

pLink 2 User Guide

Version 2.3



pFind Group

2020.01

Contents

- ❖ **Brief Introduction**
- ❖ **Installation Requirement**
- ❖ **Software Installation**
- ❖ **Quick Start**
- ❖ **Result Report**
- ❖ **Appendix**

Brief Introduction

- ❖ pLink is an engine for cross-link peptide identification, including peptides with chemical crosslinking, endogenous crosslinking and sumoylation.
- ❖ The main form of peptide to be identified is inter-linked peptide. Besides, it also supports other forms, such as mono-linked peptides, loop-linked peptides and so on.
- ❖ The current version is 2.3 which is ~40 times faster than pLink 1.

Installation Requirement

❖ Hardware

- CPU: 2.0 GHz or higher
- Memory: 4 GB or higher recommended
- Hard Disk: ~50 MB for software storage and an extra disk space to store the results and temporary files

❖ Software

- Operating system: Windows 7/8/10, **64 bit version**
- [.NET Framework 4.5.2](#)
- [MSFileReader](#), **3.0 SP2 or below**, both 32 bit and 64 bit version, pLink 2 uses MSFileReader to access RAW files
- [Java 8](#), **64 bit version**, pLink 2 needs Java environment for quantification

Software Installation

- ❖ Double click pLink2.exe and install it in the chosen directory.
- ❖ Fill in personal information in the pLink License Dialog.
- ❖ Copy and send the information to pLink@ict.ac.cn to get the license.
- ❖ Click “Import the license file” browse and import the license.
- ❖ Finally, restart pLink 2.

pLink License Dialog

*Activation code: 869958008B7BF854CBE160DA091AD9D4

*User name: First name Last name

*University / Company:

*Work email address:

*Lab leader / Supervisor:

*Supervisor's email:

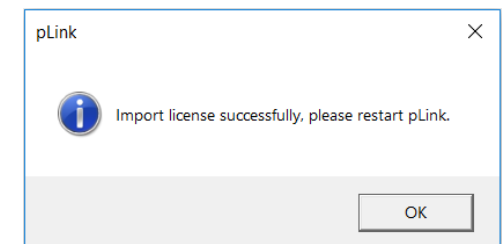
*Country / Region: China

*How do you hear about pLink?
For example, colleagues: which lab do they come from, conference: the name of the conference, publication: the title of the publication.

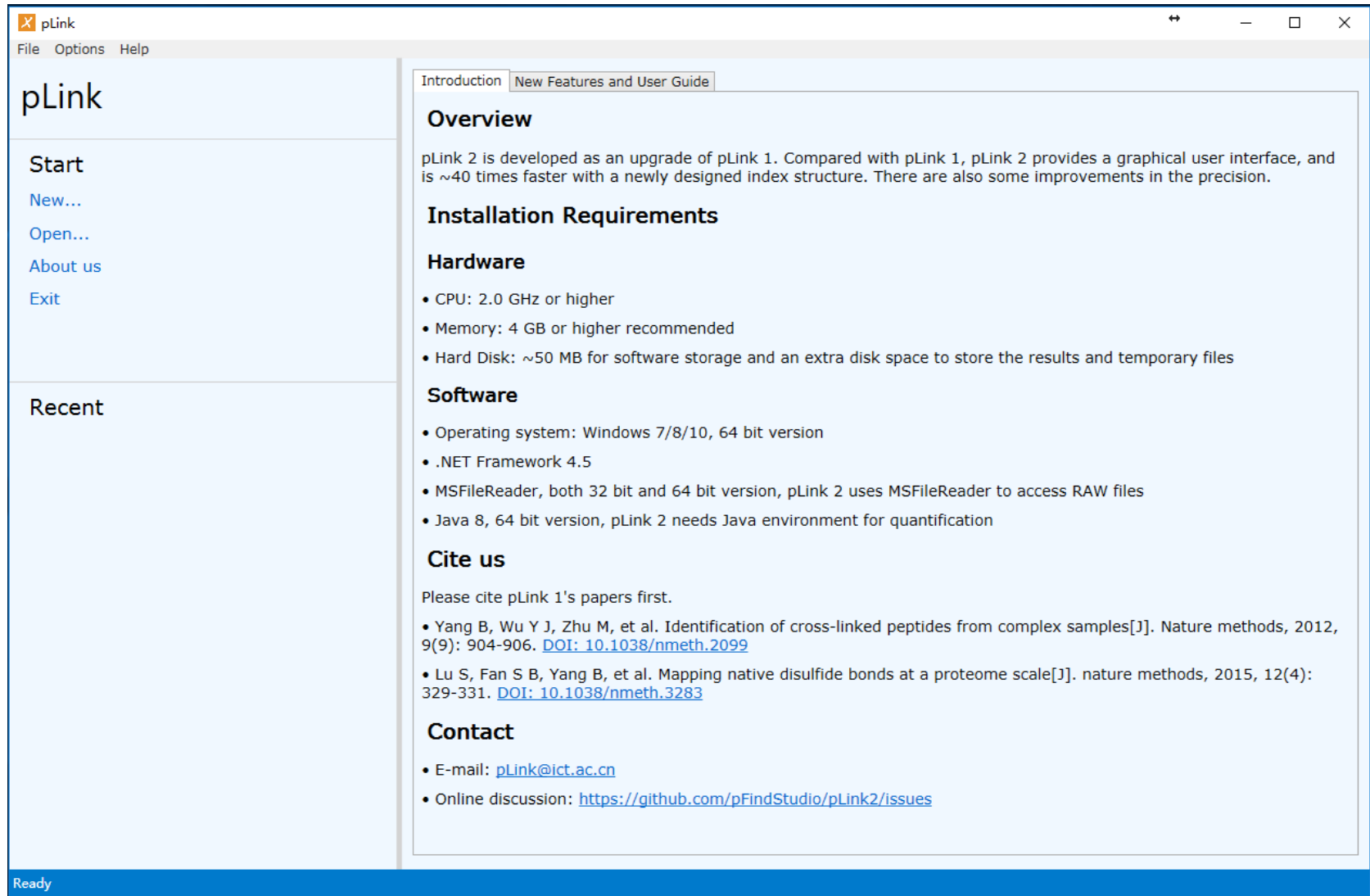
*What can pLink do for you?
You can share your interesting research with us here, for example what kind of cross-linking you are analyzing.

Notice:
1. All items must be filled.
2. For Chinese, please fill in the table with Chinese, thanks.
3. Please email this table to pLink@ict.ac.cn to get the license.
4. Please let us know if you use pLink in your publication.

Copy to clipboard Import the license file



Main interface of pLink 2



The screenshot displays the main interface of pLink 2. The window title is "pLink" and the menu bar includes "File", "Options", and "Help". The interface is divided into a left sidebar and a main content area.

Left Sidebar:

- pLink**
- Start**
 - New...
 - Open...
 - About us
 - Exit
- Recent**

Main Content Area:

Introduction | **New Features and User Guide**

Overview

pLink 2 is developed as an upgrade of pLink 1. Compared with pLink 1, pLink 2 provides a graphical user interface, and is ~40 times faster with a newly designed index structure. There are also some improvements in the precision.

Installation Requirements

Hardware

- CPU: 2.0 GHz or higher
- Memory: 4 GB or higher recommended
- Hard Disk: ~50 MB for software storage and an extra disk space to store the results and temporary files

Software

- Operating system: Windows 7/8/10, 64 bit version
- .NET Framework 4.5
- MSFileReader, both 32 bit and 64 bit version, pLink 2 uses MSFileReader to access RAW files
- Java 8, 64 bit version, pLink 2 needs Java environment for quantification

Cite us

Please cite pLink 1's papers first.

- Yang B, Wu Y J, Zhu M, et al. Identification of cross-linked peptides from complex samples[J]. Nature methods, 2012, 9(9): 904-906. DOI: [10.1038/nmeth.2099](https://doi.org/10.1038/nmeth.2099)
- Lu S, Fan S B, Yang B, et al. Mapping native disulfide bonds at a proteome scale[J]. nature methods, 2015, 12(4): 329-331. DOI: [10.1038/nmeth.3283](https://doi.org/10.1038/nmeth.3283)

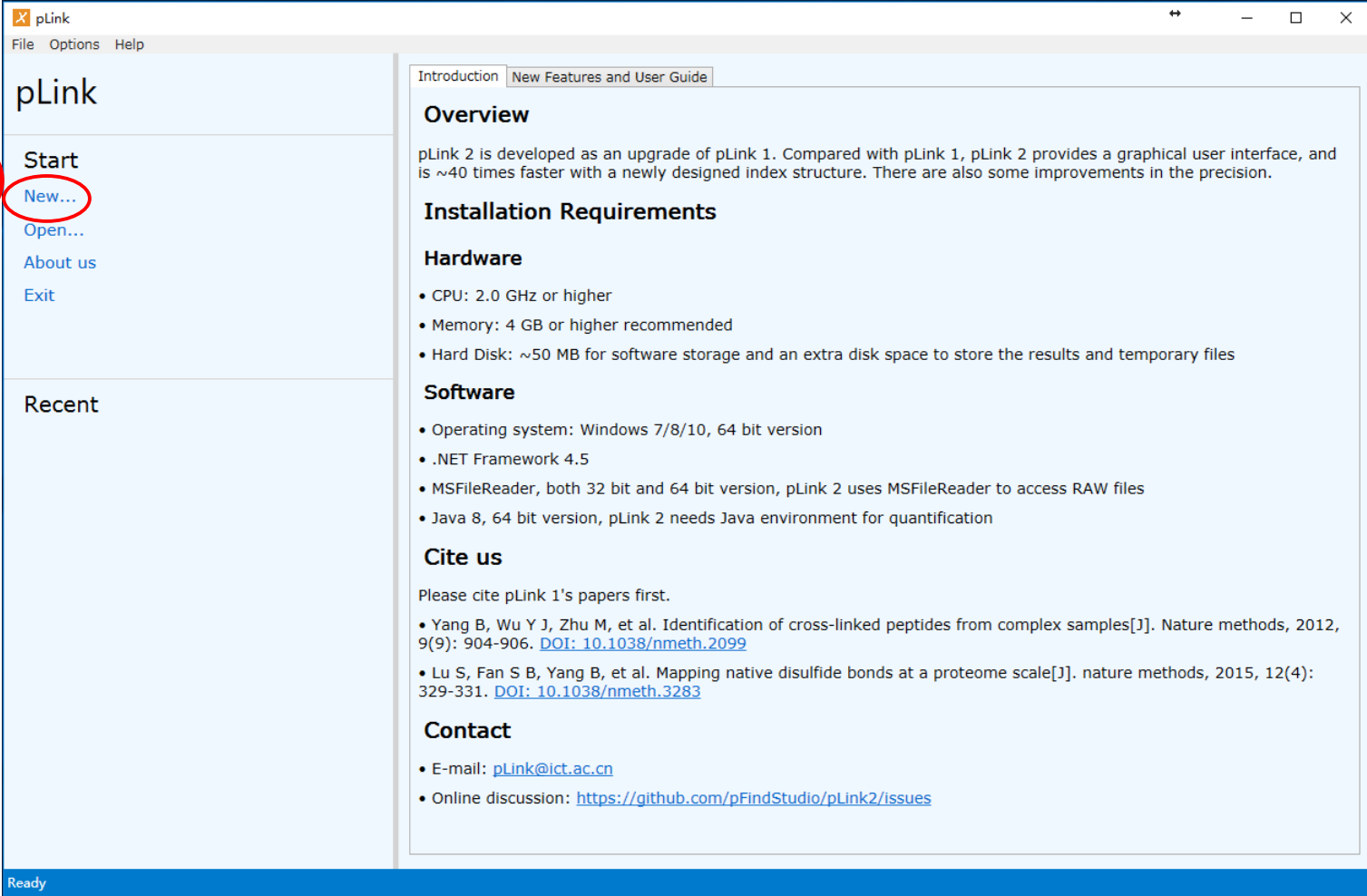
Contact

- E-mail: pLink@ict.ac.cn
- Online discussion: <https://github.com/pFindStudio/pLink2/issues>

Ready

Create a new search task

❖ Click New...



The screenshot shows the pLink application window. The title bar reads 'pLink' and the menu bar includes 'File', 'Options', and 'Help'. The main window is divided into three sections: a left sidebar, a top navigation bar, and a main content area. The sidebar has a 'Start' section with a red circle and the number '1' next to the 'New...' option, which is also circled in red. Below 'Start' are 'Open...', 'About us', and 'Exit'. The 'Recent' section is empty. The top navigation bar has 'Introduction' and 'New Features and User Guide' tabs. The main content area displays the 'Overview' section of the user guide, followed by 'Installation Requirements', 'Hardware', 'Software', 'Cite us', and 'Contact' sections.

Start

- New...
- Open...
- About us
- Exit

Recent

Ready

Introduction | New Features and User Guide

Overview

pLink 2 is developed as an upgrade of pLink 1. Compared with pLink 1, pLink 2 provides a graphical user interface, and is ~40 times faster with a newly designed index structure. There are also some improvements in the precision.

Installation Requirements

Hardware

- CPU: 2.0 GHz or higher
- Memory: 4 GB or higher recommended
- Hard Disk: ~50 MB for software storage and an extra disk space to store the results and temporary files

Software

- Operating system: Windows 7/8/10, 64 bit version
- .NET Framework 4.5
- MSFileReader, both 32 bit and 64 bit version, pLink 2 uses MSFileReader to access RAW files
- Java 8, 64 bit version, pLink 2 needs Java environment for quantification

Cite us

Please cite pLink 1's papers first.

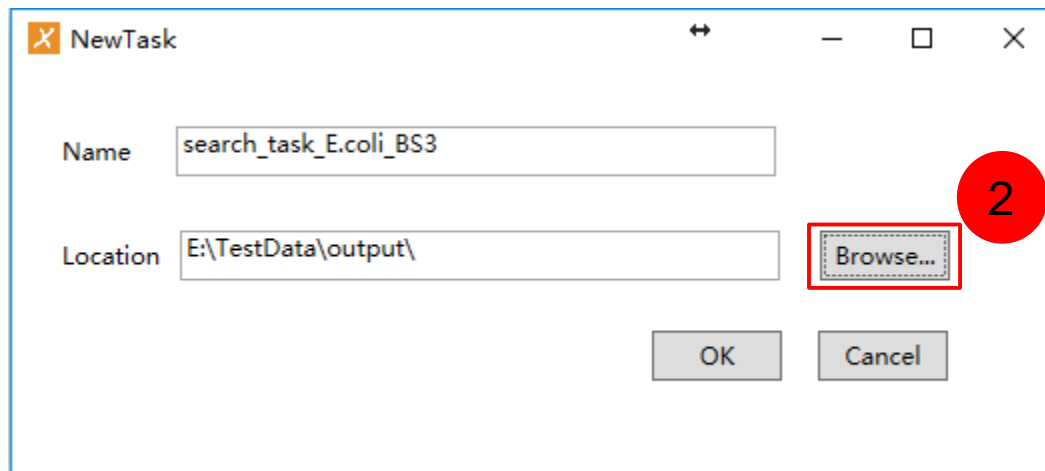
- Yang B, Wu Y J, Zhu M, et al. Identification of cross-linked peptides from complex samples[J]. Nature methods, 2012, 9(9): 904-906. DOI: [10.1038/nmeth.2099](https://doi.org/10.1038/nmeth.2099)
- Lu S, Fan S B, Yang B, et al. Mapping native disulfide bonds at a proteome scale[J]. nature methods, 2015, 12(4): 329-331. DOI: [10.1038/nmeth.3283](https://doi.org/10.1038/nmeth.3283)

Contact

- E-mail: pLink@ict.ac.cn
- Online discussion: <https://github.com/pFindStudio/pLink2/issues>

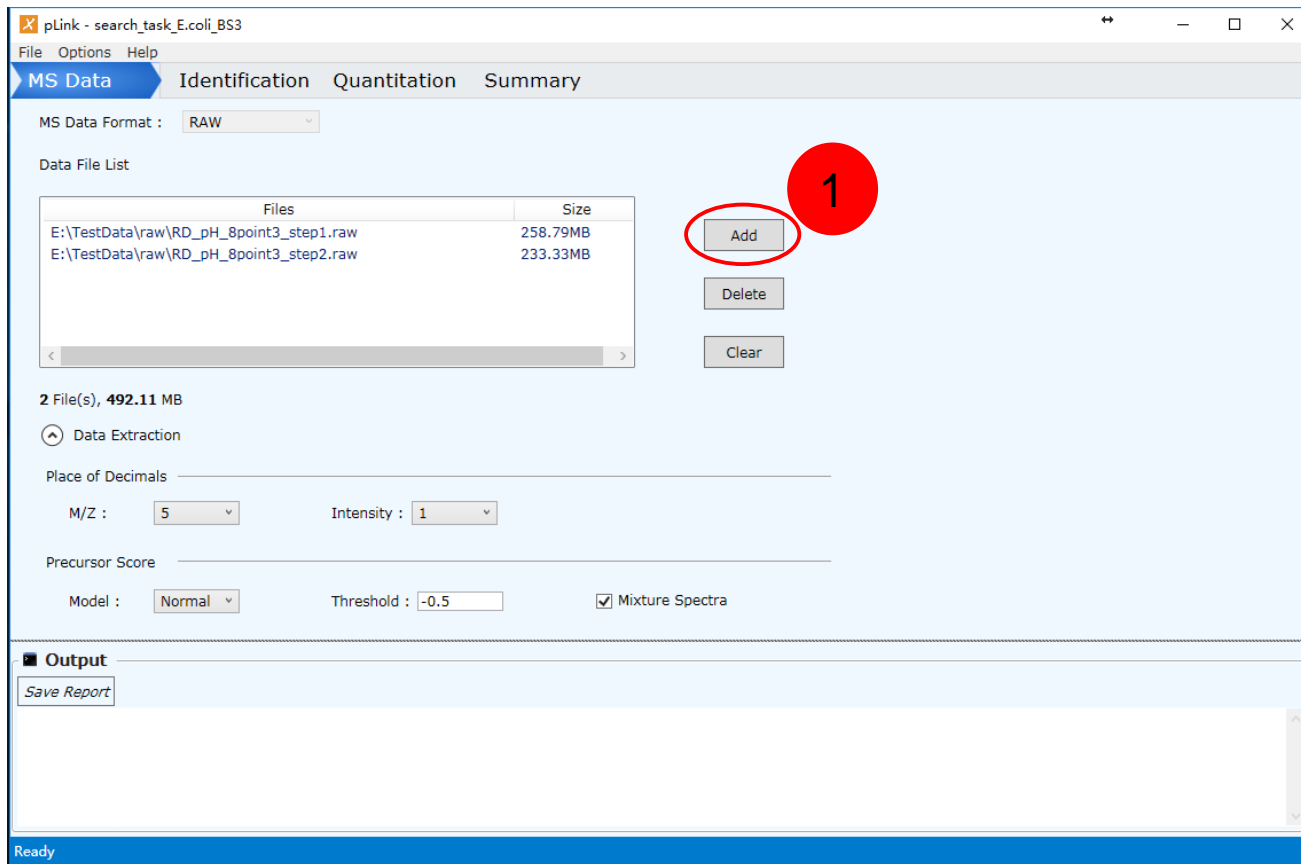
Create a new search task

- ❖ Fill the task name and browse the task location



Import data

- ❖ You can choose MS data format and other data preprocessing type in MS Data panel.



Set search parameters

❖ A) Select flow type and cross linker

The screenshot displays the pLink software interface for a search task named 'pLink - search_task_E.coli_BS3'. The 'Identification' tab is active, showing search parameters for MS Data. The 'Flow' section is expanded, revealing the 'Flow Type' dropdown menu set to 'Conventional Crosslinking (H)' and the 'Process Number' dropdown set to '4'. Below this, the 'Set Linkers' section contains two lists of linker names. The left list contains 'BS3', and the right list contains 'BS2G', 'BS2G_heavy', 'BS3_heavy', 'DSS', and 'EDC-DE'. A red circle '1' highlights the 'Flow Type' dropdown, and a red circle '2' highlights the left arrow button between the linker lists. The 'Database Search' and 'Result Filter' sections are collapsed. The 'Output' section at the bottom shows a 'Save Report' button. The status bar at the bottom indicates 'Ready'.

Set search parameters

❖ B) Select and import database.

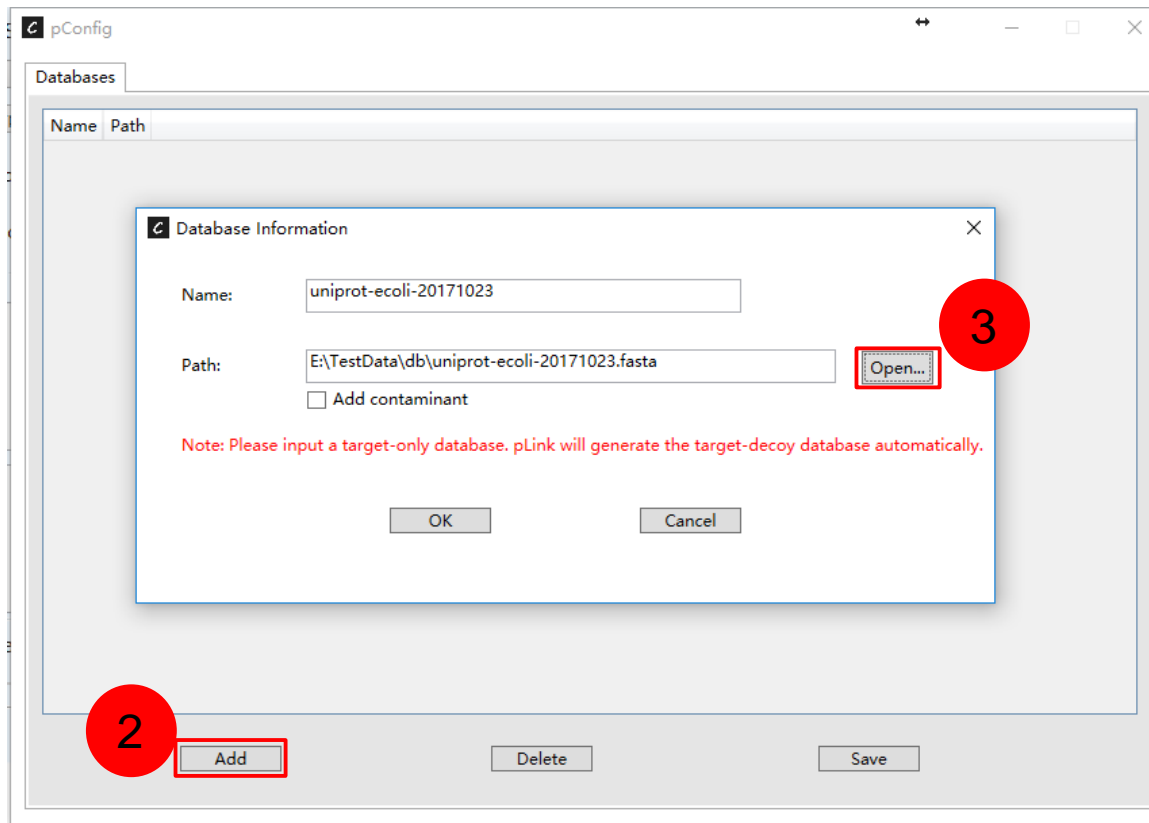
The screenshot shows the pLink software interface with the following settings:

- Flow** (expanded)
- Database Search** (expanded)
 - Database: **Customize Database...** (highlighted with a red circle and the number 1)
 - Enzyme: trypsin
 - Up to 3 missed cleavages
 - 600 ≤ Peptide Mass ≤ 6000
 - 6 ≤ Peptide Length ≤ 60
 - Precursor Tolerance ± 20 ppm
 - Fragment Tolerance ± 20 ppm
- Add Modification**
 - Fixed: (empty)
 - Variable: (empty)
 - Modification list:
 - 2-dimethylsuccinyl[C]
 - 2HPG[R]
 - 2-monomethylsuccinyl[C]
 - 2-nitrobenzyl[Y]
 - 2-succinyl[C]
 - 3-deoxyglucosone[R]
 - 3-phosphoglyceryl[K]
 - 3sulfo[AnyN-term]
 - 4AcAllylGal[C]
 - 4-ONE[C]
 - 4-ONE[H]
 - 4-ONE[K]
- Result Filter** (expanded)
- Output**
 - Save Report

Ready

Set search parameters

- ❖ B) Select and import database.
 - Add contaminated proteins to the database if it doesn't contain them.



Set search parameters

- ❖ **B) Set the appropriate peptide mass range, peptide length range, error range and modifications.**

The screenshot displays the pLink software interface for peptide identification. The window title is "pLink - search_task_E.coli_BS3". The main menu includes "File", "Options", and "Help". The "Identification" tab is active, with other tabs being "MS Data", "Quantitation", and "Summary".

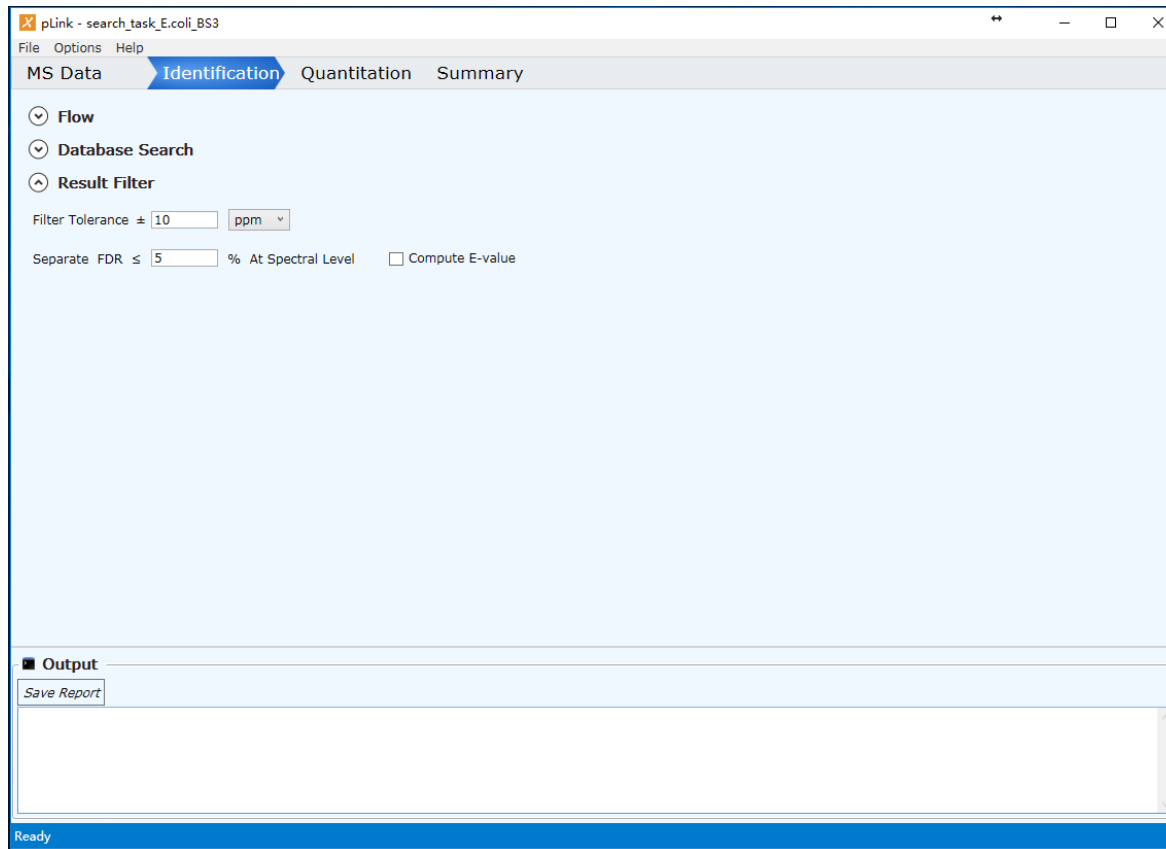
Flow (expanded):

- Database Search** (expanded):
 - Database: uniprot-ecoli-20171023
 - Enzyme: Trypsin, Up to 3 missed cleavages
 - Peptide Mass: 600 ≤ Peptide Mass ≤ 6000
 - Peptide Length: 6 ≤ Peptide Length ≤ 60
 - Precursor Tolerance: ± 20 ppm
 - Fragment Tolerance: ± 20 ppm
- Add Modification** (expanded):
 - Fixed:** Carbamidomethyl[C]
 - Variable:** Oxidation[M]
 - Available Modifications:** Oxidation[D], Oxidation[F], Oxidation[H], Oxidation[K], Oxidation[N], Oxidation[P], Oxidation[R], Oxidation[W], Oxidation[Y], Oxidation+NEM[C], OxLysBiotin[K], OxLysBiotinRed[K]
- Result Filter** (expanded)
- Output** (expanded): Save Report

The status bar at the bottom indicates "Ready".

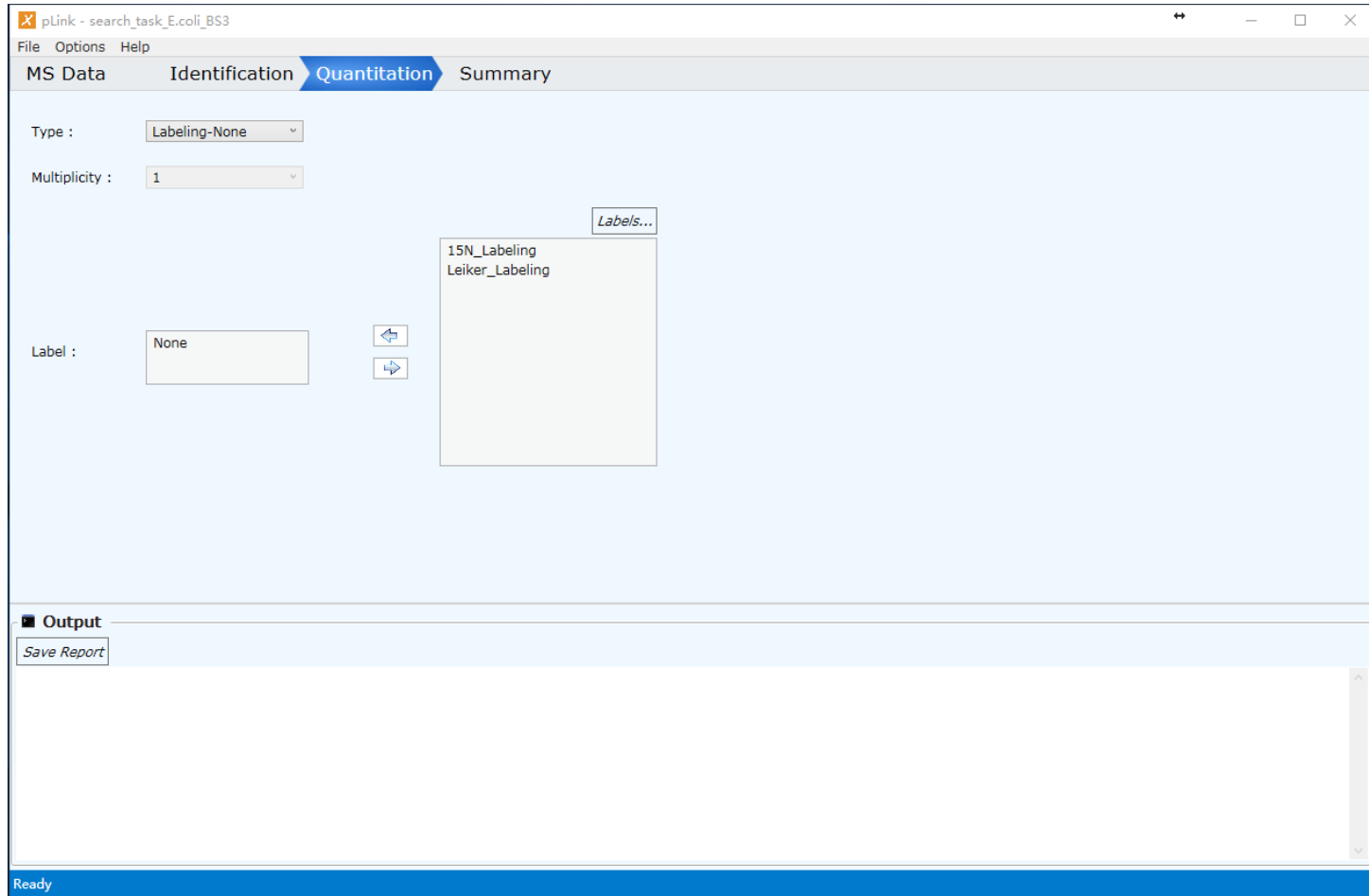
Set search parameters

- ❖ C) Set the appropriate filter tolerance and FDR.
 - As it is time-consuming to compute E-value, the default value is unchecked.



Set quantification parameters

- ❖ **15N labeling and Leiker labeling are supported if necessary**



Check parameters and run tasks

The screenshot shows the pLink software interface with the 'Summary' tab selected. The interface is divided into several sections, each with a table of properties and values. At the bottom right, there are three buttons: 'Save', 'Start', and 'Stop'. A red circle with the number '1' is placed over the 'Save' button, and a red circle with the number '2' is placed over the 'Start' button. The 'Output' section at the bottom left contains a 'Save Report' button.

MS Data

Property	Value
Format	raw
Data File List	E:\TestData\raw\RD_pH_8point3_step1.raw E:\TestData\raw\RD_pH_8point3_step2.raw
Mixture Spectra	True
Decimal Places Of M/Z	5
Decimal Places Of Intensity	1
Model	Normal
Threshold	-0.5

Search

Property	Value
Flow Type	Conventional Crosslinking (HCD)
Process Number	4
Cross-Linker(s)	BS3
Database	uniprot-ecoli-20171023
Enzymes	Trypsin
Number of Missed Cleavages	3
Peptide Mass	[600 , 6000]
Peptide Length	[6 , 60]
Precursor Tolerance	±20 ppm
Fragment Tolerance	±20 ppm
Fixed Modifications	Carbamidomethyl[C]
Variable Modifications	Oxidation[M]

Filter

Property	Value
Filter Tolerance	±10 ppm
FDR	Separate FDR ≤ 5 % At Spectral Level
Compute E-value	False

MS1 Quantitation

Property	Value
Quantitation	Labeling_None
Multiplicity	1
Label	None

Buttons: Save (1), Start (2), Stop

Output: Save Report

Ready

Searching...

```
Output
Save Report
[pLink] Welcome to use pLink v2.2.1611, it will be expired on 20190101
[pLink] Search Engine initializing...
[pLink] Generating reverse database...
[pLink] Search identifier: IPTLS
[pLink] Search Engine is ready to search.

[pLink] Start searching E:\TestData\raw\RD_pH_8point3_step1_HCDFT.pf2, Labeling None
[pLink] Total spectra: 12528
[pLink] Loaded 3133 spectra, 3133 / 12528
[pLink] Loaded 3133 spectra, 6266 / 12528
[pLink] Loaded 3133 spectra, 9399 / 12528
[pLink] Loaded 3129 spectra, 12528 / 12528
[pLink] Complete First search.

[pLink] Load File0.Tmp.All.pfd.
[pLink] there are no enough spaces to infer proteins,
try to allocate new spaces (6.10MB).
```

Running

Searching completed

```
Output
Save Report
[pLink] Load File1.Tmp.All.pfd.
[pLink] there are no enough spaces to infer proteins,
      try to allocate new spaces (6.10MB).
[pLink] Start re-ranking using SVM...
[pLink] Iteration: round 1/5
[pLink] Iteration: round 2/5
[pLink] Iteration: round 3/5
[pLink] Iteration: round 4/5
[pLink] Iteration: round 5/5
[pLink] Complete re-rank.

[pLink] Saved uniprot-ecoli-20171023_2017.12.22.File1.pfd.
[pLink] Complete Searching RD_pH_spoint3_step2_HCDFT.pfd.

[pLink] Start to generate reports...
[pLink] Complete report.
```

Ready

Contents of search results files

(E:) > TestData > output > search_task_E.coli_BS3 >

名称	修改日期	类型	大小
htmls	12/22/2017 22:53	文件夹	
images	01/05/2018 15:06	文件夹	
reports	12/26/2017 14:07	文件夹	
tmps	12/22/2017 22:53	文件夹	
general.html	12/26/2017 14:08	Chrome HTML D...	7 KB
pQuant_cfg.txt	12/23/2017 11:32	文本文档	3 KB
RD_pH_8point3_step1_HCDFT.cross-linked.BS3.plabel	12/22/2017 22:53	PLABEL 文件	8 KB
RD_pH_8point3_step1_HCDFT.loop-linked.BS3.plabel	12/22/2017 22:53	PLABEL 文件	4 KB
RD_pH_8point3_step1_HCDFT.mono-linked.BS3.plabel	12/22/2017 22:53	PLABEL 文件	22 KB
RD_pH_8point3_step1_HCDFT.regular.plabel	12/22/2017 22:53	PLABEL 文件	228 KB
RD_pH_8point3_step2_HCDFT.cross-linked.BS3.plabel	12/22/2017 22:53	PLABEL 文件	18 KB
RD_pH_8point3_step2_HCDFT.loop-linked.BS3.plabel	12/22/2017 22:53	PLABEL 文件	12 KB
RD_pH_8point3_step2_HCDFT.mono-linked.BS3.plabel	12/22/2017 22:53	PLABEL 文件	32 KB
RD_pH_8point3_step2_HCDFT.regular.plabel	12/22/2017 22:53	PLABEL 文件	205 KB
search_task_E.coli_BS3.plink	01/05/2018 15:08	PLINK 文件	1 KB

CSV results →

Web page result →

pQuant parameter file →

pLabel parameter files

Parameter file →

Web page result

❖ Click number with hyperlink to see the details.

1. Cross-Linked Results

Table 1 Identification of Cross-Linked Results Under 5% FDR Control.

#(Spectra)	#(Peptide Pairs)	#(Linked Sites)
251	46	54

2. All Results

Table 2 Identification of Spectra Under 5% FDR Control.

Types	Cross-Linked Spectra	Loop-Linked Spectra	Mono-Linked Spectra	Regular Spectra
Counts	251	163	608	5021
Percent	4.2%	2.7%	10.1%	83.1%

Table 3 Identification of Peptides (Pairs) Under 5% FDR Control.

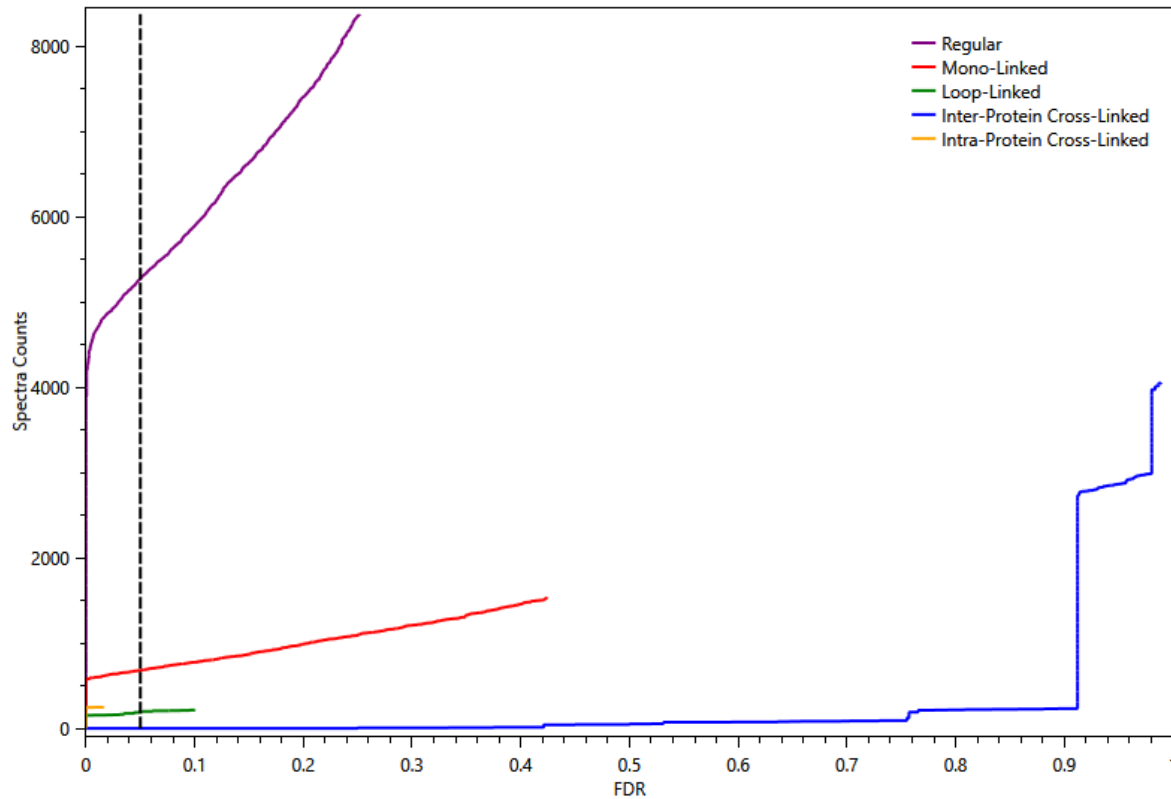
Types	Cross-Linked Peptide Pairs	Loop-Linked Peptides	Mono-Linked Peptides	Regular Peptides
Counts	46	49	166	1249
Percent	3.0%	3.2%	11.0%	82.7%

Table 4 Identification of Linked Sites Under 5% FDR Control.

Types	Cross-Linked Sites (Group)	Loop-Linked Sites (Group)
Counts	54 (41)	51 (49)
Percent	51.4%	48.6%

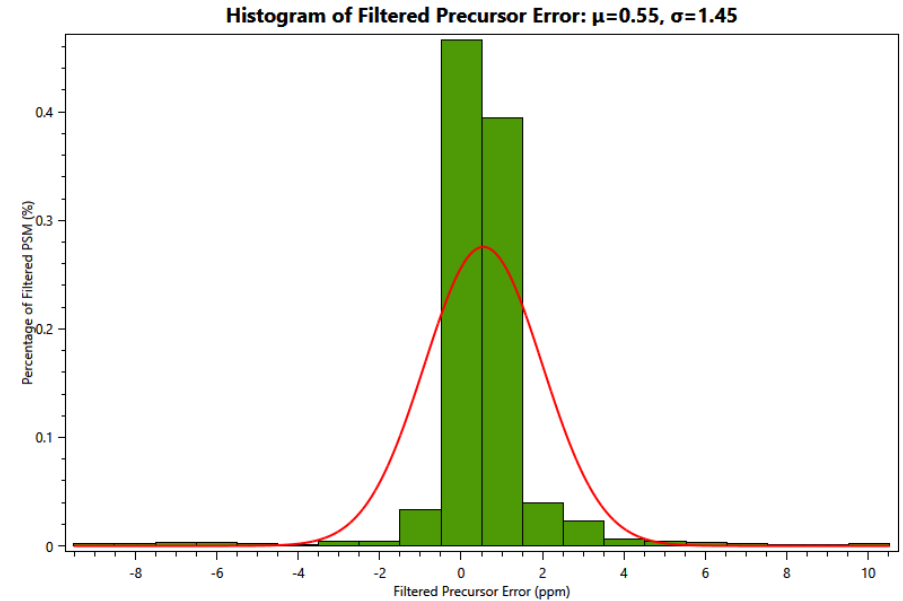
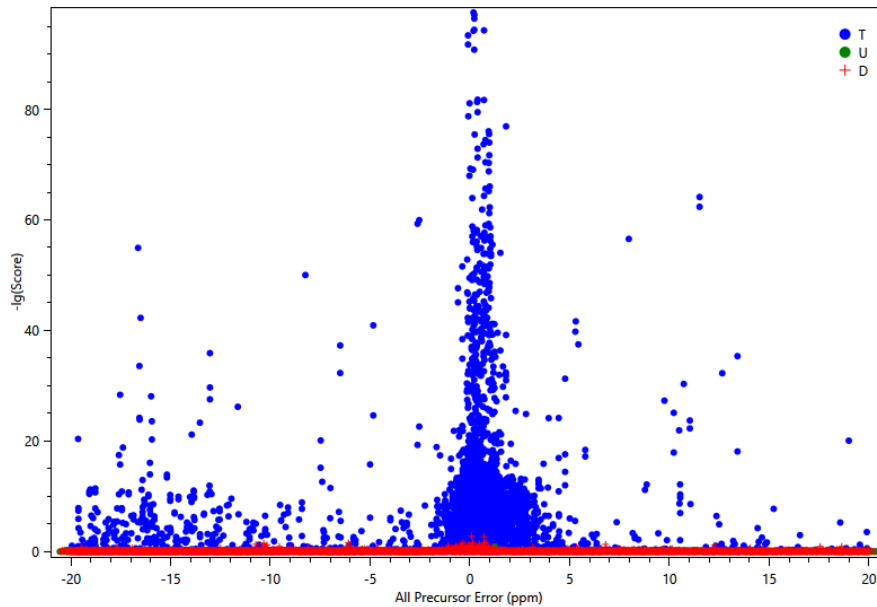
Web page result

❖ FDR Curve (Spectral Level).



Web page result









❖ Precursor Error Distribution.



CSV results

- ❖ Each peptide type has results in spectra, peptide and site(protein) level.

TestData > output > search_task_E.coli_BS3 > reports

名称	修改日期	类型	大小
 uniprot-ecoli-20171023_2017.12.22.csv	2017/12/22 22:53	Microsoft Excel ...	4,062 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_cross-linked_peptides.csv	2017/12/22 22:53	Microsoft Excel ...	32 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_cross-linked_sites.csv	2017/12/22 22:53	Microsoft Excel ...	40 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_cross-linked_spectra.csv	2017/12/22 22:53	Microsoft Excel ...	73 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_loop-linked_peptides.csv	2017/12/22 22:53	Microsoft Excel ...	21 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_loop-linked_sites.csv	2017/12/22 22:53	Microsoft Excel ...	27 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_loop-linked_spectra.csv	2017/12/22 22:53	Microsoft Excel ...	40 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_mono-linked_peptides.csv	2017/12/22 22:53	Microsoft Excel ...	73 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_mono-linked_sites.csv	2017/12/22 22:53	Microsoft Excel ...	91 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_mono-linked_spectra.csv	2017/12/22 22:53	Microsoft Excel ...	142 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_precursor_error_distribution.csv	2017/12/22 22:53	Microsoft Excel ...	184 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_regular_peptides.csv	2017/12/22 22:53	Microsoft Excel ...	581 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_regular_proteins.csv	2017/12/22 22:53	Microsoft Excel ...	707 KB
 uniprot-ecoli-20171023_2017.12.22.filtered_regular_spectra.csv	2017/12/22 22:53	Microsoft Excel ...	1,114 KB
 uniprot-ecoli-20171023_2017.12.22.precursor_error_distribution.csv	2017/12/22 22:53	Microsoft Excel ...	735 KB
 uniprot-ecoli-20171023_2017.12.22.summary.txt	2017/12/22 22:53	文本文档	2 KB

Appendix

❖ A) How to add a new linker?

1. Click Options → Meta Data Configuration
2. Select Linkers tab

Name	AlphaSites	BetaSites	LinkerMass	MonoMass	LinkerComposition	MonoComposition	LongMass	ShortMass
BS3	[K	[K	138.068	156.079	C(8)H(10)O(2)	C(8)H(12)O(3)	0	0
BS3_heavy	[K	[K	142.093	160.103	C(8)H(6)2H(4)O(2)	C(8)H(8)2H(4)O(3)	0	0
SS	C	C	-2.016	0	H(-2)	H(0)	0	0
BS2G	[K	[K	96.021	114.032	C(5)H(4)O(2)	C(5)H(6)O(3)	0	0
BS2G_heavy	[K	[K	100.046	118.057	C(5)2H(4)O(2)	C(5)H(2)2H(4)O(3)	0	0
DSS	[K	[K	138.068	156.079	C(8)H(10)O(2)	C(8)H(12)O(3)	0	0
EDC-DE	[K	DE	-18.011	0	H(-2)O(-1)	H(0)	0	0
SS_0	C	C	0	125.048	H(0)	H(125)	0	0
Azo_Leiker	[K	[K	459.179	477.19	C(26)H(25)N(3)O(5)	C(26)H(27)N(3)O(6)	0	0
Leiker_clv	[K	[K	316.142	334.153	C(17)1H(6)H(14)N(2)O(4)	C(17)1H(6)H(16)N(2)O(5)	0	0
Leiker_clv_d6	[K	[K	322.179	340.19	C(17)H(14)2H(6)N(2)O(4)	C(17)H(16)2H(6)N(2)O(5)	0	0
Leiker_bAL2	[K	[K	704.299	722.31	C(36)H(44)N(6)O(7)S(1)	C(36)H(46)N(6)O(8)S(1)	0	0
Leiker_bAL2_d6	[K	[K	710.336	728.347	C(36)H(38)N(6)O(7)S(1)	C(36)H(40)N(6)O(8)S(1)	0	0
DSSO	K	K	158.004	176.015	C(6)H(6)O(3)S(1)	C(6)H(8)O(4)S(1)	85.9826	54.0106
KArGO	[K	R	334.084	352.094	C(20)H(14)O(5)	C(20)H(16)O(6)	0	0
ArGO	R	R	334.084	370.105	C(20)H(14)O(5)	C(20)H(18)O(7)	0	0

Appendix

❖ A) How to add a new linker?

3. Click Add, fill in linker information in the dialog
4. Click Update and then Save
5. Close pConfig window, the new linker will appear in Linkers ListView

The screenshot shows a dialog box titled "Linker Information" with a close button (X) in the top right corner. It contains several input fields for linker details:

- Name: BS3_example
- Alpha Site: [K]
- Beta Site: [K]
- Linker Mass: 138.068
- Mono Mass: 156.079
- Linker Composition: C(8)H(10)O(2)
- Mono Composition: C(8)H(12)O(3)
- Long Mass: 0
- Short Mass: 0

An "Update" button is located at the bottom right of the dialog.

The screenshot shows the pLink software interface with the "Identification" tab selected. The "Flow" section is expanded, showing "Flow Type" set to "Conventional Crosslinking (F)" and "Process Number" set to "4". Below this, the "Set Linkers" section features a list of linker names: BS2G, BS2G_heavy, BS3, BS3_example (highlighted), BS3_heavy, and DSS. Navigation arrows are visible between the list and an empty box on the left. At the bottom, there are expandable sections for "Database Search" and "Result Filter".

Appendix

❖ A) Linker information needed

- Name: the name of the new linker.
- AlphaSites: the first cross-linked amino acid, parentheses “(” and “)” denote the peptide N terminus and C terminus, respectively; square brackets “[“ and “]” denote the protein N terminus and C terminus, respectively.
- BetaSites: the second cross-linked amino acid, “(”, “)”, “[“, and “]” denote the same as AlphaSites.
- LinkerMass: monoisotopic linker mass in inter/loop links.
- MonoMass: monoisotopic linker mass in mono links.
- LinkerComposition: linker composition in inter/loop links.
- MonoComposition: linker composition in mono links.
- LongMass: the longer mass in cleavable linker, 0 for uncleavable linker.
- ShortMass: the shorter mass in cleavable linker, 0 for uncleavable linker.



❖ **Thank you for using pLink 2!**

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

❖ **You can also post issues at GitHub for discussion:**

- <https://github.com/pFindStudio/pLink2/issues>
- **how to post issues at GitHub?**
 - see <http://pfind.ict.ac.cn/file/github.pdf>