

# SpotLink Manual

## Brief Introduction

SpotLink is a software for identifying site non-specific cross-links from LC-MS/MS, which can realize the identification from simple proteins to the proteomics. SpotLink has a user friendly user interface to set parameters. The cross-linking result is easily visualized by proXL.



# The recommended configuration

### Version: SpotLink 1.2

### Release Date: 2021.12.31

## ## Computer configuration

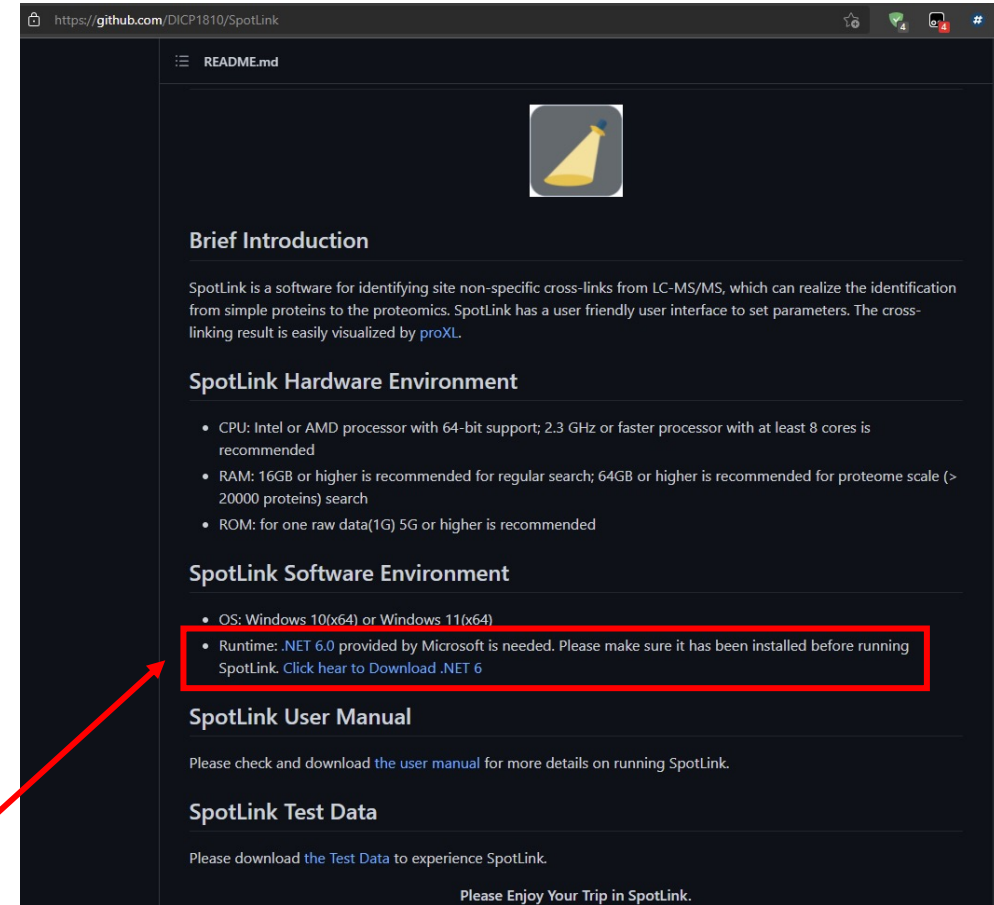
**CPU:** Intel or AMD processor with 64-bit support; 2.3 GHz or faster processor with at least 8 cores is recommended

**RAM:** 16GB or higher is recommended for regular search; 64GB or higher is recommended for proteome scale (> 20000 proteins) search

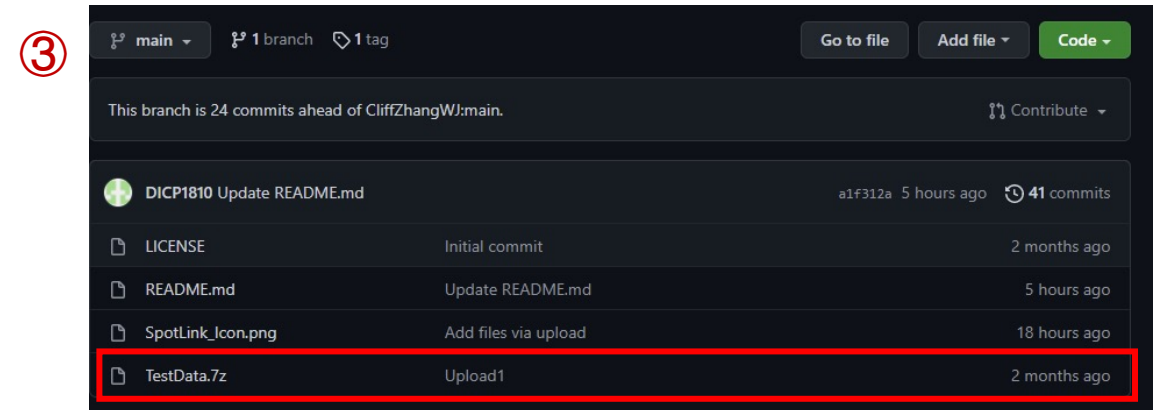
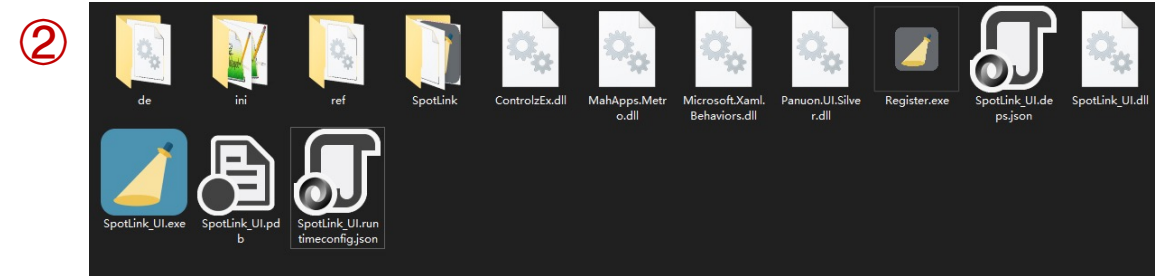
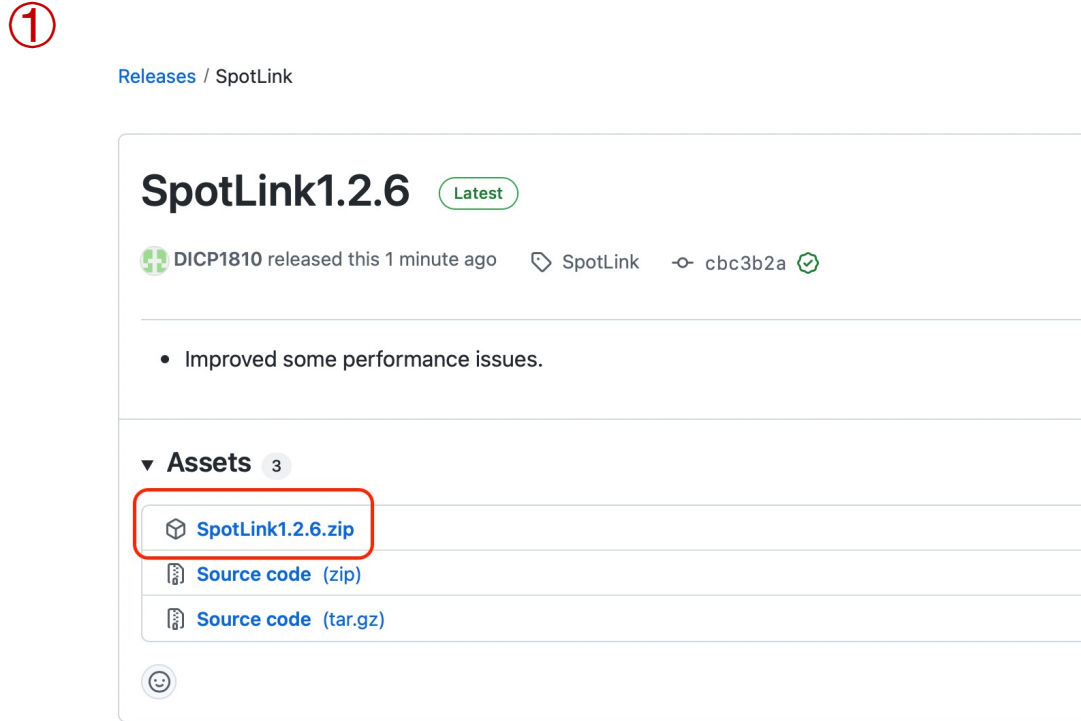
**ROM:** for one raw data (1G) 5G or higher is recommended

**OS:** Windows 10 (x64) or Windows 11 (x64)

**Other:** **.NET 6.0** is needed and 360 anti-virus software should be shut down.



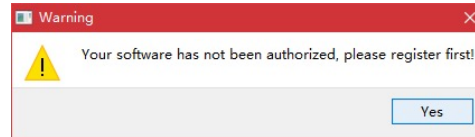
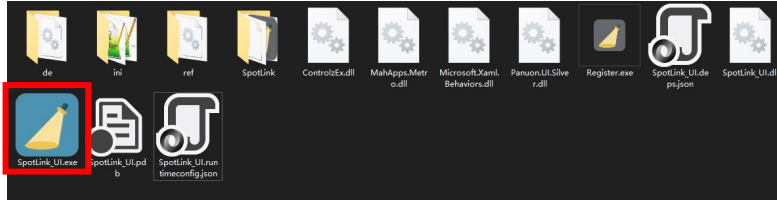
# 1. SpotLink and test data download



- ① Login <https://github.com/DICP1810/SpotLink/releases> and download **SpotLinkv1.2.\*.zip** at the bottom of this page.
- ② The unzipped SpotLink files.
- ③ Download the test data from <https://github.com/DICP1810/SpotLink/>.

## 2 SpotLink register

①



②

S/N: DC2148B6CFD2

Username:

University/Company:

Email address:

Lab Supervisor:

Supervisor's email:

Country/Region: China

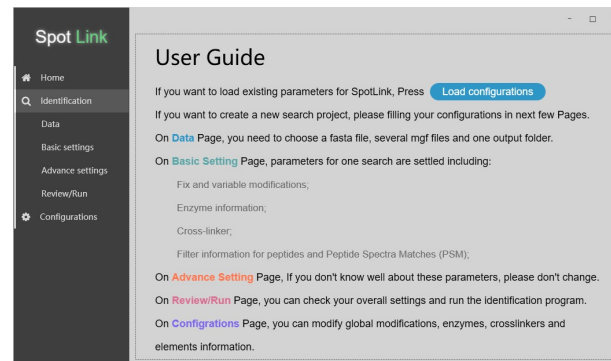
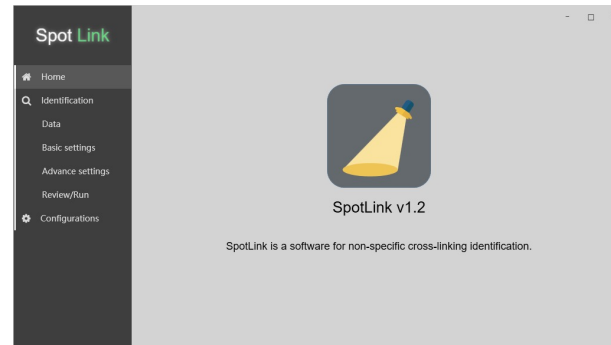
How do you hear about SpotLink?  
For example, colleagues; which lab do they come from, conference; the name of the conference, publication; the title of the publications.

What can SpotLink do for you?  
You can share your interesting research with us here, for example what kind of sample you are analyzing.

Notice:  
1. All items must be filled.  
2. For Chinese, please fill in the table with Chinese, thanks.  
3. Please connect to the Internet to upload the information.

Submit Cannel

③



- ① Double click **SpotLink\_UI.exe**, if software has not been authorized, click **Register** button, before that, make sure that your PC is linked to the Internet.
- ② Fill the register information and click **Submit**, then pGlycoQuant will be authorized.
- ③ The SpotLink User Interface.

### 3 SpotLink Identification

The screenshot displays the SpotLink web application interface. On the left is a dark sidebar with the 'Spot Link' logo and a navigation menu containing 'Home', 'Identification', 'Data' (highlighted), 'Basic settings', 'Advance settings', 'Review/Run', and 'Configurations'. The main content area is titled 'Input Files' and contains three sections: 'FASTA File' with a blue button and a text input field containing a file path; 'MGF Files' with a blue button and a red 'Delete' button, followed by a text input field with the same file path; and 'Output Folder' with a blue button and a text input field containing a folder path. The interface is clean and modern, using a light gray color scheme for the main content area.

**Spot Link**

- Home
- Identification
- Data**
- Basic settings
- Advance settings
- Review/Run
- Configurations

#### Input Files

**FASTA File**

Input FASTA file:  
E:\Code\SpotLink\TestData\uniprot-reviewed\_yes+AND+organism\_Escherichia

**MGF Files**

Input MGF files:  
E:\Code\SpotLink\TestData\uniprot-reviewed\_yes+AND+organism\_Escherichia

**Delete**

#### Output Folder

**Output Folder**

Output folder:  
E:\Code\SpotLink\TestData

In **Data** page, fill the fasta file, the mgf file and the output folder.

### 3 SpotLink Identification

**Spot Link**

Home Identification Data **Basic settings** Advance settings Review/Run Configurations

#### Modifications, Enzyme and Linker

Modification_Name	Modification_Type
2-dimethylsuccinyl[C]	None
2-monomethylsuccinyl[C]	None
2-nitrobenzyl[Y]	None
2-succinyl[C]	None
2HPG[R]	None
3-deoxyglucosone[R]	None
3-phosphoglyceryl[K]	None
3sulfo[AnyN-term]	None
4-ONE+Delta_H(-2)O(-1)[C]	None
4-ONE+Delta_H(-2)O(-1)[H]	None
4-ONE+Delta_H(-2)O(-1)[K]	None
4-ONE[C]	None
4-ONE[H]	None

Choose Enzyme: Trypsin Choose Linker: SDA

Enzyme Missed Cleavages Number sets 3.

Max Modification Number sets 3.

In **Basic settings** page, you can set the fixed and variable modification, the enzyme and the linker information.

**Spot Link**

Home Identification Data **Basic settings** Advance settings Review/Run Configurations

#### Filter

Peptide Mass Range

Max: 6000 Min: 500

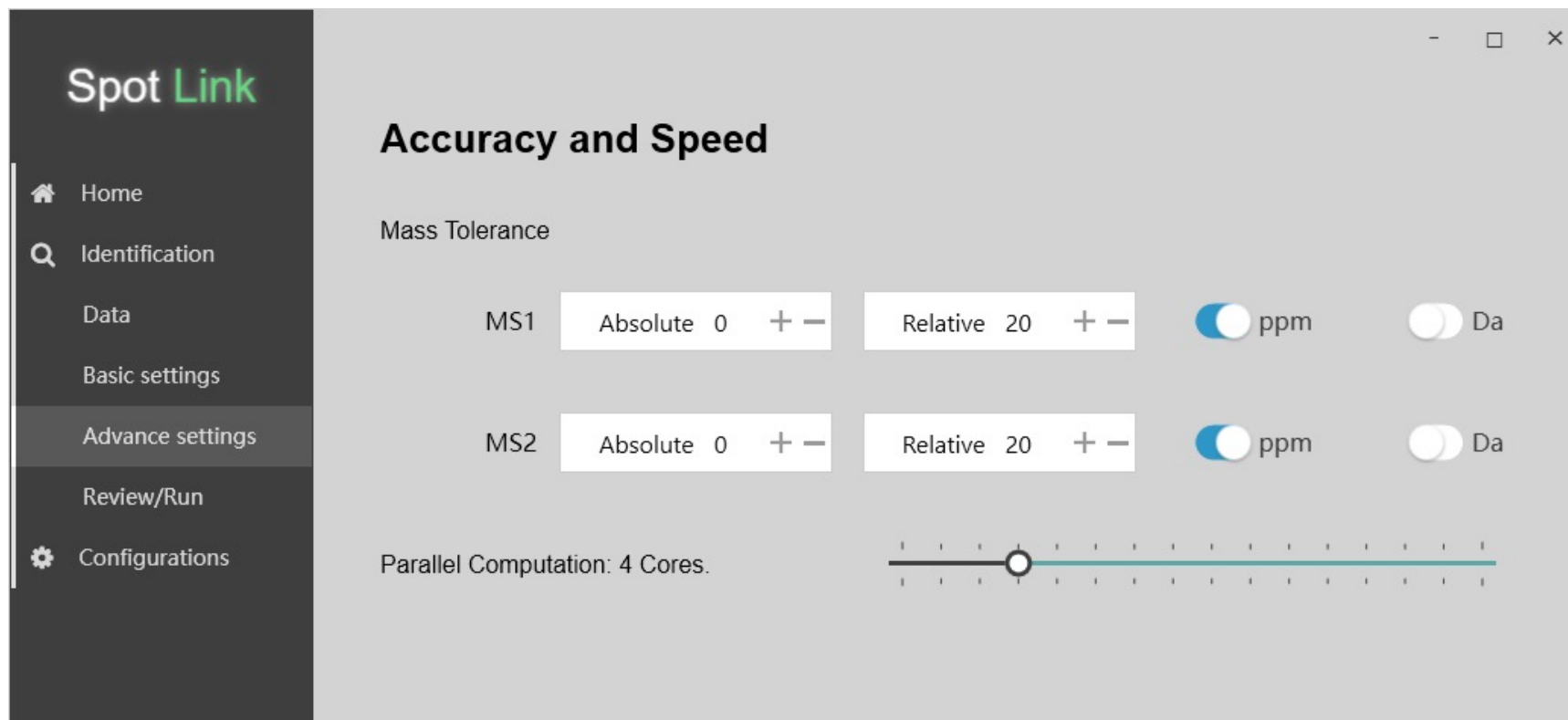
Peptide Length Range From 5 to 30.

FDR FDR less than 10%.

sFDR sFDR less than 10%.

You can set the peptide mass range, the peptide length range, the FDR and the sFDR.

### 3 SpotLink Identification



In **Advance settings** page, you can set the mass tolerance of ms1 and ms2.

Setting the parallel computation according to your PC.



### 3 SpotLink Identification

Spot Link

Home

Identification

Data

Basic settings

Advance settings

Review/Run

Configurations

Review

Core Parameters Check

100%

✓ Check Input FASTA file.

✓ Check Input MGF files.

✓ Check Input Output folder.

✓ Check Input Enzyme.

✓ Check Input Linker.

⚠ No modification for Searching.

Specific Parameters

Configuration_Name	Configuration_Value
Fasta File	E:\Code\SpotLink\TestData\uniprot-reviewed_yes+AND+org
Input mgf Files	uniprot-reviewed_yes+AND+organism__Escherichia+coli+(
OutPut Folder	E:\Code\SpotLink\TestData
Enzyme Name	Trypsin
Enzyme Miss Cleavage Number	3
Linker Name	SDA
Fixed Modifications	No Fixed Modifications
Variable Modifications	No Variable Modifications
Max Modification Number	3
Peptide Mass Range	[500,6000]
Peptide Length Range	[5,30]
MS1 Tolerance	Absolute:0ppm Relative:20ppm
MS2 Tolerance	Absolute:0ppm Relative:20ppm
FDR	Less than or equal 10%
sFDR	Less than or equal 10%
Processors Number	4
Search Preference	Balance

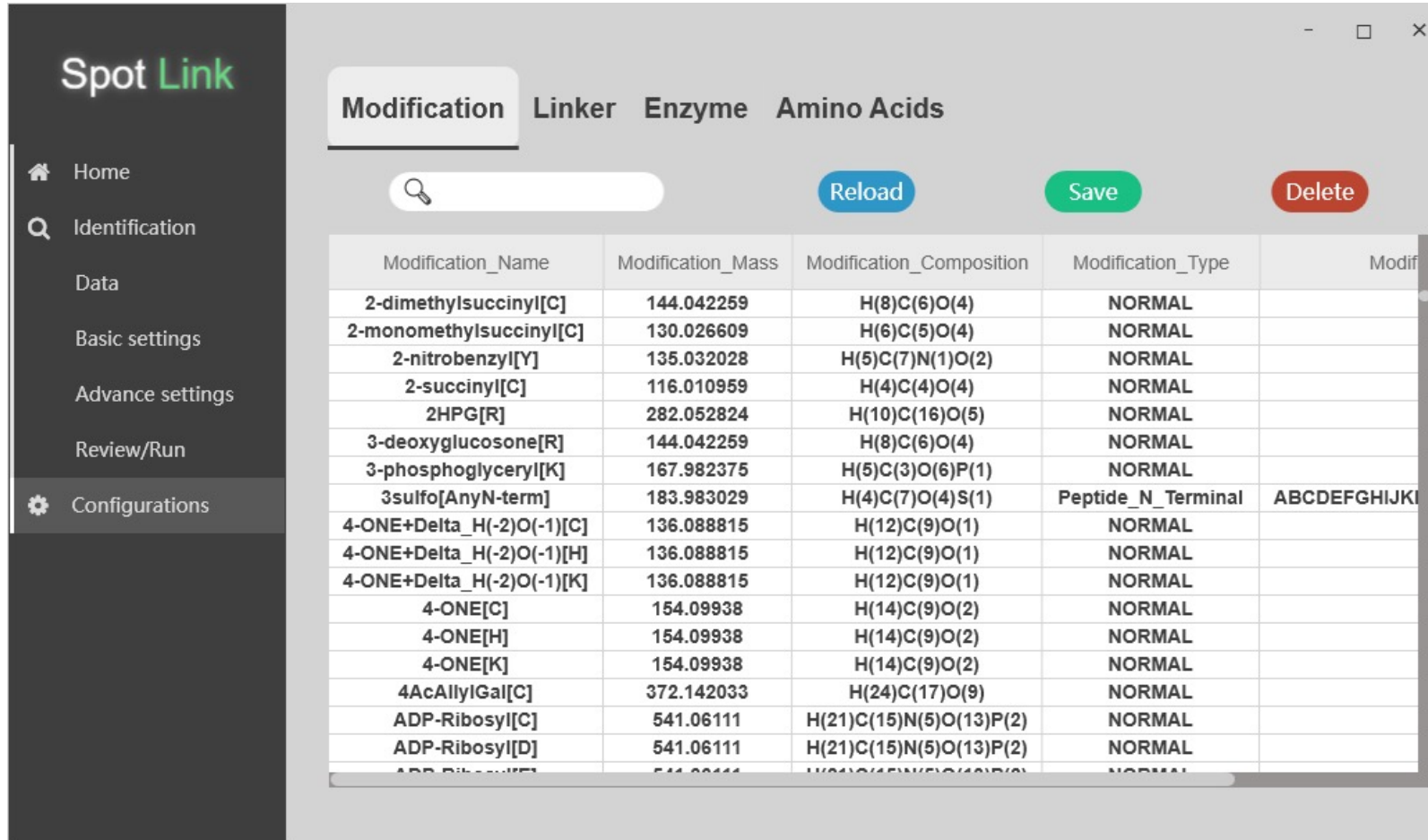
Save

Search

Stop

In **Review / Run** page, you can check the parameters, save and run the software.

### 3 SpotLink Identification



The screenshot displays the SpotLink web application interface. On the left is a dark sidebar with navigation links: Home, Identification, Data, Basic settings, Advance settings, Review/Run, and Configurations (highlighted with a gear icon). The main content area has a light gray background with a top navigation bar containing tabs for Modification, Linker, Enzyme, and Amino Acids. Below these tabs is a search bar, a Reload button, a Save button, and a Delete button. The central part of the interface features a table with the following columns: Modification\_Name, Modification\_Mass, Modification\_Composition, Modification\_Type, and a partially visible column for Modification\_Amino\_Acids. The table lists various modifications such as 2-dimethylsuccinyl[C], 2-monomethylsuccinyl[C], 2-nitrobenzyl[Y], 2-succinyl[C], 2HPG[R], 3-deoxyglucosone[R], 3-phosphoglycerol[K], 3sulfo[AnyN-term], 4-ONE+Delta\_H(-2)O(-1)[C], 4-ONE+Delta\_H(-2)O(-1)[H], 4-ONE+Delta\_H(-2)O(-1)[K], 4-ONE[C], 4-ONE[H], 4-ONE[K], 4AcAllylGal[C], ADP-Ribosyl[C], ADP-Ribosyl[D], and ADP-Ribosyl[E].

Modification_Name	Modification_Mass	Modification_Composition	Modification_Type	Modification_Amino_Acids
2-dimethylsuccinyl[C]	144.042259	H(8)C(6)O(4)	NORMAL	
2-monomethylsuccinyl[C]	130.026609	H(6)C(5)O(4)	NORMAL	
2-nitrobenzyl[Y]	135.032028	H(5)C(7)N(1)O(2)	NORMAL	
2-succinyl[C]	116.010959	H(4)C(4)O(4)	NORMAL	
2HPG[R]	282.052824	H(10)C(16)O(5)	NORMAL	
3-deoxyglucosone[R]	144.042259	H(8)C(6)O(4)	NORMAL	
3-phosphoglycerol[K]	167.982375	H(5)C(3)O(6)P(1)	NORMAL	
3sulfo[AnyN-term]	183.983029	H(4)C(7)O(4)S(1)	Peptide_N_Terminal	ABCDEFGHIJKL
4-ONE+Delta_H(-2)O(-1)[C]	136.088815	H(12)C(9)O(1)	NORMAL	
4-ONE+Delta_H(-2)O(-1)[H]	136.088815	H(12)C(9)O(1)	NORMAL	
4-ONE+Delta_H(-2)O(-1)[K]	136.088815	H(12)C(9)O(1)	NORMAL	
4-ONE[C]	154.09938	H(14)C(9)O(2)	NORMAL	
4-ONE[H]	154.09938	H(14)C(9)O(2)	NORMAL	
4-ONE[K]	154.09938	H(14)C(9)O(2)	NORMAL	
4AcAllylGal[C]	372.142033	H(24)C(17)O(9)	NORMAL	
ADP-Ribosyl[C]	541.06111	H(21)C(15)N(5)O(13)P(2)	NORMAL	
ADP-Ribosyl[D]	541.06111	H(21)C(15)N(5)O(13)P(2)	NORMAL	
ADP-Ribosyl[E]	541.06111	H(21)C(15)N(5)O(13)P(2)	NORMAL	

In **Configurations** page, you can edit the modification, linker, enzyme and amino acids information here.

# 4 Identification Results

```
E:\Code\SpotLink\004_PKG_SpotLink_20211221_WJ_1.2.4\SpotLink\SpotLink.exe

[SpotLink] Copyright © 2021. All rights reserved.
2021-12-21 21:26:09.018044

[SpotLink] SpotLink whole workflow.
2021-12-21 21:26:09.018044
[SpotLink] Start to read ini and ms2 file.
2021-12-21 21:26:09.019040
[SpotLink] Start to generate protein & peptide index.
2021-12-21 21:26:10.070438
[SpotLink] Digestion.
2021-12-21 21:26:10.070438
[SpotLink] Generating Theoretical Spectra.
2021-12-21 21:26:11.082987
[SpotLink] Saving indexes.
2021-12-21 21:26:11.935520
[SpotLink] Start searching.
2021-12-21 21:26:11.936521
[SpotLink] Start file uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.sms2.
2021-12-21 21:26:11.936521
[SpotLink] End file uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.sms2.
2021-12-21 21:28:53.838815
[SpotLink] End searching.
2021-12-21 21:28:54.363187
[SpotLink] Start Quality Control.
2021-12-21 21:28:54.363187
[SpotLink] Fine scoring on uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.rpsm.
2021-12-21 21:28:55.324158
[SpotLink] End fine scoring on uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.rpsm.
2021-12-21 21:29:15.530108
[SpotLink] Start SVM classification on PSM.
2021-12-21 21:29:15.753017
[SpotLink] SVM classification on uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.fpsm.
2021-12-21 21:29:15.754013
[SpotLink] Calculating q-Value on uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.fpsm.
2021-12-21 21:29:18.918053
[SpotLink] Start Site Scoring.
2021-12-21 21:29:19.195170
[SpotLink] Site scoring on uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.cxsvmpsm.
2021-12-21 21:29:20.228739
```



2021_12_21_21_26_07.spotlink	2021/12/21 21:26	SPOTLINK 文件	2 KB
SpotLink.log	2021/12/21 21:29	文本文档	75 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20210520.fasta	2021/5/20 15:29	FASTA 文件	134 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.cxsvmpsm	2021/12/21 21:29	CXSVMPSM 文件	2,395 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.fpsm	2021/12/21 21:29	FPSM 文件	2,272 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.mgf	2021/11/3 14:33	MGF 文件	3,466 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.rms2	2021/12/21 21:26	RMS2 文件	74,780 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.rpsm	2021/12/21 21:28	RPSM 文件	4,066 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.sfdprpsm	2021/12/21 21:29	SFDRPSM 文件	3,027 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.sms2	2021/12/21 21:26	SMS2 文件	78,985 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.spsm	2021/12/21 21:29	SPSM 文件	2,894 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX.proxl.proxl.xml	2021/12/21 21:29	XML 文档	2,089 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX_result.csv	2021/12/21 21:29	Microsoft Excel ...	499 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX_result_filtered.csv	2021/12/21 21:29	Microsoft Excel ...	499 KB
uniprot-reviewed_yes+AND+organism_Escherichia+coli+(strain+K12)_random_choose300_0_20211103_SDA_CX_site_filtered.csv	2021/12/21 21:29	Microsoft Excel ...	292 KB

The progress information and the result files.

## The Output File Description

File name and extension name	Description
_result.csv	The total unfiltered cross-links result from SpotLink
_result_filtered.csv	The FDR filtered cross-links result from SpotLink
site_filtered.csv	The sFDR filtered cross-links result from SpotLink
_proxl.proxl.xml	The xml file for ProXL to visualization

## The Error Code and the Description

Error Code	Information for users	Description
-10	Permission error!	Permission error. The software does not have the permission to perform the I/O operations.
-20	Normal error. Please check log file.	General error. The details should be found in the log file.
-30	License error! Please download the newest version of SpotLink.	The software has expired and needs to be downloaded and installed again.
-40	Empty Identification !	No cross-linking information was identified.

**Thanks!**