

CJunction User Guide

2021/06/24

Step1: Setting parameters

Step2: Importing the results of de novo sequencing of MS data

The screenshot shows the CJunction software interface with several panels highlighted by red boxes and arrows:

- Parameters Panel:** Contains settings for Enzyme (Trypsin/P), Digestion Mode (Specific), Max. Missed Cleavages (2), Precursor Tolerance (20 ppm), and a list of modifications (Acetyl, Amidated, Carbamyl, Deamidation, GlyGly, Methyl, Phospho) that can be moved between Fixed and Variable categories. The Max. Number of Variable Modifications per Peptide is set to 2.
- Input and Output Panel:** Features a list of input files (e.g., C:\Users\52615\Desktop\39_20,0) with Load and Remove buttons, a Peptide List section, and an Output Directory field set to C:\Users\52615\Desktop with a Browse button.
- Analysis Options:** Includes radio buttons for "Get Junction Peptide" (selected) and "Composition Analysis", along with a Start button.

Step3: Setting the path to the output file

Step4: Selecting the "Get Junction Peptide" option

Step5: Clicking "Start" to run the program

In the second step above, the input file needs to satisfy the following format, with the contents of each line separated by commas: name of the MS raw file, scan number of the MS2 spectrum, peptide identified by de novo sequencing, monoisotopic mass of the precursor ion, and charge, respectively.

```
39_20.0.02&0.05.csv x
0 10 20 30 40 50 60 70 80
1 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVVLALVPR,483.33044,2
2 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVLAVALPR,483.33044,2
3 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVLAVALPR,483.33044,2
4 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVVLGLLPR,483.33044,2
5 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVVLGLLPR,483.33044,2
6 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVLLAVPR,483.33044,2
7 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVLLGLPR,483.33044,2
8 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVLLVALPR,483.33044,2
9 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVLLVALPR,483.33044,2
10 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003,VVLLVALPR,483.33044,2
11 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,FPLFGVAR,453.76578,2
12 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,FPLFGVAR,453.76578,2
13 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,FPLFRAR,453.76578,2
14 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,FPLFVGAR,453.76578,2
15 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,FPLFVGAR,453.76578,2
16 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,FPLGVFAR,453.76578,2
17 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,LMLFGVAR,453.76578,2
18 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,LMLFGVAR,453.76578,2
19 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,MLLFGVAR,453.76578,2
20 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026,MLLFGVAR,453.76578,2
```

1. When the program is finished running, two files are available: Result.txt and JunctionPeptide.fasta.
2. In the Result.txt, all of the possible junction peptides corresponding to the MS2 spectrum are shown after the colon in each row.
3. JunctionPeptide.fasta provides all possible non-redundant junction peptides enumerated from the MS dataset.



```
Result.txt x
0 10 20 30 40 50 60 70
1 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10003:VWLALVPR;
2 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10026;;
3 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10036;;
4 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10039;;
5 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10040;;
6 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10043;;
7 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10044;;
8 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10047;;
9 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10052;;
10 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10057;;
11 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10065;;
12 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10076;;
13 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10083;;
14 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10087;;
15 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10093;;
16 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10096;;
17 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10102;;
18 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10120:LLPALZAFR;
19 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10136;;
20 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10145;;
21 "20150708_QE3_UPLC8_DBJ_QC_HELA_39frac_Trypsin_1.raw",10148;;
```

```
JunctionPeptide.fasta x
0 10 20
1 >JunctionPeptide_1
2 VWLALVPR
3 >JunctionPeptide_2
4 LLPALMAFR
5 >JunctionPeptide_3
6 FLLLLSGPK
7 >JunctionPeptide_4
8 LLLLLSGPK
9 >JunctionPeptide_5
10 FGMLLLFLK
11 >JunctionPeptide_6
12 APTMLLFLK
13 >JunctionPeptide_7
14 KKMLLPGR
15 >JunctionPeptide_8
16 LLPALMAFR
17 >JunctionPeptide_9
18 LLMALLPR
19 >JunctionPeptide_10
20 DHTVRSTGPAK
```