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pFind Studio

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

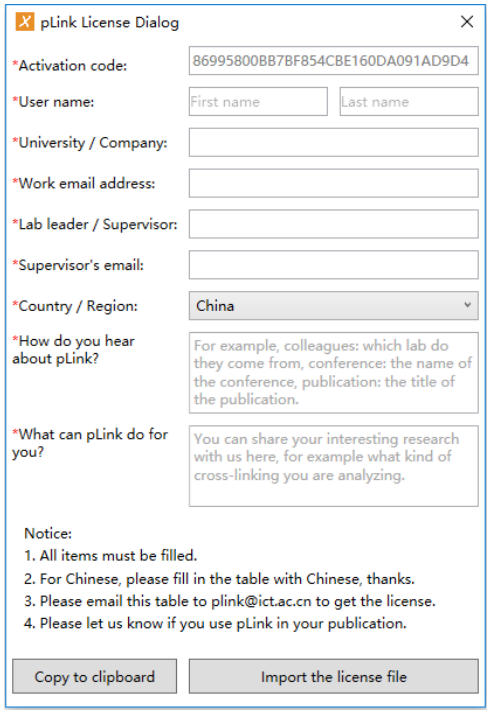
- CPU: 2.0 GHz or higher
- Memory: 4 GB or higher recommended
- Hard Disk: ~50 MB for software storage and an extra disk space to store the results and temporary files

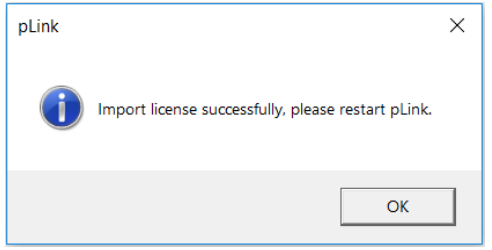
Software

- 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.





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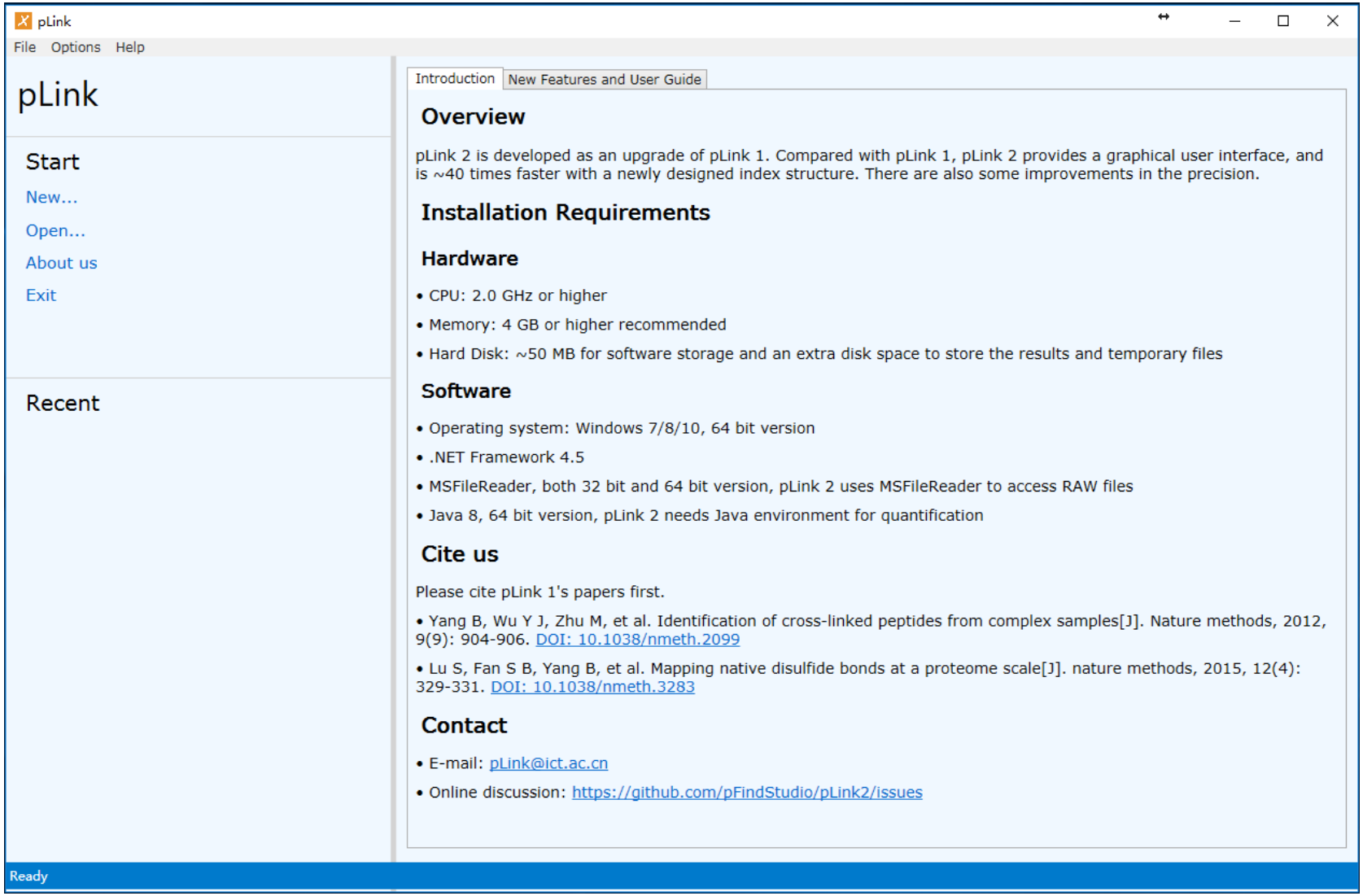
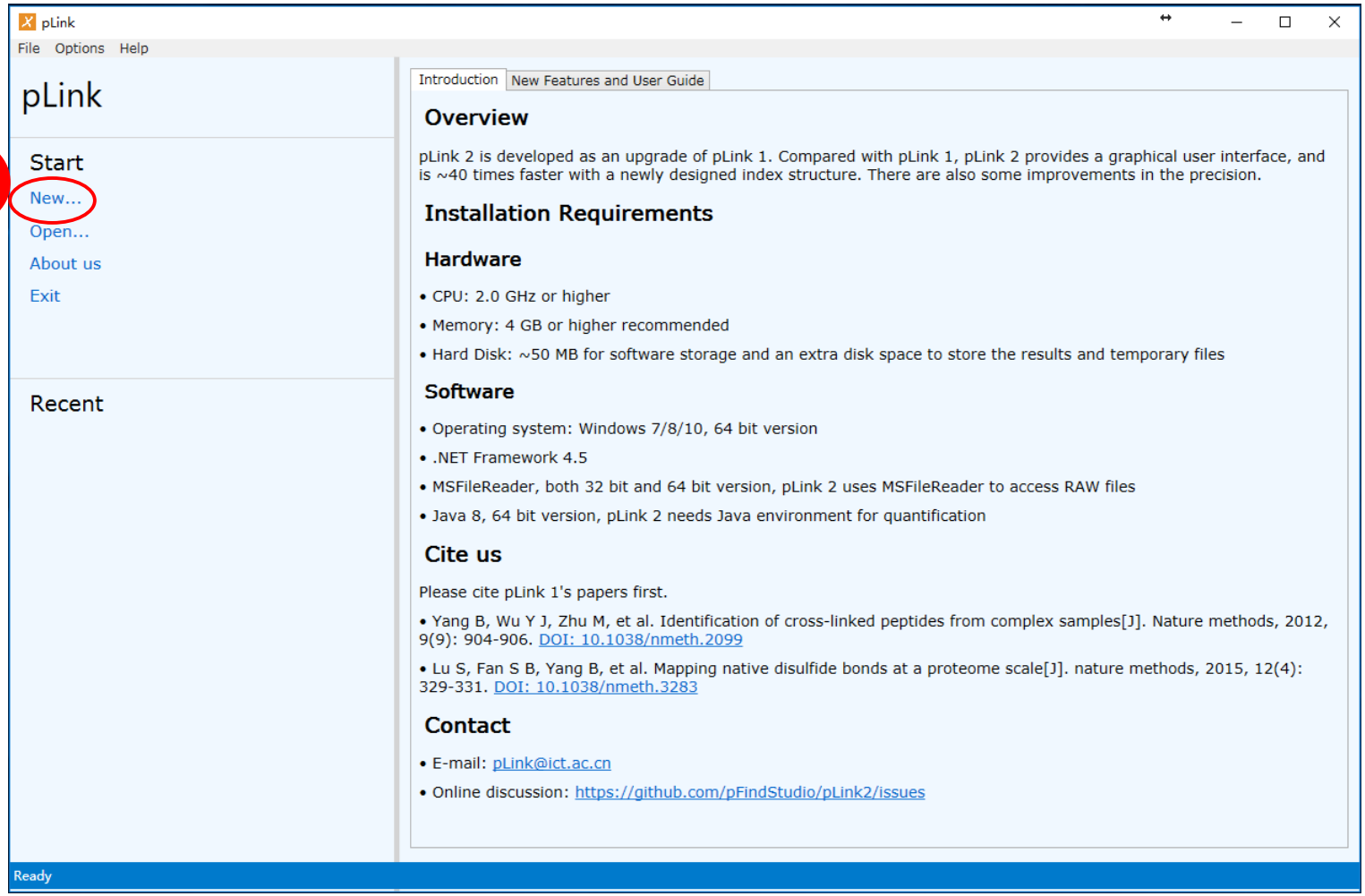
Main interface of pLink 2



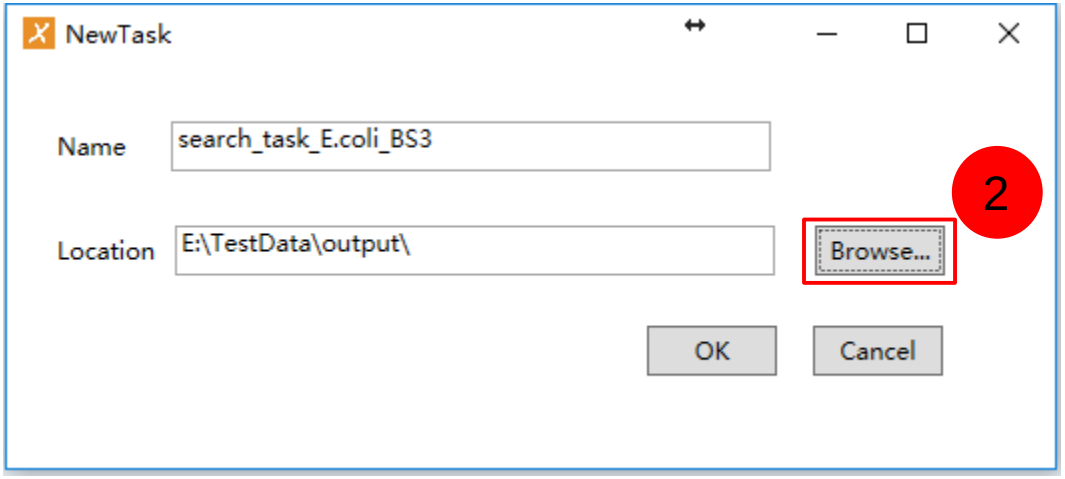
Create a new search task

❖ **Click New…**



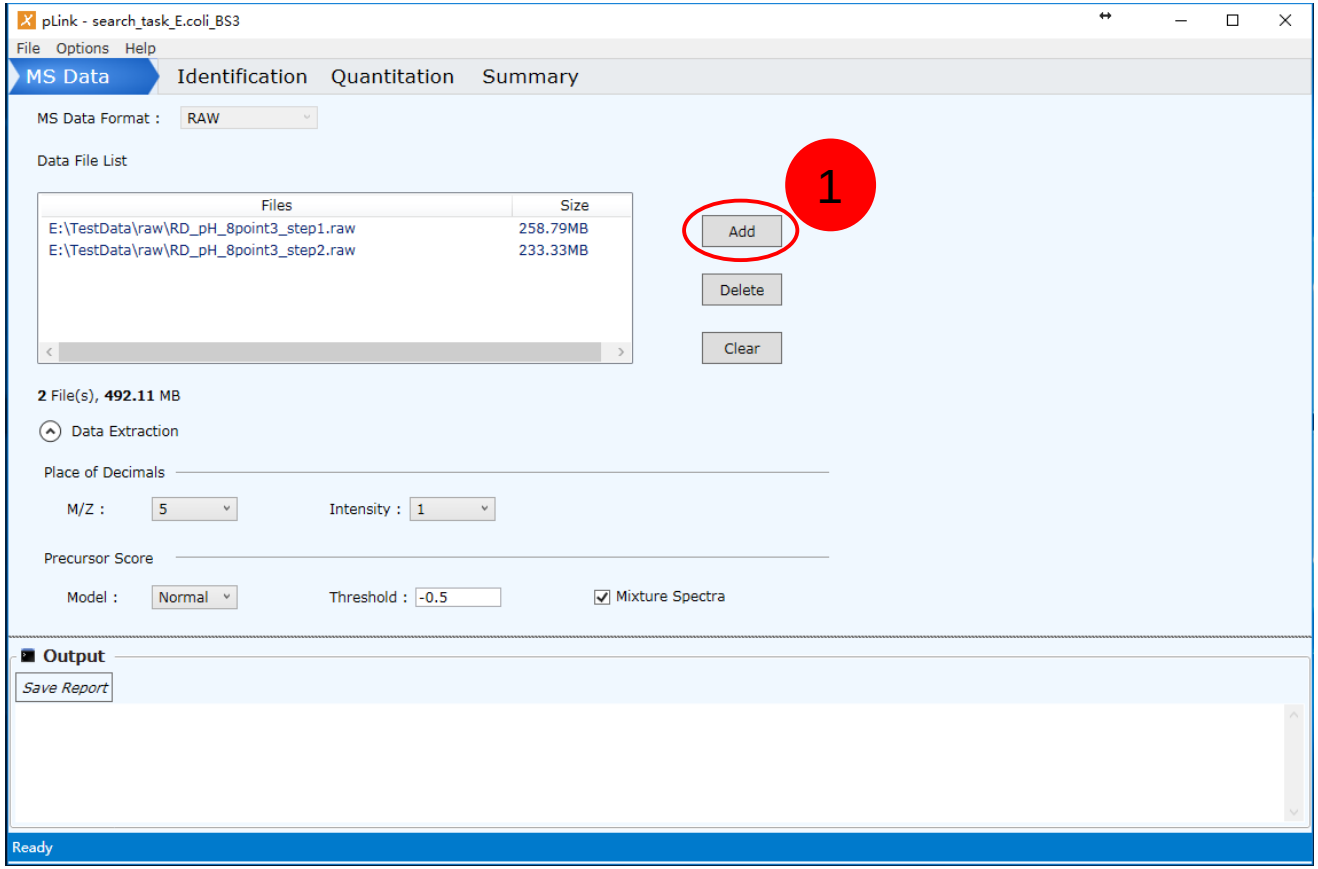
Create a new search task

- **Fill the task name and browse the task location**



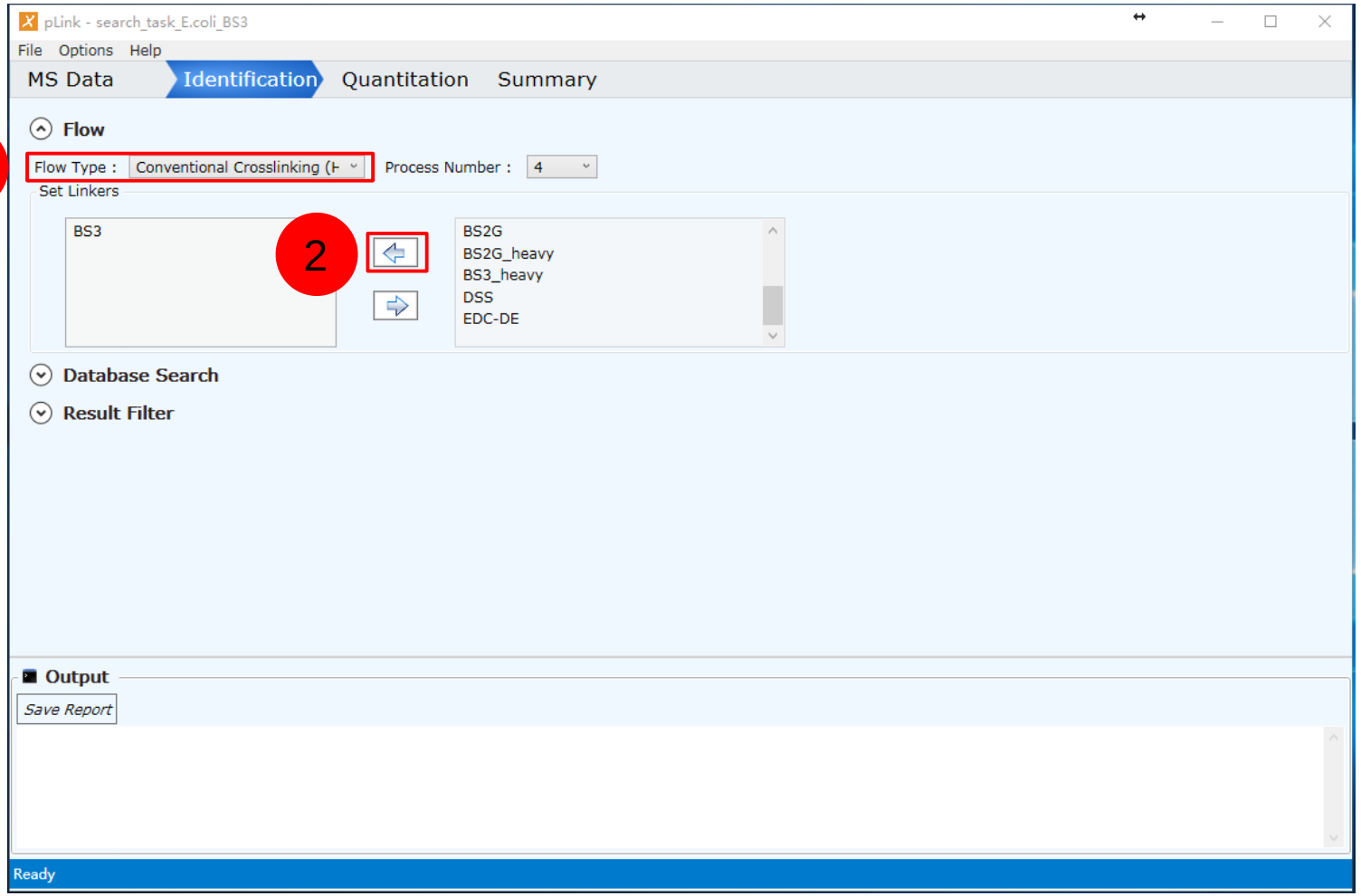
Import data

- **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



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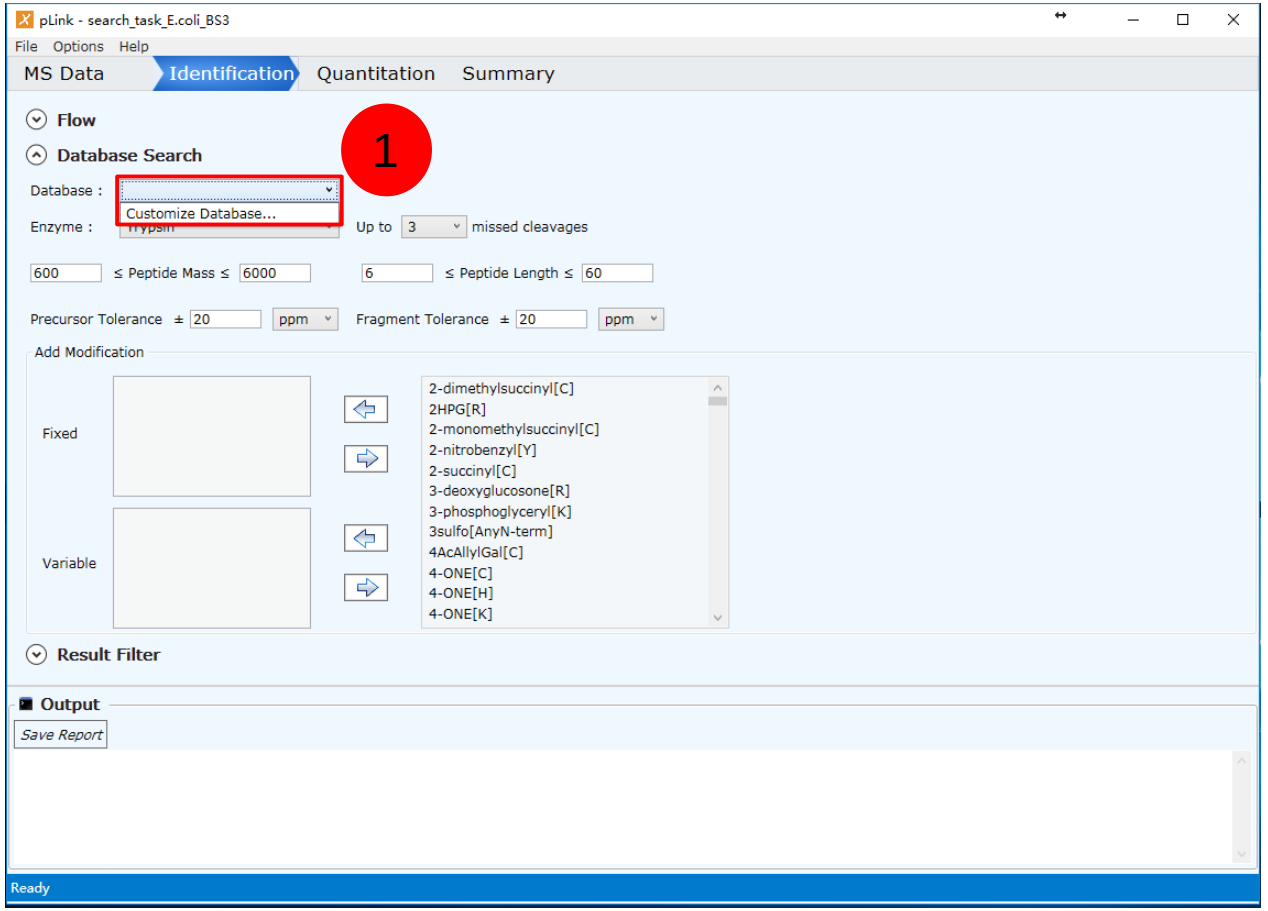
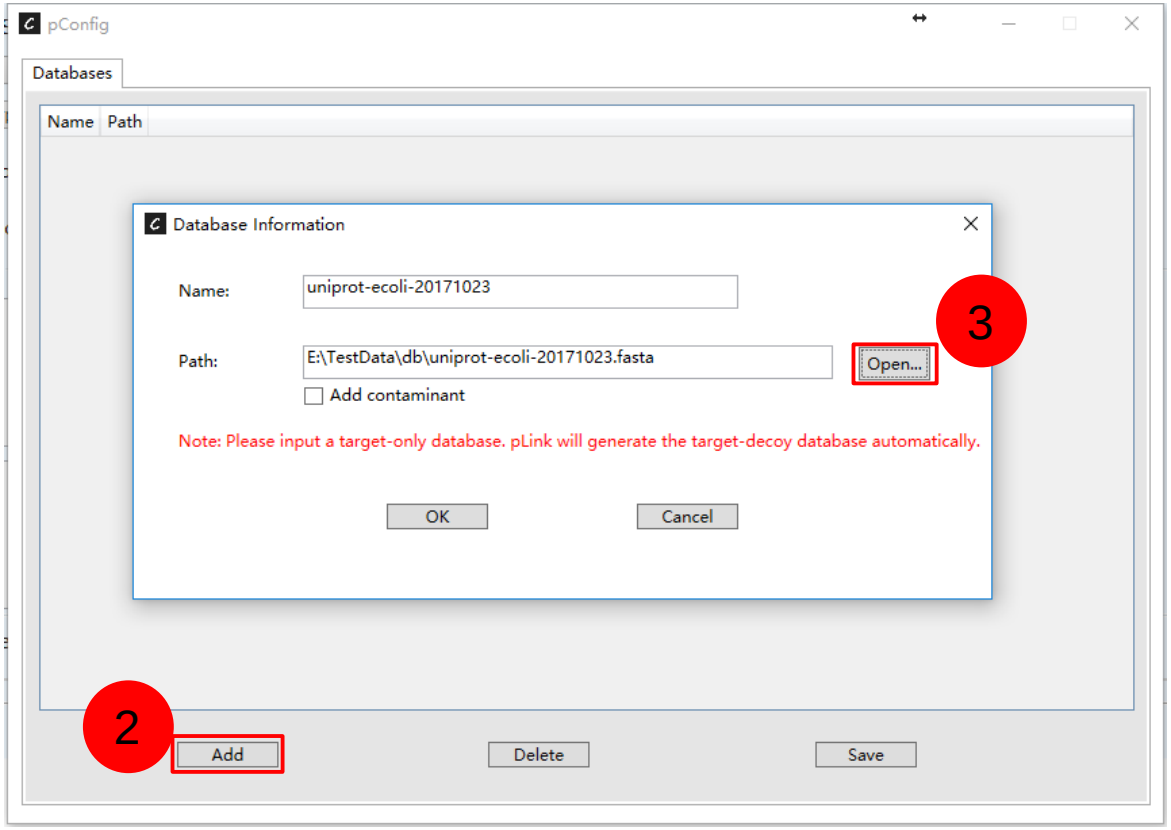
Set search parameters

❖ **B) Select and import database.**



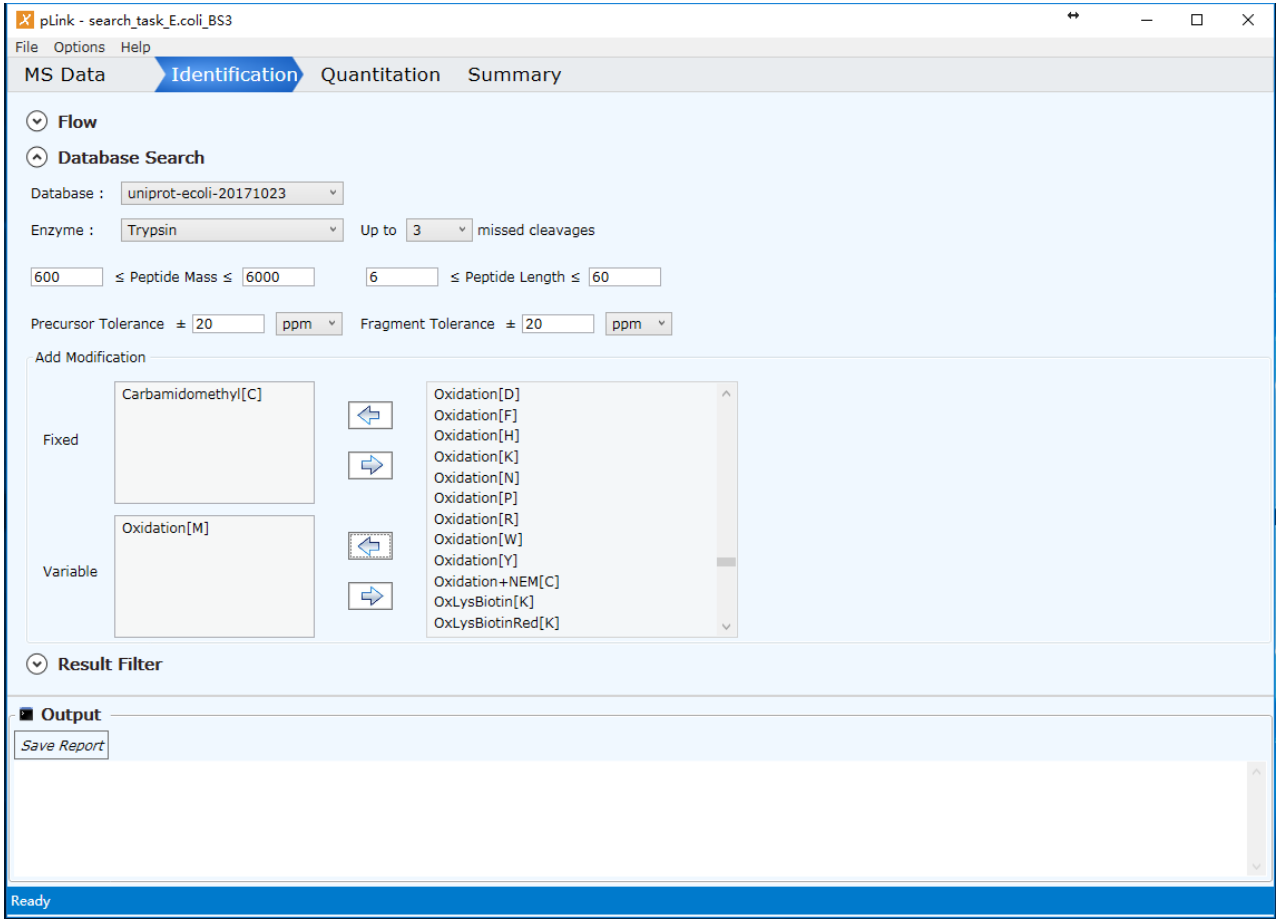
Set search parameters

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



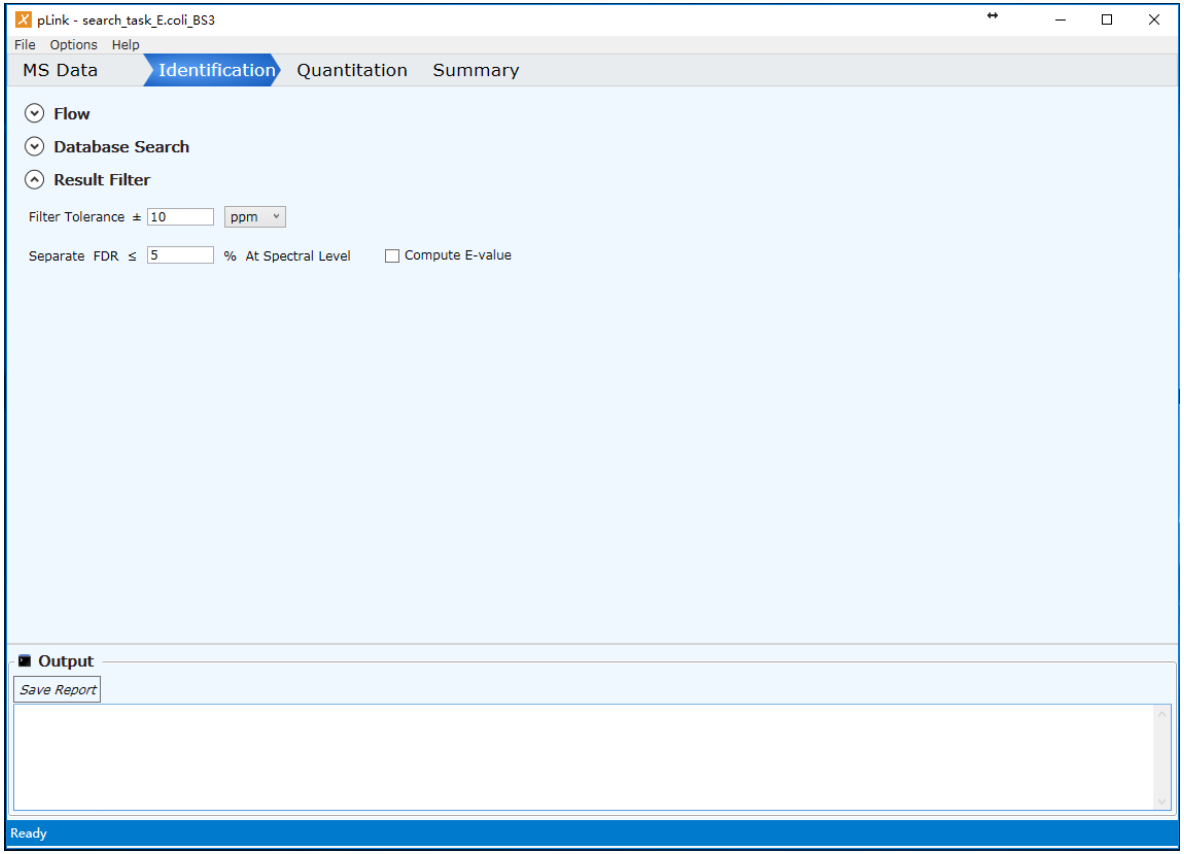
Set search parameters

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



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.**



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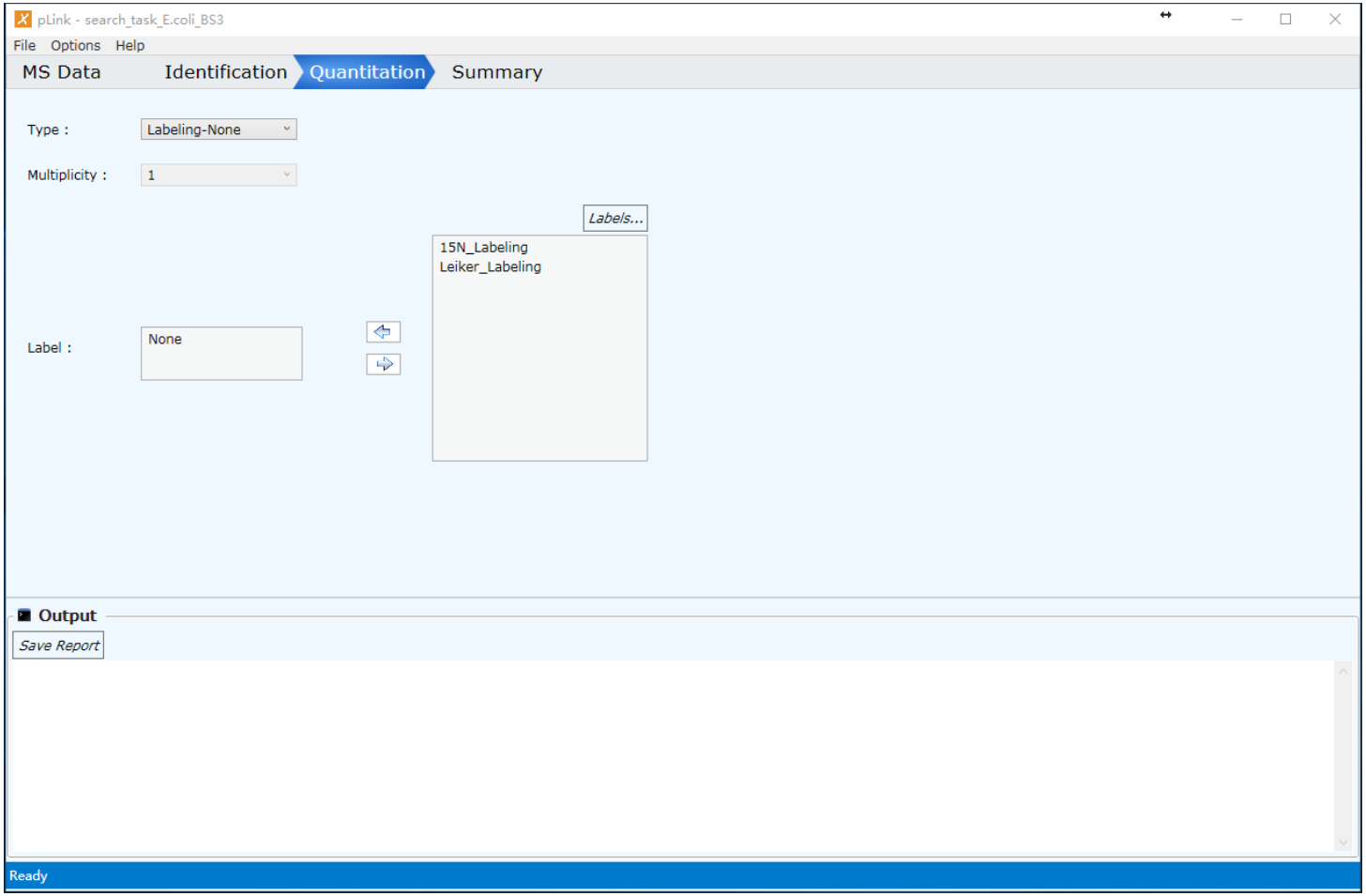
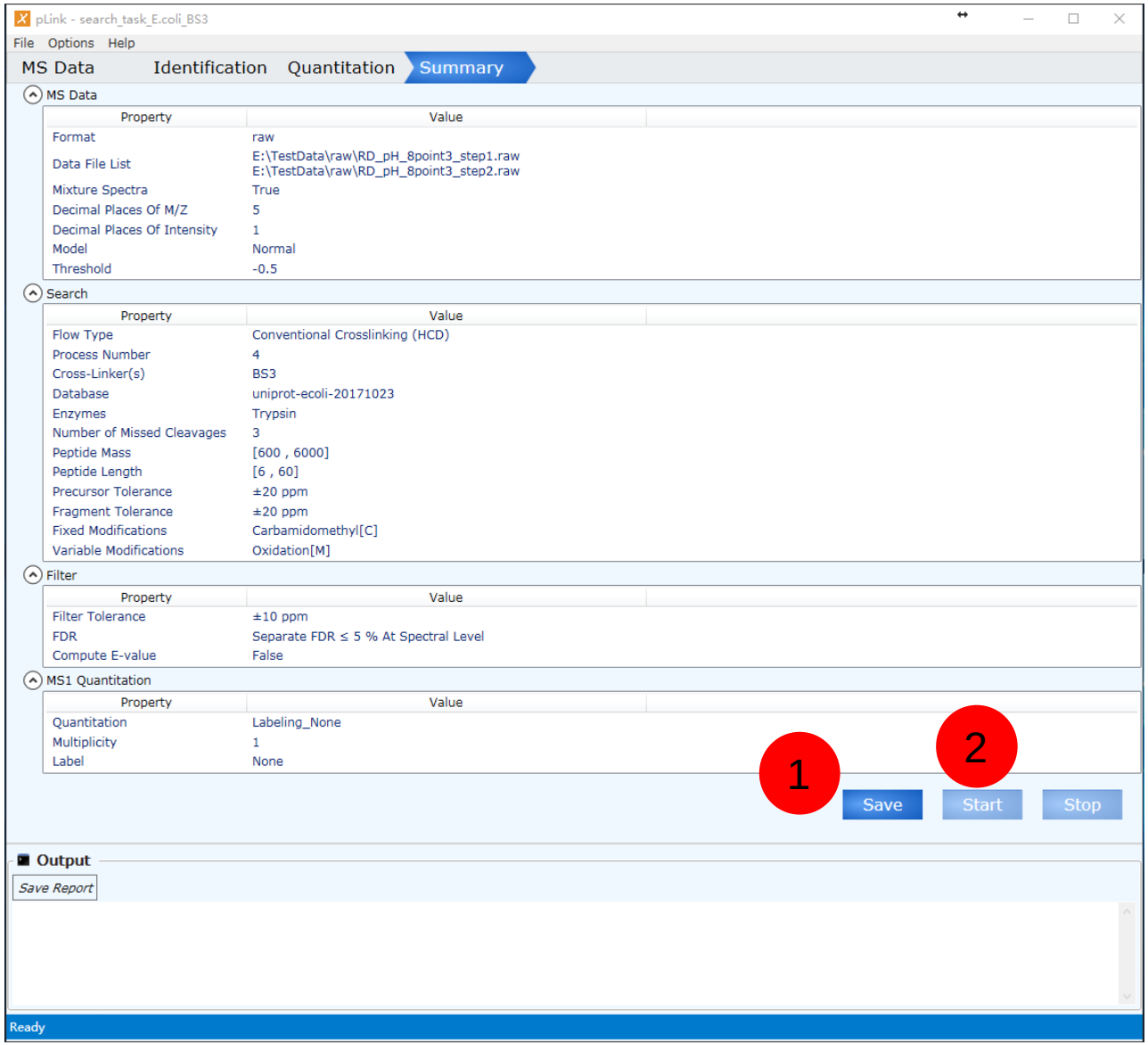
Set quantification parameters

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



Check parameters and run tasks



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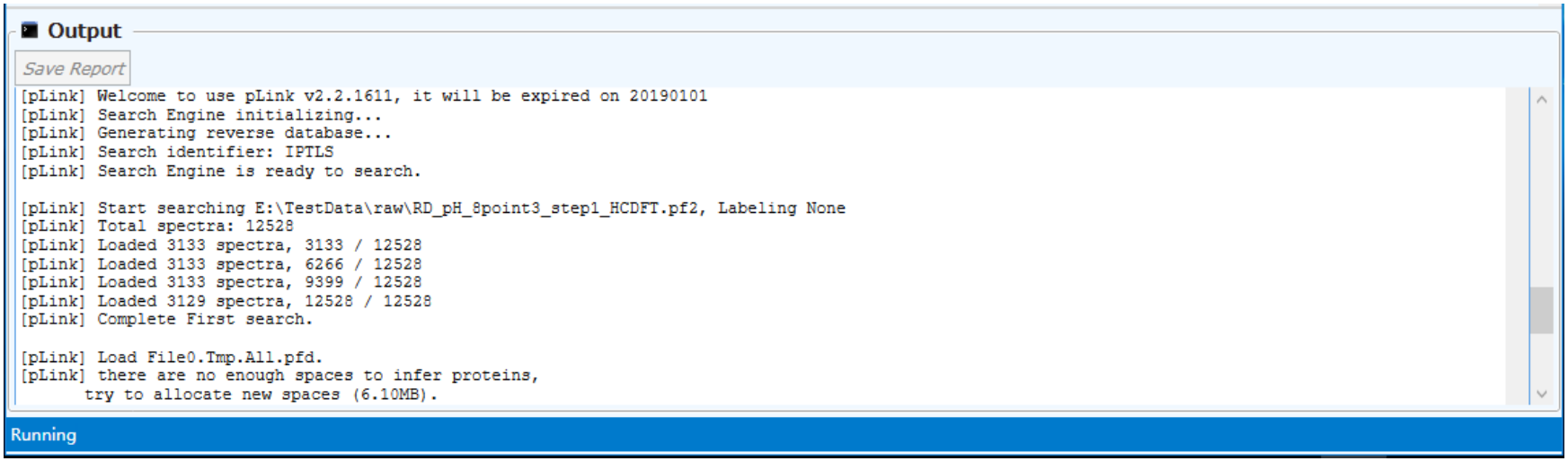
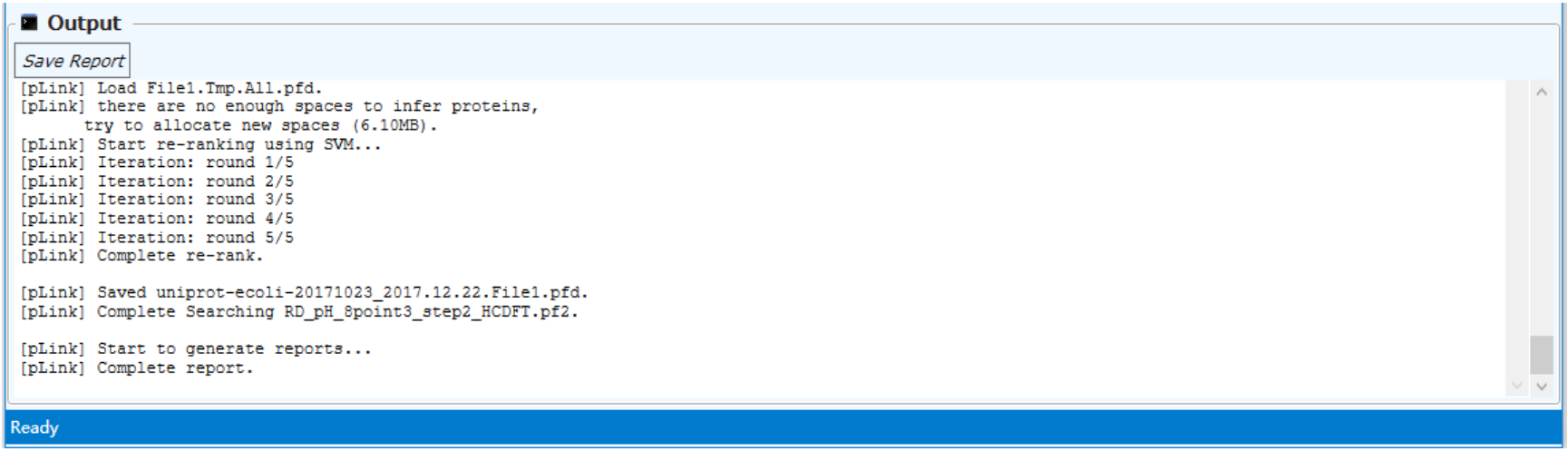
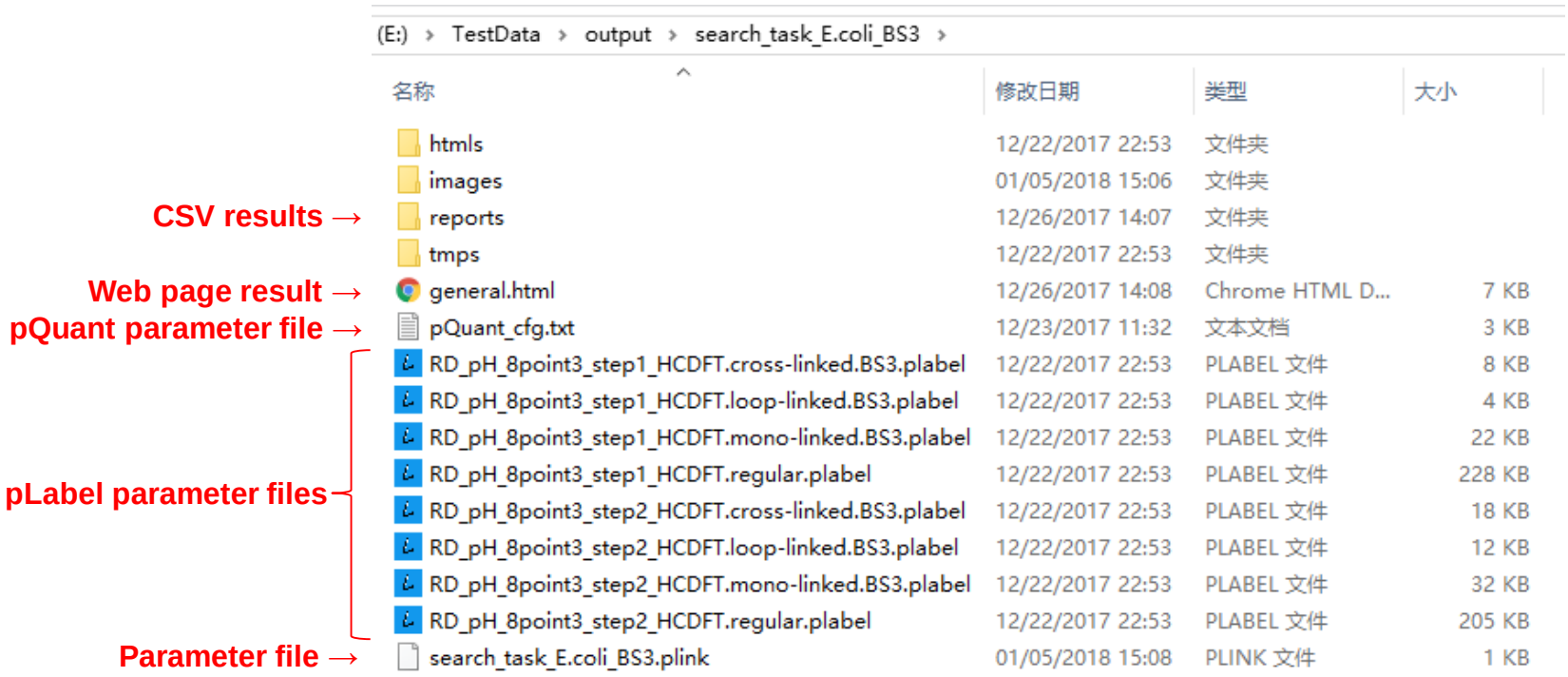
Searching…


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



Contents of search results files



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.

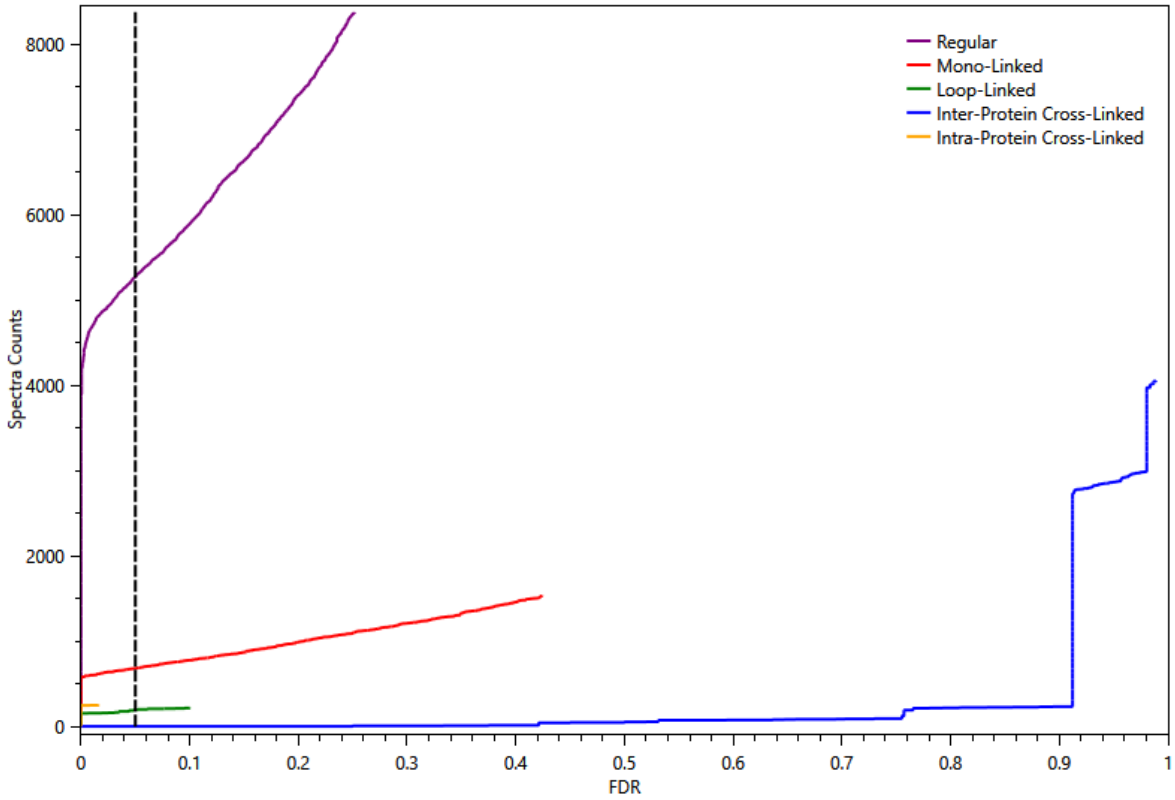
Types	Cross-Linked Peptide Pairs	Loop-Linked Peptides	Mono-Linked Peptides	Regular Peptides
Counts	46	49	166	1249
Percent	3.0%	3.2%	11.0%	82.7%

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%

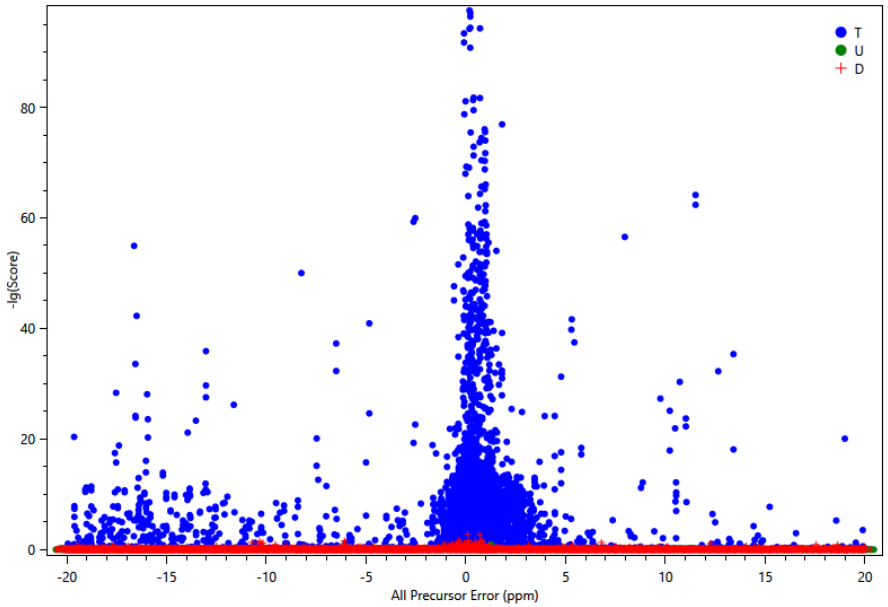
Web page result

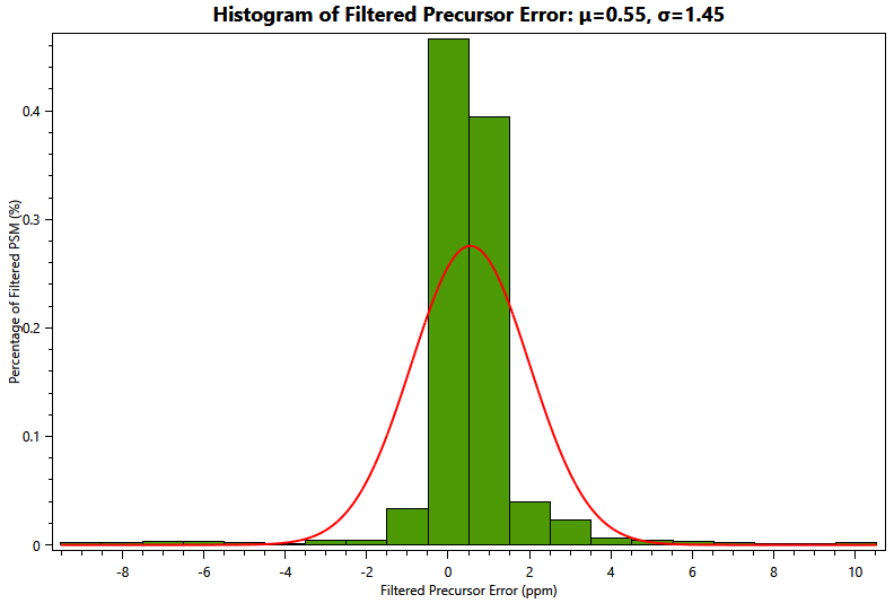
❖ **FDR Curve (Spectral Level).**



Web page result

- **Precursor Error Distribution.**





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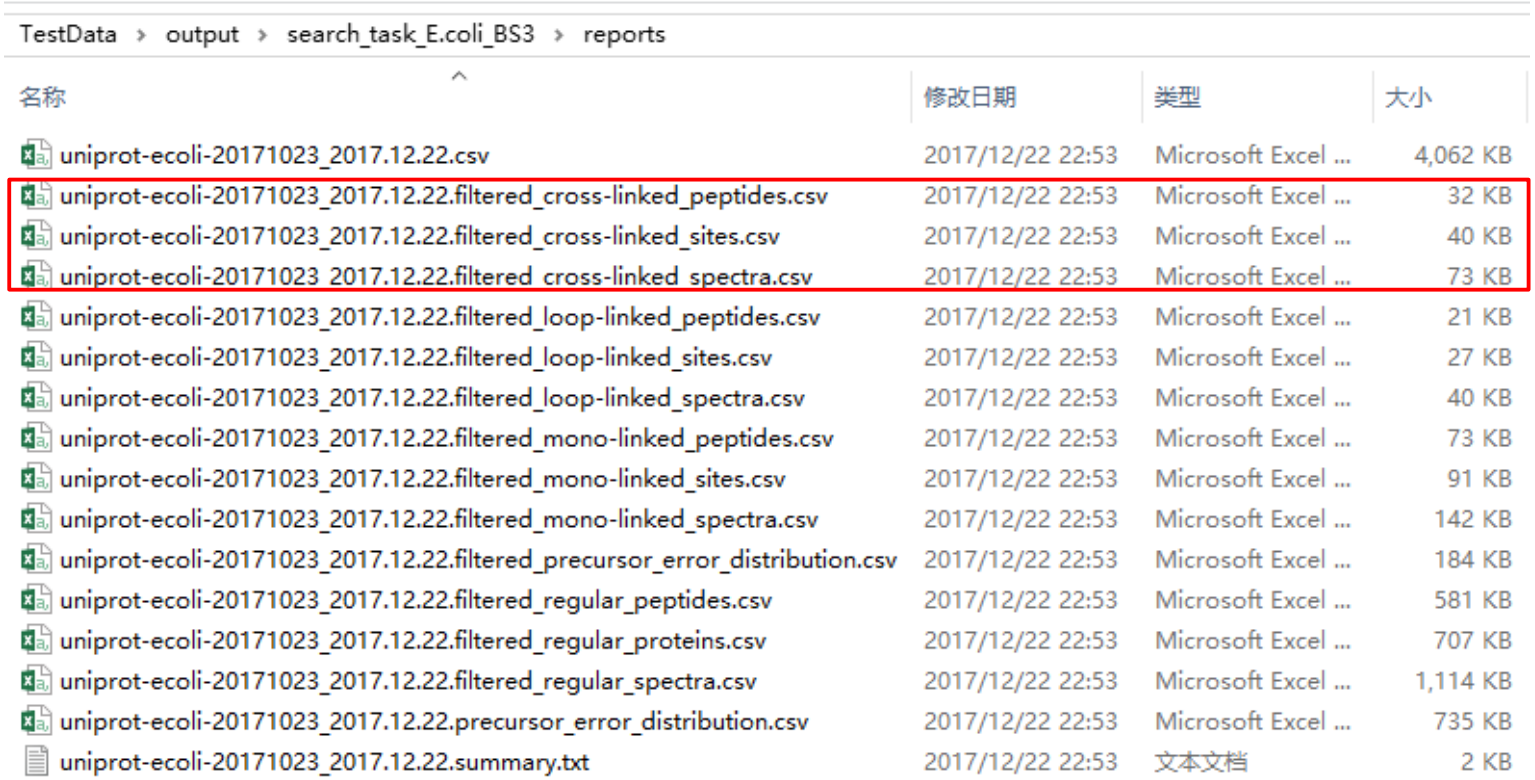
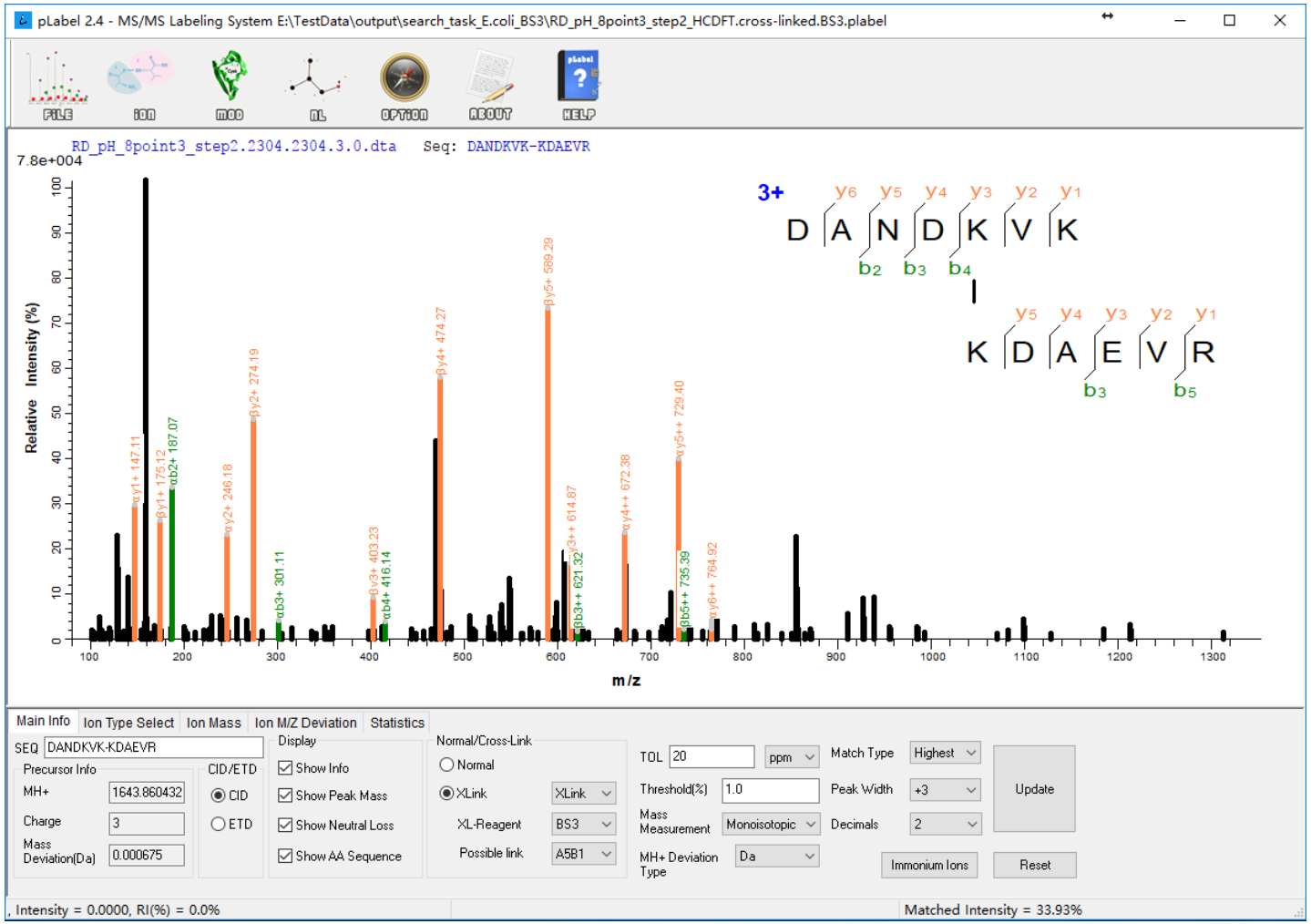
CSV results

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



View search results

- **Open *.plabel file with pLabel**



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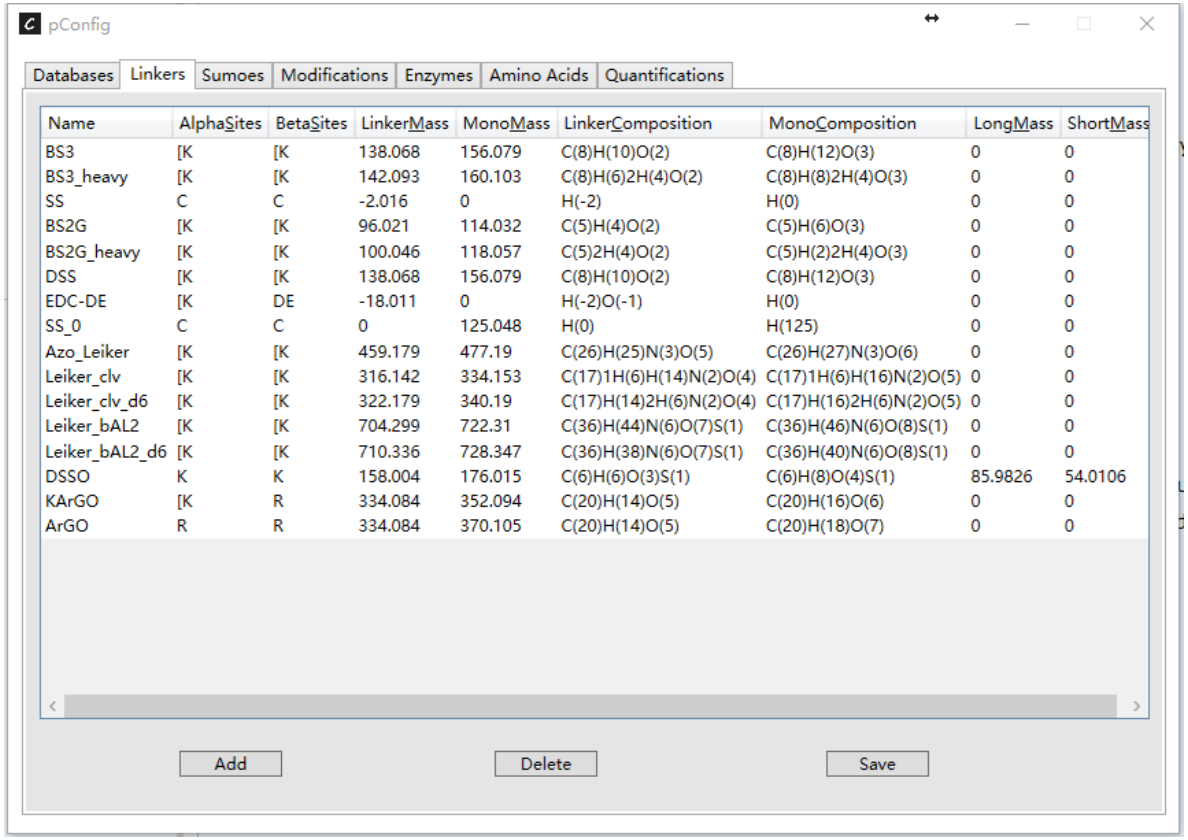
Appendix

❖ A) How to add a new linker?

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

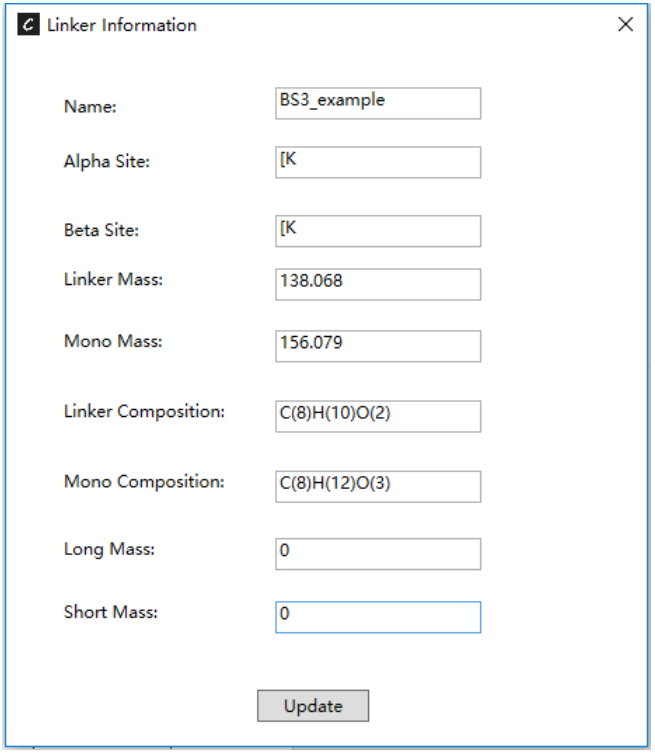


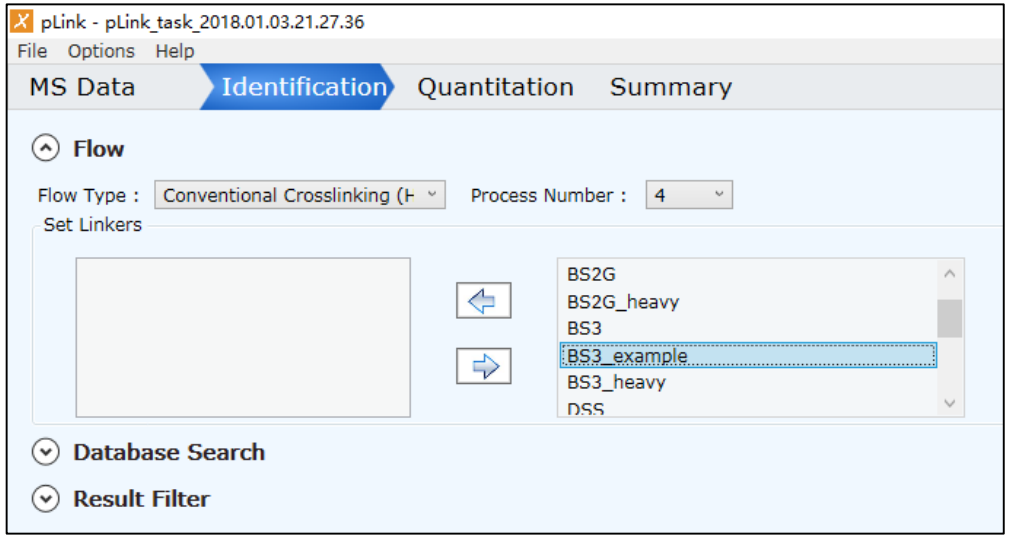
Name	AlphaSites	BetaSites	LinkerMass	MonoMass	LinkerComposition	MonoComposition	LongMass	ShortMass
BS3	[K	[K	138.068	156.079	C(8)H(10)O(2)	C(8)H(12)O(3)	0	0
BS3_heavy	[K	[K	142.093	160.103	C(8)H(6)2H(4)O(2)	C(8)H(8)2H(4)O(3)	0	0
SS	C	C	-2.016	0	H(-2)	H(0)	0	0
BS2G	[K	[K	96.021	114.032	C(5)H(4)O(2)	C(5)H(6)O(3)	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**





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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**