pNovo 3 User Guide

pFind Group

2017/12/29

Before software installation

- **Windows 7 or above**
  - **64 bit version**
  - **.Net Framework 4.5 environment or above**

pNovo 3 Introduction

- The kernel in pNovo 3 is written with C++
- The interface in pNovo 3 is written with C# / WPF
- pNovo 3 cannot run on Linux and Mac systems

How to register

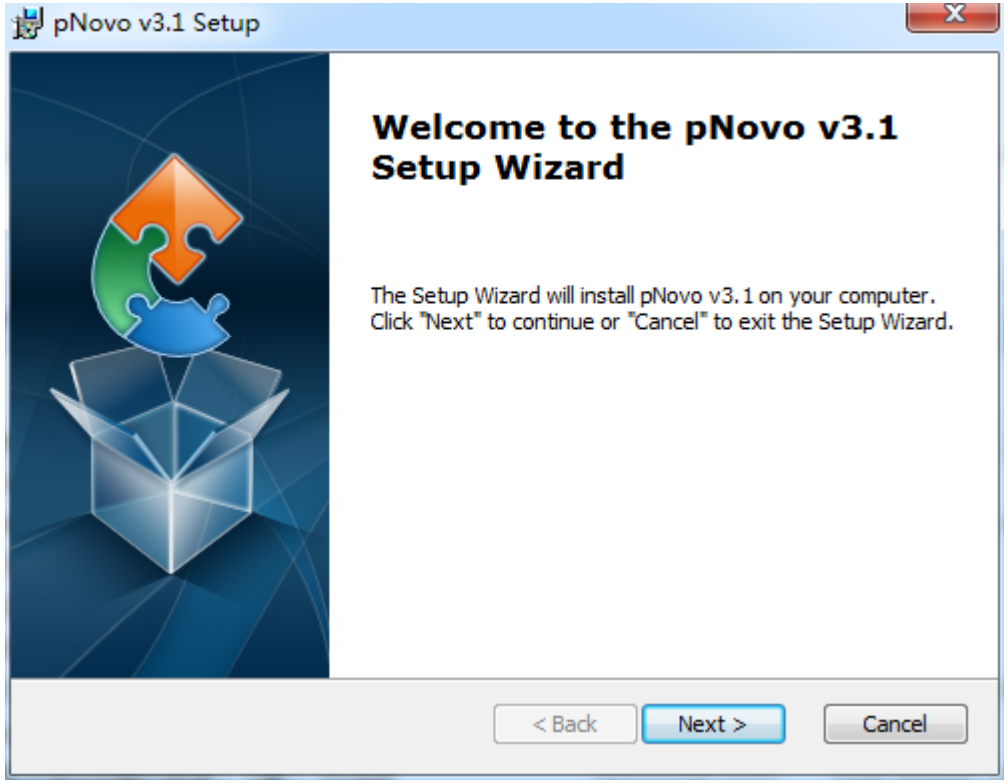
- **Please visit the website to fill in the registered table: http://pfind.ict.ac.cn/software/pNovo/index.html**
- **Please email the table to pnovo@ict.ac.cn to get the registered code**

* First name	
* Last name	
* University/Company	
* Department	
* Lab leader/Supervisor:	
* Work email address	
* Country	
City / ZIP	
Phone	
* How do you hear about pNovo?	
* What can pNovo do for you?	

Notice:
1. The item with the "*" **must be** filled.
2. **For Chinese, please fill in the table with Chinese.** Thanks.
3. Please email this table to pnovo@ict.ac.cn to get the registered code.
4. **Please let us know if you use pNovo in your publication.**

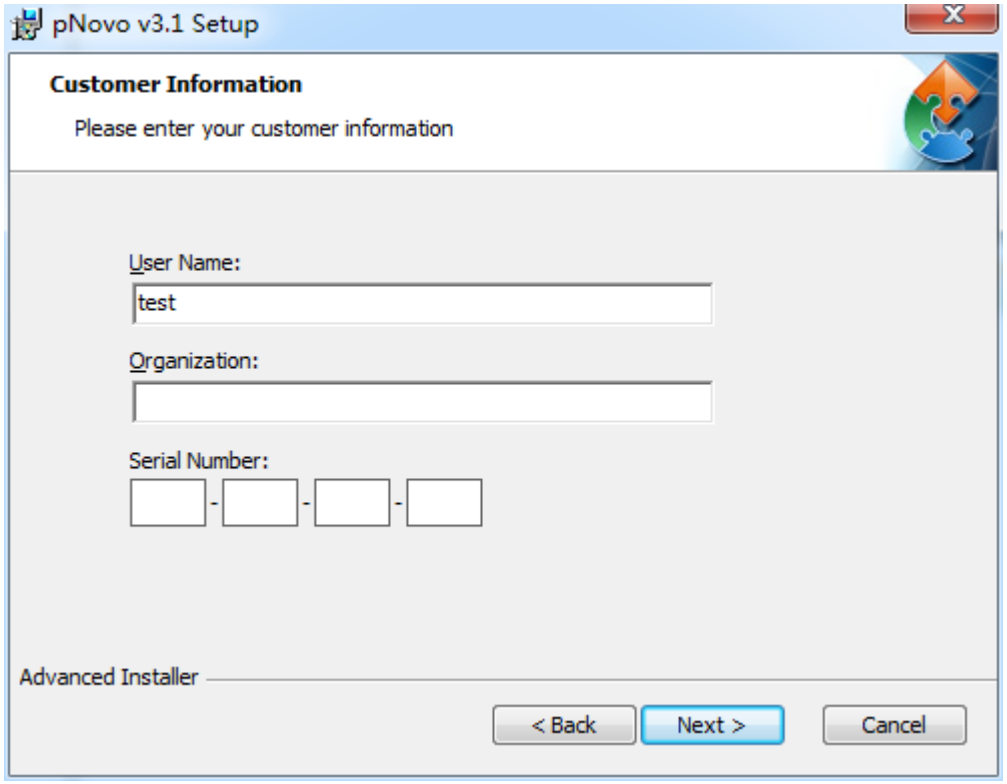
How to install

- **Double click the “EXE” setup file:**



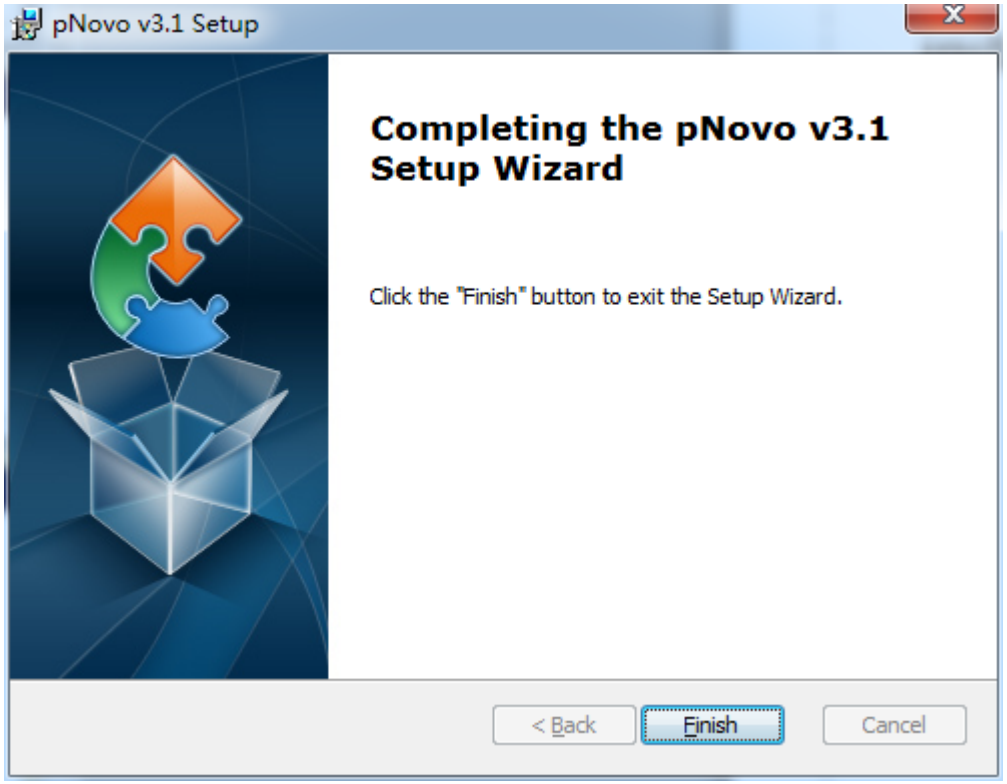
How to install

- **You should input the registered code in the installation process:**



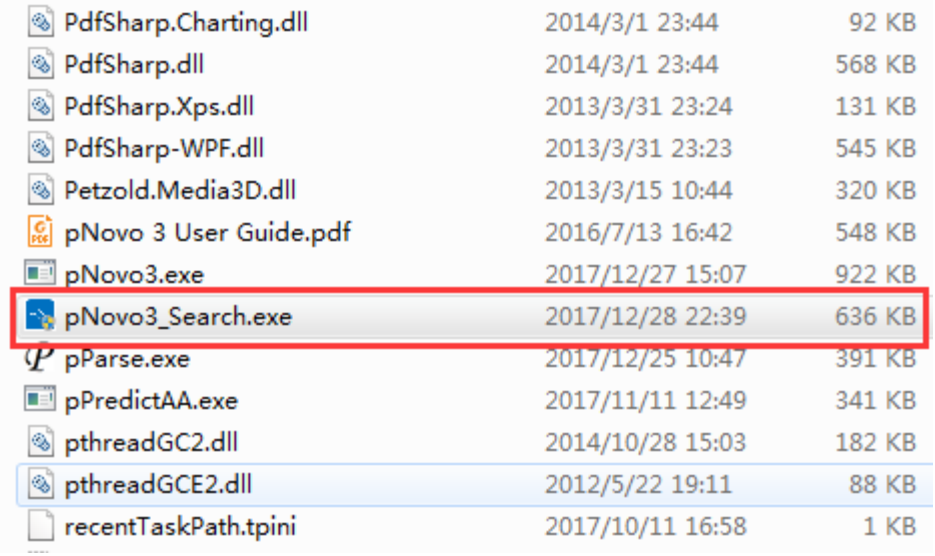
How to install

- **You can always input “Next” step in each process, pNovo will be successfully installed:**



How to run pNovo 3

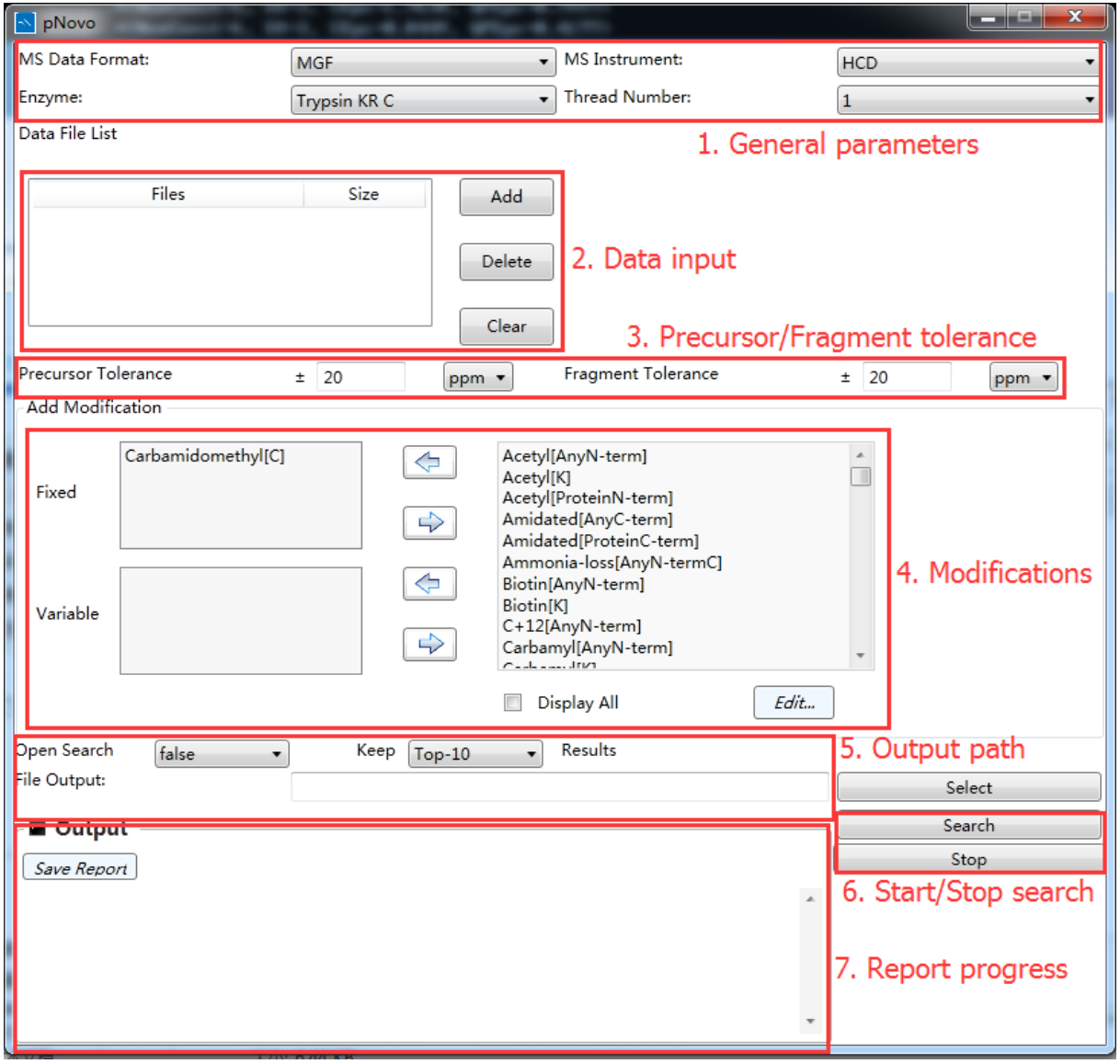
- pNovo 3 is successfully installed in your computer, the default installation directory is “C:\Program Files\ict\pNovo v3.1\”
- You can double click “pNovo3_Search.exe” file in the installation directory to start pNovo 3:



Catalog

- **Search Parameters**
- **Displaying Results**

Search Parameters



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Search Parameters

- MS Data Format
- MS Instrument
- Enzyme
- Thread Number
- Data File List
- Precursor Tolerance/Fragment Tolerance
- Fixed Modification/Variable Modification
- Open Search
- Keep Top-1/Top-10 Results
- File Output

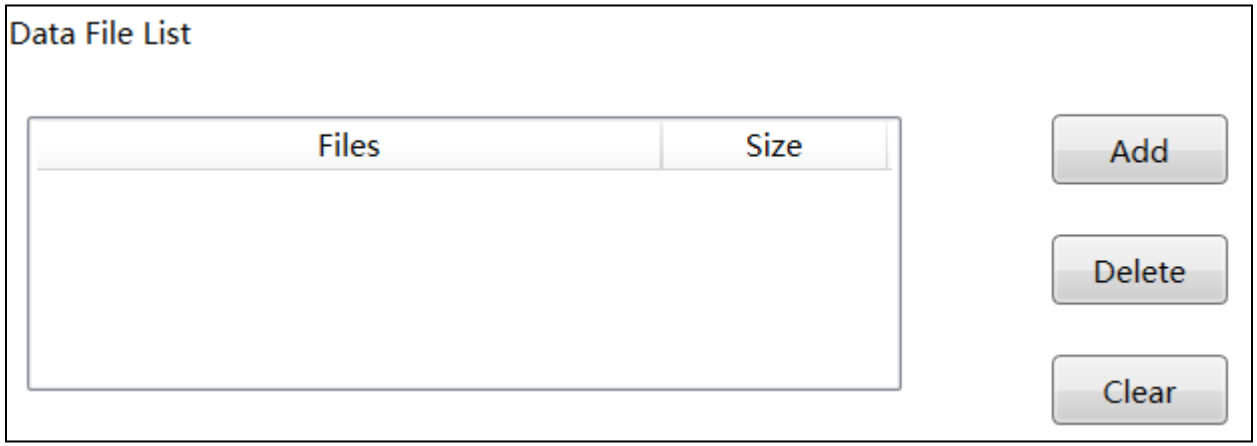
Fundamental Parameter

- MS Data Format: MGF/DTA/MS2
- MS Instrument: HCD/ETD/CID
- Enzyme: Trypsin/AspN/ArgC/GluC…
- Thread Number: 1/2/…/8

- pNovo can support all enzymes in “enzyme.ini” file, you can modify this file to support your individual enzyme.

Data File List

- You can add all data files by clicking “Add” button.
- You can delete some data files by clicking “Delete” button.
- Also, you can delete all data files by clicking “Clear” button.

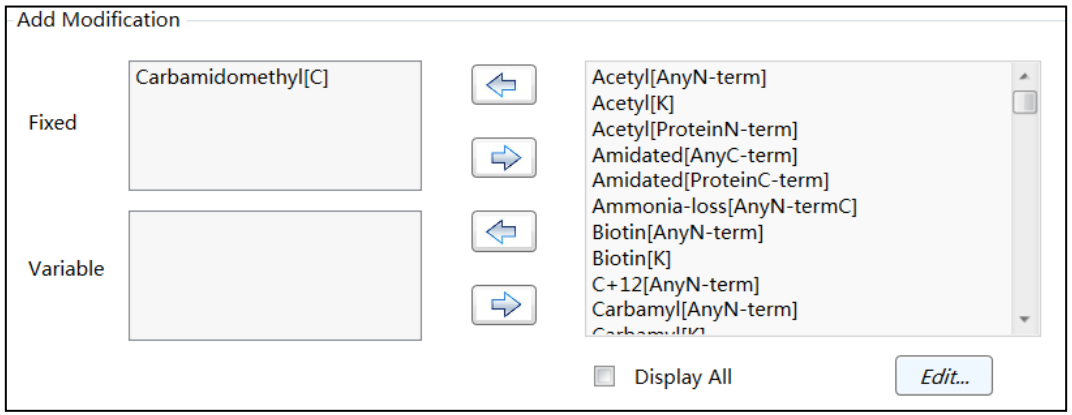


Precursor/Fragment Tolerance

- You can set the precursor tolerance and fragment tolerance in “Da” or “ppm” format.

Fixed/Variable Modification

- You can add some modifications as fixed modification or variable modification by clicking “  ” button.
- You can delete some fixed modification or variable modification by clicking “  ” button.
- Also, you can see all modifications in Unimod by clicking “Display All” checkbox while pNovo defaultly displays common modifications.
- Finally, you can modify all modifications in Unimod in “modification.ini” file.



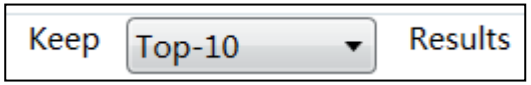
Open Search

- pNovo has a very important function of searching “unexpected” modification.
- You can only add “Carbamidomethyl[C]” as fixed modification and few modifications as variable modifications, and set the “Open Search” as “true”, then pNovo will automatically find credible “unexpected” modifications in Unimod.

Keep Top-1/Top-10 Results

- pNovo can support the very fast searching by keeping only Top-1 result when you set “Keep Top-1 Result”.

- pNovo defaultly set “Keep Top-10 Results” because the results will be much more credible although the speed is slow.



File Output

- You can select a path as the output path and you should insure that the selected path is existing.
- pNovo will create two folders (“param” and “result” folder) in the selected path. The param folder has only one file named “pNovo.param” which contains all searching parameters while the result folder has two files, one file is “pNovo.res” which contains all searching result and the other file is “time.txt” which displays the searching time.
- Finally, you can click “Search” button to start searching!!!

Catalog

- **Search Parameters**
- **Displaying Results**

Displaying Results

- **Result File Format**
- **Peptide List**
- **Peptide-Spectrum-Matching Graph**

Displaying Results

- pNovo will create a folder named “result”, which restores all result files.
- This folder contains three main result files:
  - “results.res” records the top-one PSM per spectrum
  - “pNovo.res” records the top-ten PSMs per spectrum
  - “time.txt” records the searching time in this searching task

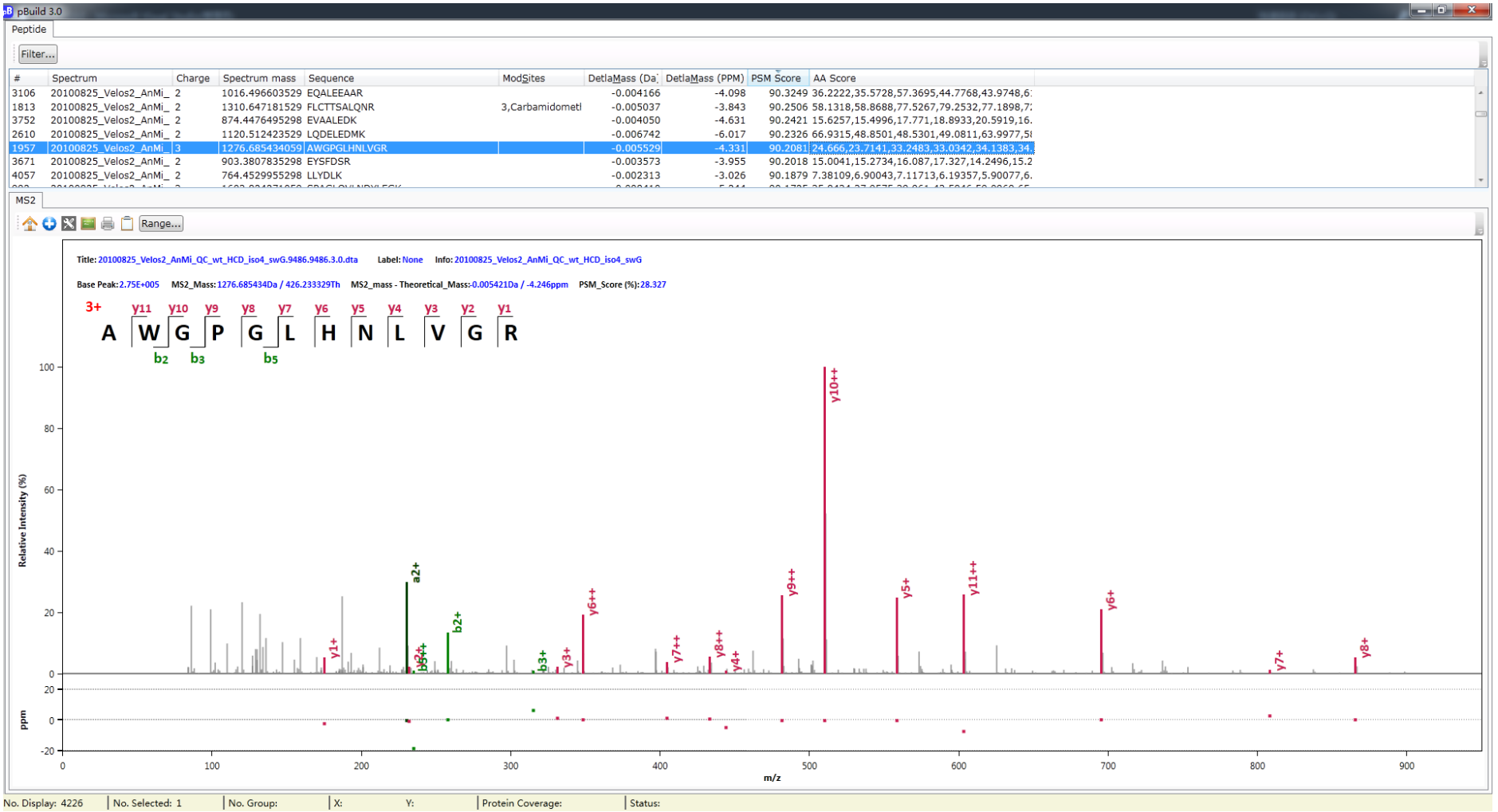
Displaying Results

- The format of “results.res” file
- Each row contains one PSM for each spectrum and it has 9 columns, the following will introduce these columns:
  - Spectrum title
  - Sequence
  - Modifications
  - Average score of all amino acids in this sequence
  - Final score of this sequence
  - All scores of amino acids (using “,” to split)
  - The best sequence in background candidates
  - Modifications of this best sequence
  - Precursor mass deviation of the original sequence

Displaying Results

- The format of "pNovo.res" file, the line starts with "S" records the spectrum title:
- The line starts with "P" records top-ten PSMs of this spectrum (from the first column to last column):
  - "P1" to "P10" means the first 1 to 10 candidate result
  - Sequence
  - Final score of this result
  - Modification abundance (if result doesn't contain modification, it is 1.0)
  - Precursor mass deviation
  - Path rank in the spectrum graph
  - Score of main ions
  - Score of internal ions
  - Continuity score of b ions
  - Continuity score of y ions
  - Enzyme score
  - Std. fragment mass deviations
  - Max. fragment mass deviations
  - Charge feature
  - Raw score of peptide-spectrum matching
  - Spearman correlation between the real and theoretical ion intensities
  - Gap feature
  - Gap feature for N-terminal sequence

Displaying Results



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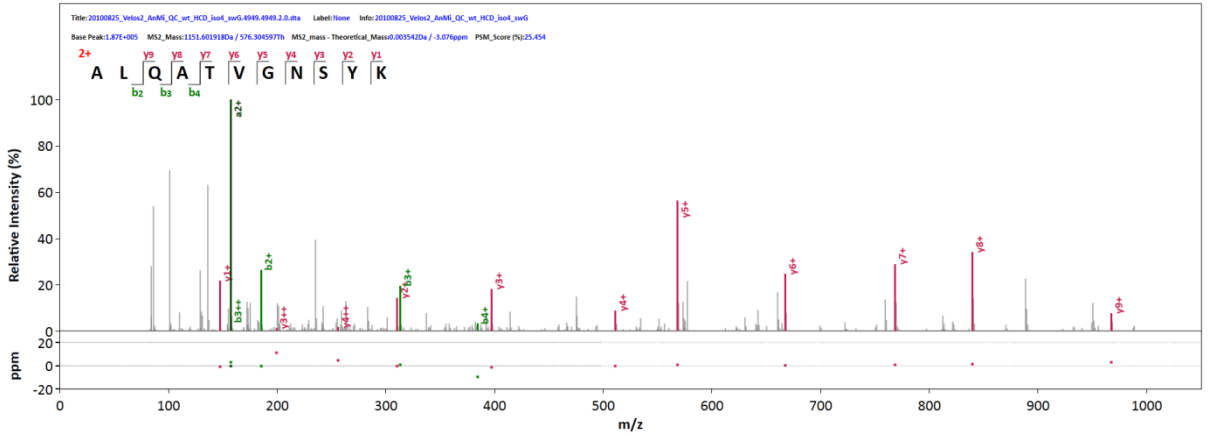
Peptide List

- pNovo will display all searching results which contain the title and charge of spectrum, the sequence and modifications of peptide.

#	Spectrum	Charge	Spectrum mass	Sequence	ModSites	DetlaMass (Da)	DetlaMass (PPM)	PSM Score	AA Score
3106	20100825_Velos2_AnMi_	2	1016.496603529	EQALEEAAR		-0.004166	-4.098	90.3249	36.2222,35.5728,57.3695,44.7768,43.9748,61.9872,58.2665,53.68,58.5509
1813	20100825_Velos2_AnMi_	2	1310.647181529	FLCTTSALQNR	3,Carbamidometl	-0.005037	-3.843	90.2506	58.1318,58.8688,77.5267,79.2532,77.1898,72.9922,68.6975,73.4063,65.1435,53.3508,64.5376
3752	20100825_Velos2_AnMi_	2	874.4476495298	EVAALEDK		-0.004050	-4.631	90.2421	15.6257,15.4996,17.771,18.8933,20.5919,16.9963,16.1662,17.4287
2610	20100825_Velos2_AnMi_	2	1120.512423529	LQDELEDMK		-0.006742	-6.017	90.2326	66.9315,48.8501,48.5301,49.0811,63.9977,58.7425,58.0835,65.027,65.7872
1957	20100825_Velos2_AnMi_	3	1276.685434059	AWGPGLHNLVGR		-0.005529	-4.331	90.2081	24.666,23.7141,33.2483,33.0342,34.1383,34.0924,28.3314,23.466,23.1274,16.7401,17.3826,20.221
3671	20100825_Velos2_AnMi_	2	903.3807835298	EYSFDSR		-0.003573	-3.955	90.2018	15.0041,15.2734,16.087,17.327,14.2496,15.2974,15.0217
4057	20100825_Velos2_AnMi_	2	764.4529955298	LLYDLK		-0.002313	-3.026	90.1879	7.38109,6.90043,7.11713,6.19357,5.90077,6.10736
992	20100825_Velos2_AnMi_	3	1603.824271059	SPAGLQVLNDYLEGK		-0.008410	-5.244	90.1725	35.8434,37.9575,39.961,43.5846,59.0969,65.3083,68.2589,68.7975,60.4357,59.0147,55.2496,44.8304
3547	20100825_Velos2_AnMi_	2	933.5056075298	MVLNESLK		-0.001800	-1.929	90.1372	25.554,24.5401,30.0771,24.5792,25.1699,17.8601,17.6659,24.8212
1355	20100825_Velos2_AnMi_	3	1441.651141059	PNMVTPGHACTEK	10,Carbamidome	-0.005162	-3.581	90.101	86.4819,76.0616,94.0065,95.0569,95.1556,92.1839,91.9984,87.0515,79.5969,67.6415,65.132,73.1309
2901	20100825_Velos2_AnMi_	2	1055.542857529	TPGPGAESALR		-0.005207	-4.933	90.1006	67.4762,68.8808,83.1992,80.1005,46.4961,45.0688,68.3379,59.4903,56.6764,60.9396,63.3482
2321	20100825_Velos2_AnMi_	3	1191.593539059	HMYHSLYLK		-0.004453	-3.737	89.9754	73.2959,70.5134,64.4153,92.3771,96.2282,95.2941,88.8753,91.5386,91.9403
2897	20100825_Velos2_AnMi_	2	1057.620351529	LLLLGAGESGK		-0.004888	-4.621	89.9011	69.2428,69.1773,87.2617,81.5448,59.8472,55.9012,58.9159,70.6328,65.3996,66.1602,71.5286
3927	20100825_Velos2_AnMi_	2	818.4399555298	VPMTSGVK		-0.004183	-5.111	89.9	37.2744,35.4711,33.915,27.5262,21.2455,19.9556,20.8822,26.7701
552	20100825_Velos2_AnMi_	3	1880.976307059	LPQFGLSTPGSDLHVNAK		-0.010248	-5.448	89.8246	40.5997,38.9631,42.3,59.0855,33.0017,32.0896,42.4111,60.0389,61.8855,62.344,56.5164,50.5773,51.
4145	20100825_Velos2_AnMi_	2	705.3912015298	LGFSGPK		-0.001829	-2.593	89.7968	29.1624,26.8303,25.4813,30.9401,21.7269,22.5209,27.4492
2431	20100825_Velos2_AnMi_	3	1167.663421059	VRHPVSTMLK		-0.003294	-2.821	89.7835	61.5504,65.2982,76.1123,92.7921,91.5943,95.2533,92.486,90.3942,85.2538,85.1227
1746	20100825_Velos2_AnMi_	3	1328.687410059	AENPGAAVTTRNK		-0.004333	-3.261	89.7615	10.8554,10.5064,10.3276,10.168,9.63623,9.15852,10.6045,12.735,12.7474,10.2398,9.62697,9.38333,
3206	20100825_Velos2_AnMi_	2	993.5682115298	PFTTLSSLK		-0.006815	-6.859	89.754	18.3093,17.8236,18.0097,19.3485,18.5556,21.0713,17.7632,17.0673,19.2455
1691	20100825_Velos2_AnMi_	3	1345.741591059	TFLALKPDQANK		-0.005893	-4.379	89.7037	56.3161,58.3302,71.2653,71.1724,78.7773,75.9969,68.1076,57.5868,50,47.9475,51.8158,59.3173
1257	20100825_Velos2_AnMi_	2	1480.697305529	LLELSGSSSEDSEK		-0.004048	-2.734	89.6978	33.9376,31.2725,30.4049,36.0227,34.5867,39.2929,38.8935,39.6679,33.8411,32.7827,32.0168,25.417
3512	20100825_Velos2_AnMi_	2	941.5012695298	PTHVNFAR		-0.006234	-6.622	89.6958	15.4567,15.0841,13.4981,14.6773,14.0355,12.1411,11.1929,13.0804
1359	20100825_Velos2_AnMi_	3	1440.665335059	PNMVTPGHACTQK	10,Carbamidome	-0.006982	-4.847	89.6765	65.8393,56.3734,65.9723,81.8404,81.458,40.6467,42.5657,64.6207,47.1105,48.3662,47.9475,50.5922
2444	20100825_Velos2_AnMi_	2	1166.599515529	AASLTSEVFNK		-0.005739	-4.920	89.6336	62.1528,62.6405,62.3087,73.7978,88.2065,82.0782,82.2277,81.6553,85.418,66.1265,79.7612
1411	20100825_Velos2_AnMi_	2	1422.660317529	NQSFCPTVNLDK	5,Carbamidometl	-0.007974	-5.605	89.6019	44.8672,46.0375,70.1314,79.5725,82.0747,74.6659,72.8987,77.0922,72.0504,66.6574,65.805,75.387
2914	20100825_Velos2_AnMi_	3	1052.548654059	YRNFDLPK		-0.003755	-3.567	89.5013	19.3465,19.9846,21.5535,19.9825,20.9617,19.731,19.7834,18.9739
2949	20100825_Velos2_AnMi_	2	1046.543949529	VLDDTNLTR		-0.003770	-3.602	89.4615	65.645,67.7372,75.9581,50,50.5008,71.9966,66.3088,65.2581,68.1723

Peptide-Spectrum-Matching Graph

- pNovo will display the peptide-spectrum-matching graph.
- You can hold down the mouse button to zoom in the graph while you can also scroll the mouse button to zoom in or zoom out.
- You can also input “Ctrl+C” to copy the graph to clipboard and “Ctrl+V” to paste the graph to word or PPT.
- Finally, there are other useful functions in pNovo. You can try it by yourself and we will wish you to have a good time.



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- **Thank you for using pNovo 3 software!**

- **If you have any questions, please contact pnovo@ict.ac.cn.**