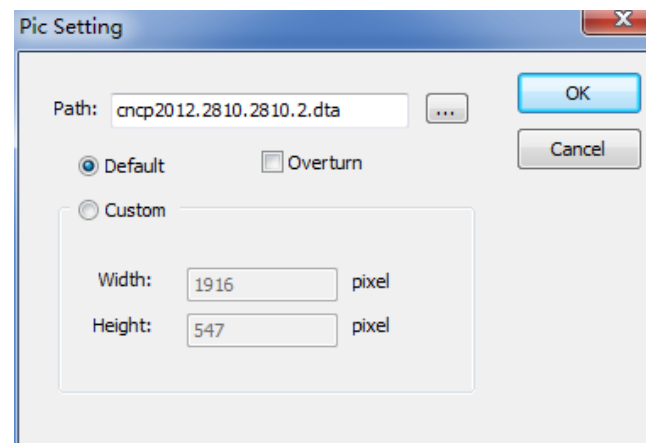
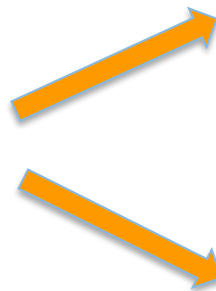
The figure shows the 'Statistics' tab in pFind Studio. It contains several input fields and buttons for generating statistical data:

- Generate Statistical File:** A text box for 'File Name' containing 'C:\Program Files (x86)\pFind\pLabel\bin\statistic.txt', followed by a browse button ('...') and a 'Make' button.
- Statistical Functions:** A text box for 'Ion Type(eg: "b")', a 'Charge' dropdown menu set to 'All', and buttons for 'Count Num' and 'Immonium Num'.
- Tolerance File:** A text box for 'Tolerance File' containing 'C:\Program Files (x86)\pFind\pLabel\bin\Tolerance.txt', followed by a browse button ('...') and a 'Make' button.
- Precursor TOL:** A button located to the right of the 'Generate Statistical File' section.

Saving Pictures



Saving one spectrum





❖ **Thank you for using pLabel.**

❖ **If you have any questions, please contact pfind@ict.ac.cn.**