User’s guideline

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# Chapter 1. Evaluation of citrullinated PSMs

# Download

**To download Citrullination analysis.ipynb:**

- Go to https://github.com/Sunghyun-Huh/Citrullination-Diagnostic-Ion-Analysis.
- Click on ‘Citrullination analysis.ipynb’ among the listed files.
- Click ‘Raw’ on the top right panel.
- Press ctrl+s and type ‘Citrullination analysis.ipynb’ to keep the ipynb extension.

# Requirements

**Python requirements**

- Python version 3.6 or greater
- Libraries needed: pandas, numpy, pyteomics, itertools, collections, statistics, re, os, glob

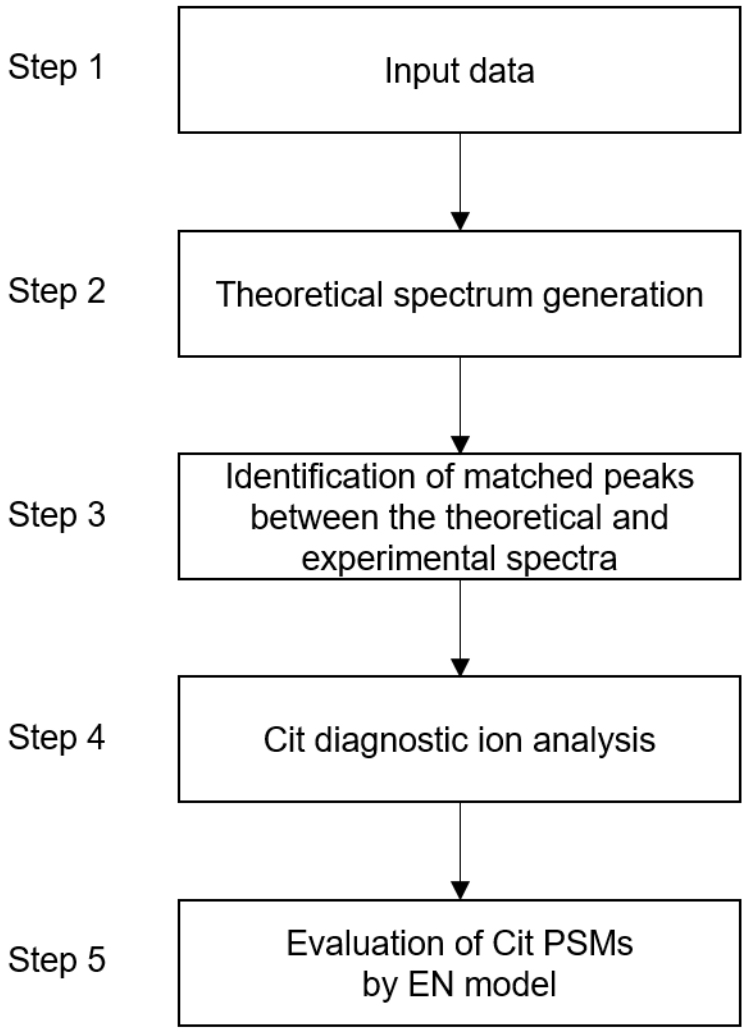
**Necessary Files**

- Input search result file: The input file must be a CSV file(s) containing the following three columns (see **Step 1** for the details):

| | |
|---|---|
| ‘Title’ | MS2 spectrum title as written in MGF file |
| ‘Peptide’ | Peptide sequence with modification delta mass rounded up to third decimal places |
| ‘Charge’ | Charge state of the peptide |

- Spectrum file: The spectrum file must be a Mascot Generic Format (MGF) file(s) containing MS2 spectra corresponding to those matched to the PSMs in the input search result file. If MS2 spectra in the input search file and spectrum file are not equivalent, only the common MS2 spectra will be retained and subsequently processed.

## Overview flowchart



# Step 1: Input data

## A. Setting initial parameters

The initial parameter settings:

### 0. Initial parameters

In [ ]:
```
# Fragmentation method used
Frag_method = 'HCD' # HCD, CID, ETD, ECD

# Ion types for theoretical spectrum generation
ion_type = ['Precur', 'y', 'b', 'a', 'z', 'c', 'INT', 'IM']

# Generate each ion type
if Frag_method == 'HCD' or Frag_method == 'CID':
    annot_yb     = True # y-, b-, a-ion
    annot_zc     = False # z-, c-ion
elif Frag_method == 'ETD' or Frag_method == 'ECD':
    annot_yb     = False # y-, b-, a-ion
    annot_zc     = True # z-, c-ion
annot_precur = True # precursor ion
annot_INT    = True # internal ion
annot_IM     = True # immonium ion
annot_dict = {
    'Precur' : annot_precur,
    'y'      : annot_yb,
    'b'      : annot_yb,
    'a'      : annot_yb,
    'z'      : annot_zc,
    'c'      : annot_zc,
    'INT'    : annot_INT,
    'IM'     : annot_IM
}

# MS2 mass tolerance (ppm)
ms2_ppm = 15

# Signal-to-noise (SNR) filter for MS2 spectrum
apply_SNR = True # Apply SNR filter
SNR       = 2 # SNR threshold
low       = 0.05 # Define low x% intensity as baseline noise level

# Maximum charge state of sequence ions
max_charge = 2 # 2, 3, ... 'max'

# Maximum number of neutral loss from a single ion
max_NL = 3
```

| | |
|---|---|
| Frag_method | Fragmentation method used in the input data (value = ‘HCD’, ‘CID’, ‘ETD’, or ‘ECD’). If set as ‘HCD’ or ‘CID’, *y*-ion, *b*-ion, and *a*-ion will be generated for theoretical spectrum. If set as ‘ETD’ or ‘ECD’, *z*-ion and *c*-ion will be generated for theoretical spectrum. Commonly, precursor, internal, and immonium ions will be generated for all these fragmentation methods. |
| ms2_ppm | MS2 level mass tolerance in ppm (default = 15 ppm). |
| apply_SNR | Determine whether to apply signal-to-noise filter to remove noise peaks (value = True or False; default = True). |
| SNR | Signal threshold level. The average intensity of noise peaks (as defined in ‘low’) multiplied by this signal threshold level will be the final signal-to-noise filter. If ‘apply_SNR’ = True, all peaks below the signal-to-noise filter will be removed (default = 2). |
| low | Proportion of MS2 peaks regarded as noise. If ‘apply_SNR’ = True, all peaks below this noise level will be treated as noise (default = 0.05). |
| max_charge | Maximum charge state of fragment ions (default = 2). |
| max_NL | Maximum number of neutral losses from a single ion (default = 3). |

# Step 1: Input data

## B. Loading input data

Example input search result file:

| | A | B | C |
|---|---|---|---|
| | Title | Peptide | Charge |
| | 20160312_02_A1.10012.10012.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1001 | YETSGIGEAR+0.984VK | 2 |
| | 20160312_02_A1.10045.10045.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1004 | NIVTPR+0.984TPPPSQGK | 2 |
| | 20160312_02_A1.10116.10116.3 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1011 | NIVTPR+0.984TPPPSQGK | 3 |
| | 20160312_02_A1.10222.10222.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1022 | NIVTPR+0.984TPPPSQGK | 2 |
| | 20160312_02_A1.10334.10334.3 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1033 | NIVTPR+0.984TPPPSQGK | 3 |
| | 20160312_02_A1.10362.10362.3 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1036 | TPSTAHLR+0.984VPK | 3 |
| | 20160312_02_A1.10418.10418.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1041 | NIVTPR+0.984TPPPSQGK | 2 |
| | 20160312_02_A1.10479.10479.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1047 | AQSR+0.984EQLAALK | 2 |
| | 20160312_02_A1.1054.1054.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1054" | DSR+0.984SGSPM+15.995AR | 2 |
| | 20160312_02_A1.10602.10602.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1060 | NIVTPR+0.984TPPPSQGK | 2 |
| | 20160312_02_A1.10646.10646.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1064 | Q+0.984KR+0.984LQ+0.984AM+15.995Q+0.984K | 2 |
| | 20160312_02_A1.10671.10671.3 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1067 | SGSEAGSPRR+0.984PRRQR | 3 |
| | 20160312_02_A1.1073.1073.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1073" | R+0.984GGGGGRR+0.984SK | 2 |
| | 20160312_02_A1.10764.10764.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1076 | R+0.984FIN+0.984DMVK | 2 |
| | 20160312_02_A1.10769.10769.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1076 | NIVTPR+0.984TPPPSQ+0.984GK | 2 |
| | 20160312_02_A1.10874.10874.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1087 | MAR+0.984EAEFEAEQER | 2 |
| | 20160312_02_A1.11026.11026.3 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1102 | RGR+0.984PPKDEK | 3 |
| | 20160312_02_A1.11286.11286.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1128 | N+0.984R+0.984Q+0.984VIC+57.021VTLK | 2 |
| | 20160312_02_A1.11398.11398.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1139 | GGTSR+0.984ALAAASSVK | 2 |
| | 20160312_02_A1.11489.11489.3 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1148 | EEFER+0.984Q+0.984N+0.984KQLR | 3 |
| | 20160312_02_A1.11557.11557.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1155 | TVEMR+0.984DGEVIK | 2 |
| | 20160312_02_A1.11735.11735.2 File:"20160312_02_A1.raw", NativeID:"controllerType=0 controllerNumber=1 scan=1173 | Q+0.984Q+0.984IADLR+0.984EDLKR | 2 |

search_result_file

Format for peptide sequences should follow that of MS-GF+ search result. Specifically, modification delta masses should be rounded up to third decimal places. Currently allowed modifications are as follows:

| Modification | Mod on peptide |
|---|---|
| Carbamidomethyl Cys | C+57.021 |
| Oxidation Met | M+15.995 |
| Deamidated Asn | N+0.984 |
| Deamidated Gln | Q+0.984 |
| Citrullinated Arg | R+0.984 |
| Pyro-Glu from Glu | E-17.027 |
| Pyro-Glu from Gln | Q-18.011 |
| iTRAQ 4plex Lys | K+144.102 |
| iTRAQ 8plex Lys | K+304.205 |
| TMT Lys | K+229.163 |
| iTRAQ 4plex N-term | +144.102 |
| iTRAQ 8plex N-term | +304.205 |
| TMT N-term | +229.163 |
| Acetyl N-term | +42.011 |

# Step 1: Input data

## B. Loading input data

A snapshot of codes for loading input files:

### 1. Input files

```
In [ ]: # Set current working directory
        PATH = "F:/Project/"
        os.chdir(PATH)
```

```
In [ ]: # Input files
        spec_files   = glob.glob('spectrum_file.mgf') # MGF file(s)
        search_files = glob.glob('search_result_file.csv') # Search result file(s)
```

Users can upload local input files via the following steps:

- Set the directory in which the input files are located.
- Type the input filenames. In case of multiple MGF or search result files in the same directory, type in '*.mgf' or '*.csv'.

A snapshot of loaded MGF file:

```
In [38]: df_exp
```

Out[38]:

| | Title | m/z array | intensity array |
|---|---|---|---|
| 0 | 20160312_02_A1.93.93.2 File:"20160312_02_A1.ra... | [113.0715067, 113.6931047, 114.1196269, 115.08... | [215.7922515869, 159.3930053711, 169.514968872... |
| 1 | 20160312_02_A1.206.206.2 File:"20160312_02_A1.... | [110.0715323, 113.0712549, 114.0551851, 115.08... | [171.9085998535, 2270.2216796875, 213.05671691... |
| 2 | 20160312_02_A1.323.323.2 File:"20160312_02_A1.... | [112.3516646, 113.0712827, 114.0551846, 115.08... | [162.815612793, 3220.46875, 206.597442627, 867... |
| 3 | 20160312_02_A1.441.441.2 File:"20160312_02_A1.... | [113.0715639, 114.0551846, 114.1746251, 115.05... | [3046.6838378906, 329.9189758301, 160.89303588... |
| 4 | 20160312_02_A1.559.559.2 File:"20160312_02_A1.... | [113.0714846, 114.0555112, 114.3805672, 114.46... | [3265.0134277344, 383.2307434082, 174.38641357... |
| ... | ... | ... | ... |
| 390 | 20160312_04_A3.27367.27367.4 File:"20160312_04... | [110.071535, 111.0363426, 112.087122, 120.0813... | [2331.1821289063, 355.9834289551, 505.66485595... |
| 391 | 20160312_04_A3.28271.28271.4 File:"20160312_04... | [110.0715594, 110.5183569, 111.074985, 114.091... | [4666.2412109375, 348.894744873, 435.891632080... |
| 392 | 20160312_04_A3.29399.29399.3 File:"20160312_04... | [110.0716261, 111.0749855, 114.0918189, 116.04... | [9665.826171875, 666.3602294922, 485.172882080... |
| 393 | 20160312_04_A3.29644.29644.3 File:"20160312_04... | [110.071646, 111.0375996, 112.2654049, 112.639... | [1841.1348876953, 159.2207641602, 165.47415161... |
| 394 | 20160312_04_A3.30248.30248.2 File:"20160312_04... | [110.0715359, 115.0868826, 115.2165788, 116.03... | [2713.2185058594, 989.6865844727, 373.98999023... |

395 rows × 3 columns

A snapshot of loaded search result file:

```
In [44]: df
```

Out[44]:

| | Title | Peptide | Charge |
|---|---|---|---|
| 0 | 20160312_02_A1.10012.10012.2 File:"20160312_02... | YETSGIGEAR+0.984VK | 2 |
| 1 | 20160312_02_A1.10045.10045.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | 2 |
| 2 | 20160312_02_A1.10116.10116.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | 3 |
| 3 | 20160312_02_A1.10222.10222.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | 2 |
| 4 | 20160312_02_A1.10334.10334.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | 3 |
| ... | ... | ... | ... |
| 94 | 20160312_02_A1.323.323.2 File:"20160312_02_A1.... | DSR+0.984SGSPM+15.995AR | 2 |
| 95 | 20160312_02_A1.32378.32378.4 File:"20160312_02... | GHIEWPDFLSHESLLLLQ+0.984Q+0.984LR+0.984PQNSLLR | 4 |
| 96 | 20160312_02_A1.3280.3280.2 File:"20160312_02_A... | VSR+0.984VASPK | 2 |
| 97 | 20160312_02_A1.33227.33227.2 File:"20160312_02... | AR+0.984HRKTGQ+0.984KVALK | 2 |
| 98 | 20160312_02_A1.33883.33883.2 File:"20160312_02... | RERVR+0.984QLR | 2 |

99 rows × 3 columns

## Step 2: Theoretical spectrum generation

Theoretical spectrum is generated for each Cit PSM in the following order:

- Singly charged sequence and internal ions (e.g., *y*- and *b*-ions for sequence or internal ions).
- Neutral loss variants of precursor, sequence, and internal ions.
- Multiply charged sequence ions.
- Immonium ions.

A snapshot of input data:

```
In [20]: df_pep
```

Out[20]:

| | Title | Peptide | mod_Peptide | seq_Peptic |
|---|---|---|---|---|
| 0 | 20160312_02_A1.10012.10012.2 File:"20160312_02... | YETSGIGEAR+0.984VK | YETSGIGEArVK | YETSGIGEArV |
| 1 | 20160312_02_A1.10045.10045.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 2 | 20160312_02_A1.10116.10116.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 3 | 20160312_02_A1.10222.10222.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 4 | 20160312_02_A1.10334.10334.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| ... | ... | ... | ... | |
| 94 | 20160312_02_A1.323.323.2 File:"20160312_02_A1.... | DSR+0.984SGSPM+15.995AR | DSrSGSPmAR | DSrSGSPmA |
| 95 | 20160312_02_A1.32378.32378.4 File:"20160312_02... | GHIEWPDFLSHESLLLLQ+0.984Q+0.984LR+0.984PQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLL |
| 96 | 20160312_02_A1.3280.3280.2 File:"20160312_02_A... | VSR+0.984VASPK | VSrVASPK | VSrVASP |
| 97 | 20160312_02_A1.33227.33227.2 File:"20160312_02... | AR+0.984HRKTGQ+0.984KVALK | ArHRKTGqKVALK | ArHRKTGqKVAL |
| 98 | 20160312_02_A1.33883.33883.2 File:"20160312_02... | RERVR+0.984QLR | RERVrQLR | RERVrQL |

99 rows × 8 columns

A snapshot of output data:

In [35]:
```
df_pep_mz
```

Out[35]:

| | Title | Peptide | mod_Peptide | seq_Peptic |
|---|---|---|---|---|
| 0 | 20160312_02_A1.10012.10012.2 File:"20160312_02... | YETSGIGEAR+0.984VK | YETSGIGEArVK | YETSGIGEArV |
| 1 | 20160312_02_A1.10045.10045.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 2 | 20160312_02_A1.10116.10116.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 3 | 20160312_02_A1.10222.10222.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 4 | 20160312_02_A1.10334.10334.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| ... | ... | ... | ... | |
| 94 | 20160312_02_A1.323.323.2 File:"20160312_02_A1.... | DSR+0.984SGSPM+15.995AR | DSrSGSPmAR | DSrSGSPmA |
| 95 | 20160312_02_A1.32378.32378.4 File:"20160312_02... | GHIEWPDFLSHESLLLLQ+0.984Q+0.984LR+0.984PQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLL |
| 96 | 20160312_02_A1.3280.3280.2 File:"20160312_02_A... | VSR+0.984VASPK | VSrVASPK | VSrVASF |
| 97 | 20160312_02_A1.33227.33227.2 File:"20160312_02... | AR+0.984HRKTGQ+0.984KVALK | ArHRKTGqKVALK | ArHRKTGqKVAl |
| 98 | 20160312_02_A1.33883.33883.2 File:"20160312_02... | RERVR+0.984QLR | RERVrQLR | RERVrQL |

99 rows × 20650 columns

In [36]:
```
df_pep_label
```

Out[36]:

| | Title | Peptide | mod_Peptide | seq_Peptic |
|---|---|---|---|---|
| 0 | 20160312_02_A1.10012.10012.2 File:"20160312_02... | YETSGIGEAR+0.984VK | YETSGIGEArVK | YETSGIGEArV |
| 1 | 20160312_02_A1.10045.10045.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 2 | 20160312_02_A1.10116.10116.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 3 | 20160312_02_A1.10222.10222.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| 4 | 20160312_02_A1.10334.10334.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | NIVTPrTPPPSQG |
| ... | ... | ... | ... | |
| 94 | 20160312_02_A1.323.323.2 File:"20160312_02_A1.... | DSR+0.984SGSPM+15.995AR | DSrSGSPmAR | DSrSGSPmA |
| 95 | 20160312_02_A1.32378.32378.4 File:"20160312_02... | GHIEWPDFLSHESLLLLQ+0.984Q+0.984LR+0.984PQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLL |
| 96 | 20160312_02_A1.3280.3280.2 File:"20160312_02_A... | VSR+0.984VASPK | VSrVASPK | VSrVASF |
| 97 | 20160312_02_A1.33227.33227.2 File:"20160312_02... | AR+0.984HRKTGQ+0.984KVALK | ArHRKTGqKVALK | ArHRKTGqKVAl |
| 98 | 20160312_02_A1.33883.33883.2 File:"20160312_02... | RERVR+0.984QLR | RERVrQLR | RERVrQL |

99 rows × 20650 columns

# Step 3: Identification of matched peaks between the theoretical and experimental spectra

Theoretical and experimental spectra are compared, and only the matched ions with less than *m/z* difference of 15 ppm are retained.

A snapshot of input data:

```
In [53]: df_exp_mz
```

```
Out[53]: 0      [115.0868788, 129.1025438, 130.050427, 148.604...
         1      [113.0712549, 115.0870324, 129.1025438, 130.05...
         2      [113.0712827, 115.0869625, 129.1025433, 130.05...
         3      [113.0715639, 115.0507616, 115.0869447, 116.09...
         4      [113.0714846, 115.0507612, 115.0872086, 129.10...
                                    ...
         390    [110.071535, 120.0813037, 121.0843465, 129.102...
         391    [110.0715594, 115.0868819, 120.0813009, 121.08...
         392    [110.0716261, 111.0749855, 120.0813701, 143.11...
         393    [110.071646, 120.0808807, 129.1025478, 130.086...
         394    [110.0715359, 120.0813789, 121.0842901, 129.06...
         Name: m/z array, Length: 395, dtype: object
```

```
In [50]: df_theo_mz
```

Out[50]:

| | mz_Precursor | mz_y_1 | mz_y_2 | mz_y_3 | mz_y_4 | mz_y_5 | mz_y_6 | mz_y_7 | mz_y_8 | mz_y_9 | ... | mz_IM-NH3_21 | mz_IM-NH3_22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 655.833519 | 147.113353 | 246.181767 | 403.266894 | 474.304008 | 603.346601 | 660.368065 | 773.452129 | 830.473593 | 917.505621 | ... | NaN | NaN |
| 1 | 746.910097 | 147.113353 | 204.134817 | 332.193395 | 419.225423 | 516.278187 | 613.330951 | 710.383715 | 811.431394 | 968.516521 | ... | NaN | NaN |
| 2 | 498.276006 | 147.113353 | 204.134817 | 332.193395 | 419.225423 | 516.278187 | 613.330951 | 710.383715 | 811.431394 | 968.516521 | ... | NaN | NaN |
| 3 | 746.910097 | 147.113353 | 204.134817 | 332.193395 | 419.225423 | 516.278187 | 613.330951 | 710.383715 | 811.431394 | 968.516521 | ... | NaN | NaN |
| 4 | 498.276006 | 147.113353 | 204.134817 | 332.193395 | 419.225423 | 516.278187 | 613.330951 | 710.383715 | 811.431394 | 968.516521 | ... | NaN | NaN |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 94 | 540.741111 | 175.119501 | 246.156615 | 393.192015 | 490.244779 | 577.276807 | 634.298271 | 721.330299 | 878.415426 | 965.447454 | ... | NaN | NaN |
| 95 | 836.443032 | 175.119501 | 288.203565 | 401.287629 | 488.319657 | 602.362584 | 730.421162 | 827.473926 | 984.559053 | 1097.643117 | ... | 113.071488 | 53.039125 |
| 96 | 422.748533 | 147.113353 | 244.166117 | 331.198145 | 402.235259 | 501.303673 | 658.388800 | 745.420828 | NaN | NaN | ... | NaN | NaN |
| 97 | 747.947348 | 147.113353 | 260.197417 | 331.234531 | 430.302945 | 558.397908 | 687.440502 | 744.461966 | 845.509645 | 973.604608 | ... | NaN | NaN |
| 98 | 557.334162 | 175.119501 | 288.203565 | 416.262143 | 573.347270 | 672.415684 | 828.516795 | 957.559388 | NaN | NaN | ... | NaN | NaN |

99 rows × 20178 columns

```
In [51]: df_theo_label
```

Out[51]:

| | mz_Precursor | mz_y_1 | mz_y_2 | mz_y_3 | mz_y_4 | mz_y_5 | mz_y_6 | mz_y_7 | mz_y_8 | mz_y_9 | ... | mz_IM-NH3_21 | mz_IM-NH3_22 | mz_IM-NH3_23 | mz_IM-NH3_24 | mz_IM-NH3_25 | mz_IM-NH3_26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| 1 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| 2 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| 3 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| 4 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 94 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| 95 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | IM(r)-NH3 | IM(P)-NH3 | IM(Q)-NH3 | IM(N)-NH3 | IM(S)-NH3 | IM(L)-NH3 |
| 96 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | NaN | NaN | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| 97 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | NaN | NaN | NaN | NaN | NaN | NaN |
| 98 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | NaN | NaN | ... | NaN | NaN | NaN | NaN | NaN | NaN |

99 rows × 20178 columns

A snapshot of output data (matched ions):

In [39]:
```
df_mz_label
```

Out[39]:

| | Title | Peptide | mod_Peptide | Pep_length | Charge | mz_Precurs |
|---|---|---|---|---|---|---|
| 0 | 20160312_02_A1.10012.10012.2 File:"20160312_02... | YETSGIGEAR+0.984VK | YETSGIGEArVK | 12 | 2 | 655.8335 |
| 1 | 20160312_02_A1.10045.10045.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 2 | 746.9100 |
| 2 | 20160312_02_A1.10116.10116.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 3 | 498.2760 |
| 3 | 20160312_02_A1.10222.10222.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 2 | 746.9100 |
| 4 | 20160312_02_A1.10334.10334.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 3 | 498.2760 |
| ... | ... | ... | ... | ... | ... | |
| 94 | 20160312_02_A1.323.323.2 File:"20160312_02_A1.... | DSR+0.984SGSPM+15.995AR | DSrSGSPmAR | 10 | 2 | 540.7411 |
| 95 | 20160312_02_A1.32378.32378.4 File:"20160312_02... | GHIEWPDFLSHESLLLLQ+0.984Q+0.984LR+0.984PQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLLR | 28 | 4 | 836.4430 |
| 96 | 20160312_02_A1.3280.3280.2 File:"20160312_02_A... | VSR+0.984VASPK | VSrVASPK | 8 | 2 | 422.7485 |
| 97 | 20160312_02_A1.33227.33227.2 File:"20160312_02... | AR+0.984HRKTGQ+0.984KVALK | ArHRKTGqKVALK | 13 | 2 | 747.9473 |
| 98 | 20160312_02_A1.33883.33883.2 File:"20160312_02... | RERVR+0.984QLR | RERVrQLR | 8 | 2 | 557.3341 |

99 rows × 289 columns

# Step 4: Cit diagnostic ion analysis

Annotations and occurrence numbers of citrullination diagnostic ions are reported for each Cit PSM.

A snapshot of input data:

```
In [41]: df_mz_label_uniq
```

Out[41]:

| | Title | Peptide | mod_Peptide | Pep_length | Charge | mz_Precurs |
|---|---|---|---|---|---|---|
| 0 | 20160312_02_A1.10012.10012.2 File:"20160312_02... | YETSGIGEAR+0.984VK | YETSGIGEArVK | 12 | 2 | 655.8335 |
| 1 | 20160312_02_A1.10045.10045.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 2 | 746.9100 |
| 2 | 20160312_02_A1.10116.10116.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 3 | 498.2760 |
| 3 | 20160312_02_A1.10222.10222.2 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 2 | 746.9100 |
| 4 | 20160312_02_A1.10334.10334.3 File:"20160312_02... | NIVTPR+0.984TPPPSQGK | NIVTPrTPPPSQGK | 14 | 3 | 498.2760 |
| ... | ... | ... | ... | ... | ... | |
| 94 | 20160312_02_A1.323.323.2 File:"20160312_02_A1.... | DSR+0.984SGSPM+15.995AR | DSrSGSPmAR | 10 | 2 | 540.7411 |
| 95 | 20160312_02_A1.32378.32378.4 File:"20160312_02... | GHIEWPDFLSHESLLLLQ+0.984Q+0.984LR+0.984PQNSLLR | GHIEWPDFLSHESLLLLqqLrPQNSLLR | 28 | 4 | 836.4430 |
| 96 | 20160312_02_A1.3280.3280.2 File:"20160312_02_A... | VSR+0.984VASPK | VSrVASPK | 8 | 2 | 422.7485 |
| 97 | 20160312_02_A1.33227.33227.2 File:"20160312_02... | AR+0.984HRKTGQ+0.984KVALK | ArHRKTGqKVALK | 13 | 2 | 747.9473 |
| 98 | 20160312_02_A1.33883.33883.2 File:"20160312_02... | RERVR+0.984QLR | RERVrQLR | 8 | 2 | 557.3341 |

99 rows × 289 columns

A snapshot of output data (binary occurrence for the immonium ion and occurrence numbers for the other diagnostic ions):

```
In [66]: Final_result
```

Out[66]:

| el | precNL_label | seqNL_label | ... | Total_NL_count | precNL_count | seqNL_count | intNL_count | Total_INT_count | Dipeptide_count | Tripeptide_count | IM_NH3_count |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-<br>ır-<br>ır-<br>... | | y5-43-NH3++,y3-43,y4-43,y10-43-H2O-H2O++,y10-4... | ... | 48 | 0 | 23 | 25 | 10 | 4 | 6 | 0 |
| T-<br>'r-<br>... | | y10-43-H2O++,y10-43++,y11-43-H2O++,y11-43++,y1... | ... | 38 | 0 | 15 | 23 | 14 | 8 | 6 | 0 |
| T-<br>'r-<br>... | | b7-43-H2O | ... | 20 | 0 | 1 | 19 | 15 | 9 | 6 | 0 |
| T-<br>'r-<br>... | | y9-43-H2O++,y9-43++,y10-43-H2O++,y10-43++,y11-... | ... | 56 | 0 | 23 | 33 | 17 | 11 | 6 | 0 |
| T-<br>'r-<br>... | | b7-43-H2O | ... | 26 | 0 | 1 | 25 | 14 | 7 | 7 | 0 |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

# Step 5: Evaluation of Cit PSMs by EN model

The EN model developed in the study is applied to evaluate the validity of each Cit PSM.

A snapshot of input data:

In [66]:
```
Final_result
```

Out[66]:

| el | precNL_label | seqNL_label | ... | Total_NL_count | precNL_count | seqNL_count | intNL_count | Total_INT_count | Dipeptide_count | Tripeptide_count | IM_NH3_count |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-<br>ır-<br>ır-<br>... | | y5-43-NH3++,y3-43,y4-43,y10-43-H2O-H2O++,y10-4... | ... | 48 | 0 | 23 | 25 | 10 | 4 | 6 | 0 |
| T-<br>'r-<br>... | | y10-43-H2O++,y10-43++,y11-43-H2O++,y11-43++,y1... | ... | 38 | 0 | 15 | 23 | 14 | 8 | 6 | 0 |
| T-<br>'r-<br>... | | b7-43-H2O | ... | 20 | 0 | 1 | 19 | 15 | 9 | 6 | 0 |
| T-<br>'r-<br>... | | y9-43-H2O++,y9-43++,y10-43-H2O++,y10-43++,y11-... | ... | 56 | 0 | 23 | 33 | 17 | 11 | 6 | 0 |
| T-<br>'r-<br>... | | b7-43-H2O | ... | 26 | 0 | 1 | 25 | 14 | 7 | 7 | 0 |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

A snapshot of output data (Cit_probability, probability that a PSM contains Cit; and Cit_prediction = 1 when Cit_probability > 0.5):

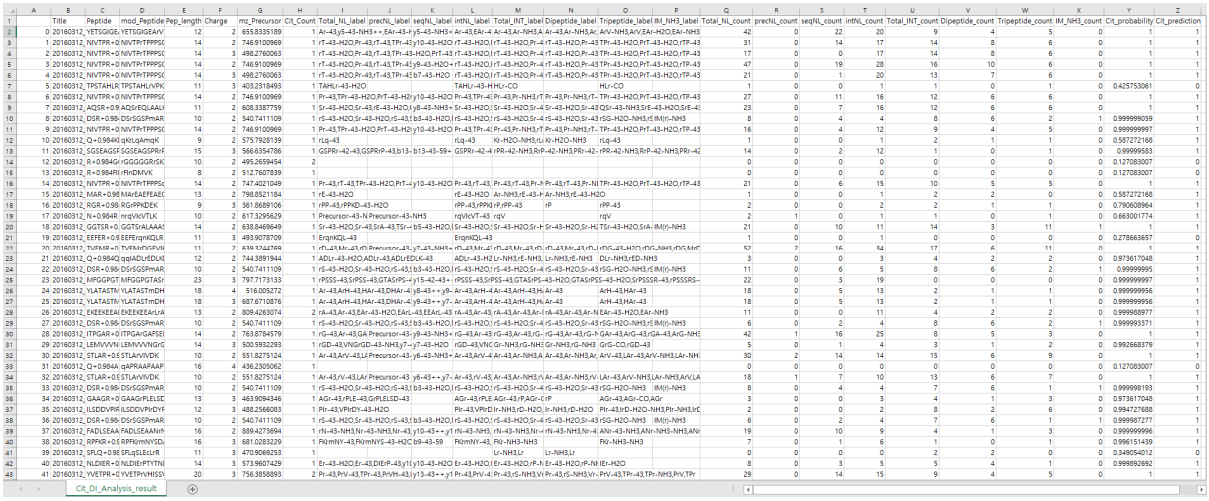
In [65]:
```
Final_result
```

Out[65]:

| ... | Total_NL_count | precNL_count | seqNL_count | intNL_count | Total_INT_count | Dipeptide_count | Tripeptide_count | IM_NH3_count | Cit_probability | Cit_prediction |
|---|---|---|---|---|---|---|---|---|---|---|
| ... | 48 | 0 | 23 | 25 | 10 | 4 | 6 | 0 | 1.000000 | 1 |
| ... | 38 | 0 | 15 | 23 | 14 | 8 | 6 | 0 | 1.000000 | 1 |
| ... | 20 | 0 | 1 | 19 | 15 | 9 | 6 | 0 | 1.000000 | 1 |
| ... | 56 | 0 | 23 | 33 | 17 | 11 | 6 | 0 | 1.000000 | 1 |
| ... | 26 | 0 | 1 | 25 | 14 | 7 | 7 | 0 | 1.000000 | 1 |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

# Step 5: Evaluation of Cit PSMs by EN model

Example output CSV file including the prediction results (Cit_probabilty and Cit_prediction):



Descriptions of the columns in the output CSV file:

| Column | Description |
|---|---|
| Title | MS2 spectrum title |
| Peptide | Original peptide sequence |
| mod_peptide | Simplified peptide sequence with a predefined set of symbols for modifications |
| Pep_length | Peptide length |
| mz_Precursor | Theoretical precursor *m/z* |
| Cit_Count | Number of citrullinated sites |
| Total_NL_label | Annotations of all diagnostic neutral loss ions |
| precNL_label | Annotations of precursor neutral losses |
| seqNL_label | Annotations of sequence ion neutral losses |
| intNL_label | Annotations of internal ion neutral losses |
| Total_INT_label | Annotations of all diagnostic internal ions |
| Dipeptide_label | Annotations of diagnostic dipeptides |
| Tripeptide_label | Annotations of diagnostic tripeptides |
| IM_NH3_label | Annotation of IM(Cit)-$NH_3$ |
| Total_NL_count | Number of all diagnostic neutral loss ions |
| precNL_count | Number of precursor neutral losses |
| seqNL_count | Number of sequence ion neutral losses |
| intNL_count | Number of internal ion neutral losses |
| Total_INT_count | Number of all diagnostic internal ions |
| Dipeptide_count | Number of diagnostic dipeptides |
| Tripeptide_count | Number of diagnostic tripeptides |
| IM_NH3_count | Number of IM(Cit)-$NH_3$ |
| Cit_probability | Probability (P) of citrullination status calculated by the EN model (HCD data only) |
| Cit_prediction | Classification of citrullination status using a P cutoff >0.5 (HCD data only) |

# Chapter 2. Prediction of false negative citrullinated PSMs

## Download

**To download Citrullination analysis.ipynb:**

- Go to https://github.com/Sunghyun-Huh/Citrullination-Diagnostic-Ion-Analysis.
- Click on ‘False negative prediction.ipynb’ among the listed files.
- Click ‘Raw’ on the top right panel.
- Press ctrl+s and type ‘False negative prediction.ipynb’ to keep the ipynb extension.

## Requirements

**Python requirements**

- Python version 3.6 or greater
- Libraries needed: pandas, numpy, pyteomics, itertools, collections, statistics, re, os, glob, scipy.stats

**Necessary Files**

- Unmodified peptide file: The input file must be a CSV file containing the following column (see **Step 1** for the details):

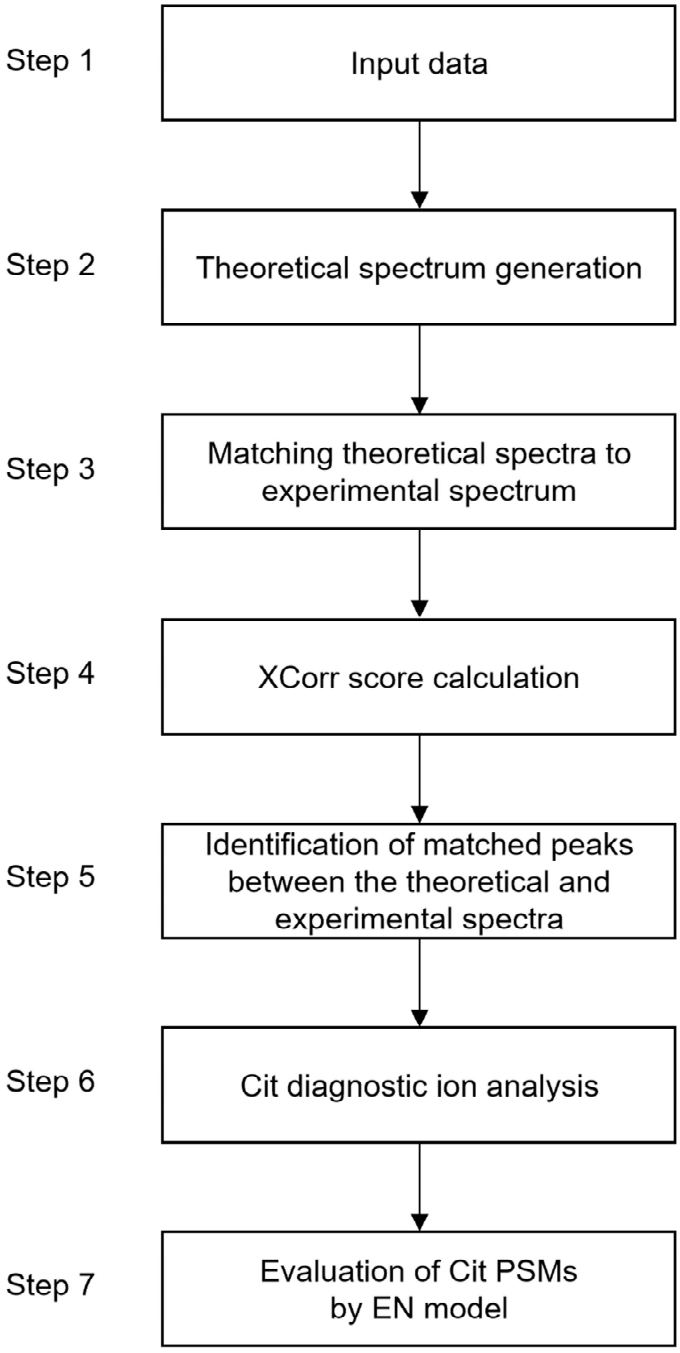
  | | |
  |---|---|
  | ‘Peptide’ | Peptide sequence with modification delta mass rounded up to third decimal places |

- Scan title file: The input file must be a CSV file containing the following column (see **Step 1** for the details):

  | | |
  |---|---|
  | ‘Title’ | MS2 spectrum scan title (scan number) as written in the MGF file |

- Spectrum file: The spectrum file must be a Mascot Generic Format (MGF) file(s) containing either total MS2 spectra or MS2 spectra unassigned by database searching only. For the former case, the codes will automatically search for unassigned MS2 spectra.

## Overview flowchart



# Step 1: Input data

## A. Setting initial parameters

The initial parameter settings:

### 0. Initial parameters

In [95]:
```
# Fragmentation method used
Frag_method = 'HCD' # HCD, CID, ETD, ECD

# Ion types for theoretical spectrum generation
ion_type = ['Precur', 'y', 'b', 'a', 'z', 'c', 'INT', 'IM']

# Generate each ion type
if Frag_method == 'HCD' or Frag_method == 'CID':
    annot_yb     = True # y-, b-, a-ion
    annot_zc     = False # z-, c-ion
elif Frag_method == 'ETD' or Frag_method == 'ECD':
    annot_yb     = False # y-, b-, a-ion
    annot_zc     = True # z-, c-ion
annot_precur = True # precursor ion
annot_INT    = True # internal ion
annot_IM     = True # immonium ion
annot_dict = {
    'Precur' : annot_precur,
    'y'      : annot_yb,
    'b'      : annot_yb,
    'a'      : annot_yb,
    'z'      : annot_zc,
    'c'      : annot_zc,
    'INT'    : annot_INT,
    'IM'     : annot_IM
}

# Mass tolerance (ppm)
ms1_ppm = 10 # MS1 level
ms2_ppm = 15 # MS2 level

# Signal-to-noise (SNR) filter for MS2 spectrum
apply_SNR = True # Apply SNR filter
SNR       = 2 # SNR threshold
low       = 0.05 # Define low x% intensity as baseline noise level

# Maximum charge state of sequence ions
max_charge = 2

# Maximum number of neutral loss from a single ion
max_NL = 3

# Threshold for intensity coverage filter
intensity_coverage = 0.2 # Filter out spectra with sum of annotated intensities < x% of sum of total intensities
```

| | |
|---|---|
| Frag_method | Fragmentation method used in the input data (value = ‘HCD’, ‘CID’, ‘ETD’, or ‘ECD’). If set as ‘HCD’ or ‘CID’, *y*-ion, *b*-ion, and *a*-ion will be generated for theoretical spectrum. If set as ‘ETD’ or ‘ECD’, *z*-ion and *c*-ion will be generated for theoretical spectrum. Commonly, precursor, internal, and immonium ions will be generated for all these fragmentation methods. |
| ms1_ppm | MS1 level mass tolerance in ppm (default = 10 ppm). |
| ms2_ppm | MS2 level mass tolerance in ppm (default = 15 ppm). |
| apply_SNR | Determine whether to apply signal-to-noise filter to remove noise peaks (value = True or False; default = True). |
| SNR | Signal threshold level. The average intensity of noise peaks (as defined in ‘low’) multiplied by this signal threshold level will be the final signal-to-noise filter. If ‘apply_SNR’ = True, all peaks below the signal-to-noise filter will be removed (default = 2). |
| low | Proportion of MS2 peaks regarded as noise. If ‘apply_SNR’ = True, all peaks below this noise level will be treated as noise (default = 0.05). |
| max_charge | Maximum charge state of fragment ions (default = 2). |
| max_NL | Maximum number of neutral losses from a single ion (default = 3). |
| intensity_coverage | Threshold for intensity coverage filter. Spectra with [sum of annotated intensities / sum of total intensities] smaller than this threshold will be filtered out (default = 0.2). |

# Step 1: Input data

### B. Loading input data

Example unmodified peptide file:

| A |
|---|
| Peptide |
| TQEEAIVK |
| TSFADGK |
| KYEGDIK |
| KYEGDIK |
| KYEGDIK |
| SKDQGATYQK |
| TQEEAIVK |
| TQEEAIVK |
| YTPVEEK |
| TSFADGK |
| SKDQGATYQK |
| NGAAQAVTAENK |
| LYENTQDYDK |
| ITTAQEM+15.995YDK |

Format for peptide sequences should follow that of MS-GF+ search result. Specifically, modification delta masses should be rounded up to third decimal places. Currently allowed modifications are as follows:

| Modification | Mod on peptide |
|---|---|
| Carbamidomethyl Cys | C+57.021 |
| Oxidation Met | M+15.995 |
| Deamidated Asn | N+0.984 |
| Deamidated Gln | Q+0.984 |
| Citrullinated Arg | R+0.984 |
| Pyro-Glu from Glu | E-17.027 |
| Pyro-Glu from Gln | Q-18.011 |
| iTRAQ 4plex Lys | K+144.102 |
| iTRAQ 8plex Lys | K+304.205 |
| TMT Lys | K+229.163 |
| iTRAQ 4plex N-term | +144.102 |
| iTRAQ 8plex N-term | +304.205 |
| TMT N-term | +229.163 |
| Acetyl N-term | +42.011 |

# Step 1: Input data

## B. Loading input data

Example scan title file:

| A |
| --- |
| Title |
| QEHF1_10122_DNL.4368.4368.6 File:"QEHF1_10122_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4368" |
| QEHF1_10122_DNL.4396.4396.6 File:"QEHF1_10122_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4396" |
| QEHF1_10123_DNL.4596.4596.6 File:"QEHF1_10123_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4596" |
| QEHF1_10123_DNL.4434.4434.6 File:"QEHF1_10123_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4434" |
| QEHF1_10124_DNL.4668.4668.6 File:"QEHF1_10124_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4668" |
| QEHF1_10125_DNL.4356.4356.6 File:"QEHF1_10125_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4356" |
| QEHF1_10125_DNL.4383.4383.6 File:"QEHF1_10125_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4383" |
| QEHF1_10125_DNL.4601.4601.6 File:"QEHF1_10125_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4601" |
| QEHF1_10247_DNL.4239.4239.6 File:"QEHF1_10247_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4239" |
| QEHF1_10250_DNL.4480.4480.6 File:"QEHF1_10250_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4480" |
| QEHF1_10250_DNL.4272.4272.6 File:"QEHF1_10250_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4272" |
| QEHF1_10275_DNL.3539.3539.6 File:"QEHF1_10275_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=3539" |
| QEHF1_10280_DNL.4501.4501.6 File:"QEHF1_10280_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4501" |
| QEHF1_10280_DNL.4474.4474.6 File:"QEHF1_10280_DNL.raw", NativeID:"controllerType=0 controllerNumber=1 scan=4474" |

# Step 1: Input data

## B. Loading input data

A snapshot of codes for loading input files:

### 1. Input files

In [3]:
```
# Set current working directory
PATH = "F:/Project/"
os.chdir(PATH)
```

In [4]:
```
# Input files
spec_files = glob.glob('*.mgf')[0:5] # MGF file(s)
df_UnmodR  = pd.read_csv("UnmodR_peptide.csv") # UnmodR peptides w/o Cit counterpart peptides
df_total   = pd.read_csv("Total_peptide.csv") # Total identified peptides
```

Users can upload local input files via the following steps:

- Set the directory in which the input files are located.
- Type the input filenames. In case of multiple MGF files in the same directory, type in '*.mgf'.

A snapshot of loaded MGF file:

In [105]:
```
df_exp
```

Out[105]:

| | m/z array | intensity array | Title | exp_Precursor | Charge |
|---|---|---|---|---|---|
| 0 | [108.9069509, 108.9075611, 108.9081714, 111.03... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 18287.58203125,... | QEHF1_10098_DNL.2.2.1 File:"QEHF1_10098_DNL.ra... | 371.101932 | 1 |
| 1 | [108.9068349, 108.9074451, 108.9080554, 110.10... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 1604.9095458984... | QEHF1_10098_DNL.3.3.1 File:"QEHF1_10098_DNL.ra... | 445.121060 | 1 |
| 2 | [108.9068088, 108.907419, 108.9080293, 121.956... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 2023.1558837891... | QEHF1_10098_DNL.4.4.1 File:"QEHF1_10098_DNL.ra... | 536.166752 | 1 |
| 3 | [108.9067553, 108.9073656, 108.9079758, 149.03... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 61633.01953125,... | QEHF1_10098_DNL.5.5.1 File:"QEHF1_10098_DNL.ra... | 462.147507 | 1 |
| 4 | [108.9068558, 108.907466, 108.9080763, 116.332... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 1553.5465087891... | QEHF1_10098_DNL.6.6.1 File:"QEHF1_10098_DNL.ra... | 593.159294 | 1 |
| ... | ... | ... | ... | ... | ... |
| 34469 | [108.9067848, 108.907395, 108.9080053, 110.335... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 2188.8542480469... | QEHF1_10102_DNL.8264.8264.1 File:"QEHF1_10102_... | 751.511179 | 1 |
| 34470 | [108.9067921, 108.9074023, 108.9080126, 112.55... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 3034.580078125,... | QEHF1_10102_DNL.8265.8265.1 File:"QEHF1_10102_... | 1143.789214 | 1 |
| 34471 | [108.9068589, 108.9074691, 108.9080794, 111.03... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 2754.2233886719... | QEHF1_10102_DNL.8266.8266.1 File:"QEHF1_10102_... | 359.314999 | 1 |
| 34472 | [108.9067433, 108.9073536, 108.9079638, 121.02... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 1498.1771240234... | QEHF1_10102_DNL.8267.8267.1 File:"QEHF1_10102_... | 407.300978 | 1 |
| 34473 | [108.9066313, 108.9072416, 108.9078518, 111.03... | [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 2158.365234375,... | QEHF1_10102_DNL.8268.8268.1 File:"QEHF1_10102_... | 387.192683 | 1 |

34474 rows × 5 columns

A snapshot of loaded unmodified peptide file:

In [108]:
```
df_UnmodR
```

Out[108]:

| | Peptide |
|---|---|
| 0 | TQEEAIVK |
| 1 | TSFADGK |
| 2 | KYEGDIK |
| 3 | KYEGDIK |
| 4 | KYEGDIK |
| ... | ... |
| 994 | THNISEGM+15.995MGYDTYPK |
| 995 | THNISEGMMGYDTYPK |
| 996 | GAEVSVVNMEALQAER |
| 997 | EADGSVTHTFVIK |
| 998 | THNISEGMM+15.995GYDTYPK |

999 rows × 1 columns

A snapshot of loaded scan title file:

```
df_total
```

| | Title |
|---|---|
| 0 | QEHF1_10122_DNL.4368.4368.6 File:"QEHF1_10122_... |
| 1 | QEHF1_10122_DNL.4396.4396.6 File:"QEHF1_10122_... |
| 2 | QEHF1_10123_DNL.4596.4596.6 File:"QEHF1_10123_... |
| 3 | QEHF1_10123_DNL.4434.4434.6 File:"QEHF1_10123_... |
| 4 | QEHF1_10124_DNL.4668.4668.6 File:"QEHF1_10124_... |
| ... | ... |
| 31391 | QEHF3_06513_DNL.9237.9237.1 File:"QEHF3_06513_... |
| 31392 | QEHF3_06513_DNL.6988.6988.1 File:"QEHF3_06513_... |
| 31393 | QEHF3_06513_DNL.9195.9195.1 File:"QEHF3_06513_... |
| 31394 | QEHF3_06513_DNL.3945.3945.1 File:"QEHF3_06513_... |
| 31395 | QEHF3_06513_DNL.6917.6917.1 File:"QEHF3_06513_... |

31396 rows × 1 columns

# Step 2: Theoretical spectrum generation

Theoretical spectrum is generated for each Cit peptide as described in **Chapter 1**:

A snapshot of input data:

```
df_pep
```

| | Peptide | mod_Peptide | seq_Peptide | Charge | Pep_length | mz_Precursor | Cit_Count |
|---|---|---|---|---|---|---|---|
| 0 | VPAPVDGER+0.984 | VPAPVDGEr | VPAPVDGEr | 1 | 9 | 940.474 | 1 |
| 1 | NHNLYIAR+0.984 | NHNLYIAr | NHNLYIAr | 1 | 8 | 1001.52 | 1 |
| 2 | NHNLYIAR+0.984 | NHNLYIAr | NHNLYIAr | 2 | 8 | 501.262 | 1 |
| 3 | TAIIR+0.984YNYASGK | TAIIrYNYASGK | TAIIrYNYASGK | 1 | 12 | 1357.71 | 1 |
| 4 | TAIIR+0.984YNYASGK | TAIIrYNYASGK | TAIIrYNYASGK | 2 | 12 | 679.36 | 1 |
| ... | ... | ... | ... | ... | ... | ... | ... |
| 204 | YTFTMRR+0.984 | YTFTMRr | YTFTMRr | 2 | 7 | 488.24 | 1 |
| 205 | TLEDNVALR+0.984ER | TLEDNVALrER | TLEDNVALrER | 1 | 11 | 1316.68 | 1 |
| 206 | TLEDNVALR+0.984ER | TLEDNVALrER | TLEDNVALrER | 2 | 11 | 658.844 | 1 |
| 207 | TLEDNVALRER+0.984 | TLEDNVALREr | TLEDNVALREr | 1 | 11 | 1316.68 | 1 |
| 208 | TLEDNVALRER+0.984 | TLEDNVALREr | TLEDNVALREr | 2 | 11 | 658.844 | 1 |

209 rows × 7 columns

A snapshot of output data:

In [97]:
```
df_pep_mz
```

Out[97]:

| | Peptide | mod_Peptide | seq_Peptide | Charge | Pep_length | mz_Precursor | Cit_Count | seq_y_1 | seq_y_2 | seq_y_3 | ... | mz_IM-NH3_14 | mz_IM-NH3_15 | m NH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | VPAPVDGER+0.984 | VPAPVDGEr | VPAPVDGEr | 1 | 9 | 940.473987 | 1 | r | rE | rEG | ... | NaN | NaN | |
| 1 | NHNLYIAR+0.984 | NHNLYIAr | NHNLYIAr | 1 | 8 | 1001.516845 | 1 | r | rA | rAI | ... | NaN | NaN | |
| 2 | NHNLYIAR+0.984 | NHNLYIAr | NHNLYIAr | 2 | 8 | 501.262335 | 1 | r | rA | rAI | ... | NaN | NaN | |
| 3 | TAIIR+0.984YNYASGK | TAIIrYNYASGK | TAIIrYNYASGK | 1 | 12 | 1357.711574 | 1 | K | KG | KGS | ... | NaN | NaN | |
| 4 | TAIIR+0.984YNYASGK | TAIIrYNYASGK | TAIIrYNYASGK | 2 | 12 | 679.359699 | 1 | K | KG | KGS | ... | NaN | NaN | |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | |
| 204 | YTFTMRR+0.984 | YTFTMRr | YTFTMRr | 2 | 7 | 488.240015 | 1 | r | rR | rRM | ... | NaN | NaN | |
| 205 | TLEDNVALR+0.984ER | TLEDNVALrER | TLEDNVALrER | 1 | 11 | 1316.681019 | 1 | R | RE | REr | ... | NaN | NaN | |
| 206 | TLEDNVALR+0.984ER | TLEDNVALrER | TLEDNVALrER | 2 | 11 | 658.844422 | 1 | R | RE | REr | ... | NaN | NaN | |
| 207 | TLEDNVALRER+0.984 | TLEDNVALREr | TLEDNVALREr | 1 | 11 | 1316.681019 | 1 | r | rE | rER | ... | NaN | NaN | |
| 208 | TLEDNVALRER+0.984 | TLEDNVALREr | TLEDNVALREr | 2 | 11 | 658.844422 | 1 | r | rE | rER | ... | NaN | NaN | |

209 rows × 6560 columns

In [98]:
```
df_pep_label
```

Out[98]:

| | Peptide | mod_Peptide | seq_Peptide | Charge | Pep_length | mz_Precursor | Cit_Count | seq_y_1 | seq_y_2 | seq_y_3 | ... | mz_IM-NH3_14 | mz_IM-NH3_15 | m NH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | VPAPVDGER+0.984 | VPAPVDGEr | VPAPVDGEr | 1 | 9 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 1 | NHNLYIAR+0.984 | NHNLYIAr | NHNLYIAr | 1 | 8 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 2 | NHNLYIAR+0.984 | NHNLYIAr | NHNLYIAr | 2 | 8 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 3 | TAIIR+0.984YNYASGK | TAIIrYNYASGK | TAIIrYNYASGK | 1 | 12 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 4 | TAIIR+0.984YNYASGK | TAIIrYNYASGK | TAIIrYNYASGK | 2 | 12 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | |
| 204 | YTFTMRR+0.984 | YTFTMRr | YTFTMRr | 2 | 7 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 205 | TLEDNVALR+0.984ER | TLEDNVALrER | TLEDNVALrER | 1 | 11 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 206 | TLEDNVALR+0.984ER | TLEDNVALrER | TLEDNVALrER | 2 | 11 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 207 | TLEDNVALRER+0.984 | TLEDNVALREr | TLEDNVALREr | 1 | 11 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |
| 208 | TLEDNVALRER+0.984 | TLEDNVALREr | TLEDNVALREr | 2 | 11 | Precursor | 1 | y1 | y2 | y3 | ... | NaN | NaN | |

209 rows × 6560 columns

# Step 3: Matching theoretical spectra to experimental spectrum

Precursor masses of theoretical and experimental spectra are compared, and only the matched theoretical spectra with less than precursor mass difference of 10 ppm are retained.

A snapshot of input data:

```
In [113]: Mass_theo
```

Out[113]:

| | 0 |
|---|---|
| 0 | 940.473987 |
| 1 | 1001.516845 |
| 2 | 1002.524670 |
| 3 | 1357.711574 |
| 4 | 1358.719399 |
| ... | ... |
| 204 | 976.480030 |
| 205 | 1316.681019 |
| 206 | 1317.688844 |
| 207 | 1316.681019 |
| 208 | 1317.688844 |

209 rows × 1 columns

```
In [114]: Mass_exp
```

Out[114]:

| | 0 |
|---|---|
| 0 | 371.101932 |
| 1 | 445.121060 |
| 2 | 536.166752 |
| 3 | 462.147507 |
| 4 | 593.159294 |
| ... | ... |
| 33123 | 751.511179 |
| 33124 | 1143.789214 |
| 33125 | 359.314999 |
| 33126 | 407.300978 |
| 33127 | 387.192683 |

33128 rows × 1 columns

A snapshot of output data:

```
In [119]: df_unID_scan['Mass_Check']
```

Out[119]:

```
0        0
1        0
2        0
3        0
4        0
        ..
33123    0
33124    0
33125    0
33126    0
33127    0
Name: Mass_Check, Length: 33128, dtype: int64
```

# Step 4: XCorr score calculation

For an experimental spectrum and its matched theoretical spectrum (or spectra), XCorr score is calculated and only the theoretical spectrum with the greatest XCorr score is retained.

A snapshot of input data:

```
In [123]: df_unID_scan_filter
```

| mz_intensity | Mass_Check | matched_theo_spectrum | theo_mz_array | Base_peak | norm intensity array | XCorr | Best_matched_theo_spectrum |
|---|---|---|---|---|---|---|---|
| [[110.0720135, 42529.13671875], [116.0712452, ... | 1 | [11] | [[110.07182241250999, 113.07148831149999, 115.... | 5.778410e+04 | [0.7360007068337424, 0.06188990719697581, 0.58... | [0.4645181088909756] | 11 |
| [[110.0718416, 39672.3046875], [113.0715189, 6... | 1 | [12] | [[110.07182241250999, 113.07148831149999, 115.... | 6.839465e+05 | [0.058004982388973406, 0.00889303027882122 4, 0... | [1.4735176389345732] | 12 |
| [[110.071755, 149039.875], [113.0714059, 94425... | 1 | [95] | [[110.07182241250999, 113.07148831149999, 117.... | 1.271601e+06 | [0.11720645524687869, 0.0742574138807271 5, 0.0... | [7.076248489273531] | 95 |
| [[126.0549671, 38965.9609375], [167.0820595, 7... | 1 | [97] | [[113.07148831149999, 119.0496813115, 130.0980... | 4.437064e+06 | [0.008781925464330181, 0.0016454831627381488, ... | [0.02522510458252917] | 97 |

A snapshot of output data:

```
In [116]: df_pep_label_match
```

Out[116]:

| | Index | Peptide | mod_Peptide | Charge | Pep_length | mz_Precursor | Cit_Count | label array | Best_matched_theo_spectrum |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 11 | GGTHDPLQSVR+0.984 | GGTHDPLQSVr | 1 | 11 | Precursor | 1 | [Precursor, y1, y2, y3, y4, y5, y6, y7, y8, y9... | 11 |
| 1 | 12 | GGTHDPLQSVR+0.984 | GGTHDPLQSVr | 2 | 11 | Precursor | 1 | [Precursor, y1, y2, y3, y4, y5, y6, y7, y8, y9... | 12 |
| 2 | 95 | GSHEPVADNSTVAGR+0.984 | GSHEPVADNSTVAGr | 2 | 15 | Precursor | 1 | [Precursor, y1, y2, y3, y4, y5, y6, y7, y8, y9... | 95 |
| 3 | 97 | YTPVEEKQNGR+0.984 | YTPVEEKQNGr | 2 | 11 | Precursor | 1 | [Precursor, y1, y2, y3, y4, y5, y6, y7, y8, y9... | 97 |
| 4 | 97 | YTPVEEKQNGR+0.984 | YTPVEEKQNGr | 2 | 11 | Precursor | 1 | [Precursor, y1, y2, y3, y4, y5, y6, y7, y8, y9... | 97 |
| | | | | | | | | [Precursor, y1, | |

# Step 5: Identification of matched peaks between the theoretical and experimental spectra

Theoretical and experimental spectra are compared, and only the matched ions with less than *m/z* difference of 15 ppm are retained.

A snapshot of input data:

```
In [125]: df_exp_mz
```

```
Out[125]: 0     [110.0720135, 116.0712452, 128.0707251, 130.05...
          1     [110.0718416, 113.0715189, 114.0552962, 115.02...
          2     [110.071755, 113.0714059, 114.0552635, 115.087...
          3     [126.0549671, 167.0820595, 167.1178537, 181.06...
          4     [126.0550001, 167.0809289, 167.1177837, 169.13...
          5     [123.9887742, 129.1026059, 137.0712778, 178.05...
          6     [110.0719872, 129.1024763, 130.086277, 147.112...
          7     [110.0607183, 110.0718668, 113.0715497, 114.05...
          8     [110.0719087, 113.0716098, 115.0871659, 116.07...
          9     [110.0601176, 110.0719138, 112.0220986, 115.02...
          10    [110.071834, 115.0214849, 115.0507414, 116.034...
          11    [113.0713727, 114.0551653, 115.0869482, 116.07...
          12    [113.0714384, 114.0552416, 115.0870154, 116.07...
          13    [110.0719918, 116.0705729, 127.0871257, 128.09...
          14    [110.0718952, 127.0871186, 128.0897561, 129.10...
          15    [110.0719309, 127.086452, 129.1025519, 130.086...
          16    [110.0719915, 116.071178, 127.087176, 129.1024...
          17    [110.0720804, 112.0219593, 127.0872803, 128.05...
          18    [110.0719435, 127.0871958, 128.0900352, 129.10...
          19    [110.0716643, 111.0918748, 112.0759924, 113.07...
          20    [110.0717597, 111.0920319, 113.0714655, 115.08...
          21    [110.0719004, 116.0710182, 122.0719581, 123.98...
          22    [110.071825, 115.0871277, 127.0871408, 130.050...
          23    [110.0718381, 115.0870345, 116.071251, 127.086...
          24    [110.0719391, 113.0715399, 114.0553174, 115.08...
          Name: m/z array, dtype: object
```

```
In [126]: df_theo_mz
```

Out[126]:

| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | ... | 1005 | 1006 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1167.575827 | 176.103517 | 275.171931 | 362.203959 | 490.262537 | 603.346601 | 700.399365 | 815.426308 | 952.485220 | 1053.532899 | ... | NaN | NaN | |
| 1 | 584.291826 | 176.103517 | 275.171931 | 362.203959 | 490.262537 | 603.346601 | 700.399365 | 815.426308 | 952.485220 | 1053.532899 | ... | NaN | NaN | |
| 2 | 749.350600 | 176.103517 | 233.124981 | 304.162095 | 403.230509 | 504.278188 | 591.310216 | 705.353143 | 820.380086 | 891.417200 | ... | 55.054775 | 27.023475 | 71. |
| 3 | 661.323318 | 176.103517 | 233.124981 | 347.167908 | 475.226486 | 603.321449 | 732.364042 | 861.406635 | 960.475049 | 1057.527813 | ... | NaN | NaN | |
| 4 | 661.323318 | 176.103517 | 233.124981 | 347.167908 | 475.226486 | 603.321449 | 732.364042 | 861.406635 | 960.475049 | 1057.527813 | ... | NaN | NaN | |
| 5 | 372.521397 | 147.113353 | 244.166117 | 373.208711 | 530.293838 | 627.346602 | 790.409922 | 927.468834 | 1014.500862 | 102.055504 | ... | NaN | NaN | |
| 6 | 372.521397 | 147.113353 | 244.166117 | 373.208711 | 530.293838 | 627.346602 | 790.409922 | 927.468834 | 1014.500862 | 102.055504 | ... | NaN | NaN | |
| 7 | 556.776343 | 176.103517 | 277.151196 | 348.188310 | 405.209774 | 533.268352 | 632.336766 | 733.384445 | 870.443357 | 984.486284 | ... | NaN | NaN | |
| 8 | 556.776343 | 176.103517 | 277.151196 | 348.188310 | 405.209774 | 533.268352 | 632.336766 | 733.384445 | 870.443357 | 984.486284 | ... | NaN | NaN | |
| 9 | 861.431787 | 176.103517 | 263.135545 | 320.157009 | 449.199602 | 506.221066 | 619.305130 | 732.389194 | 130.050418 | 243.134482 | ... | NaN | NaN | |
| 10 | 861.431787 | 176.103517 | 263.135545 | 320.157009 | 449.199602 | 506.221066 | 619.305130 | 732.389194 | 130.050418 | 243.134482 | ... | NaN | NaN | |
| 11 | 559.788584 | 176.103517 | 291.130460 | 404.214524 | 532.273102 | 633.320781 | 761.415744 | 874.499808 | 989.526751 | 130.050418 | ... | NaN | NaN | |
| 12 | 559.788584 | 176.103517 | 291.130460 | 404.214524 | 532.273102 | 633.320781 | 761.415744 | 874.499808 | 989.526751 | 130.050418 | ... | NaN | NaN | |
| 13 | 928.456229 | 176.103517 | 247.140631 | 378.181116 | 435.202580 | 506.239694 | 603.292458 | 716.376522 | 813.429286 | 116.034768 | ... | NaN | NaN | |
| 14 | 928.456229 | 176.103517 | 247.140631 | 378.181116 | 435.202580 | 506.239694 | 603.292458 | 716.376522 | 813.429286 | 116.034768 | ... | NaN | NaN | |
| 15 | 928.456229 | 176.103517 | 247.140631 | 378.181116 | 435.202580 | 506.239694 | 603.292458 | 716.376522 | 813.429286 | 116.034768 | ... | NaN | NaN | |
| 16 | 928.456229 | 176.103517 | 247.140631 | 378.181116 | 435.202580 | 506.239694 | 603.292458 | 716.376522 | 813.429286 | 116.034768 | ... | NaN | NaN | |
| 17 | 928.456229 | 176.103517 | 247.140631 | 378.181116 | 435.202580 | 506.239694 | 603.292458 | 716.376522 | 813.429286 | 116.034768 | ... | NaN | NaN | |
| 18 | 928.456229 | 176.103517 | 247.140631 | 378.181116 | 435.202580 | 506.239694 | 603.292458 | 716.376522 | 813.429286 | 116.034768 | ... | NaN | NaN | |
| 19 | 835.434765 | 176.103517 | 307.144002 | 378.181116 | 479.228795 | 594.255738 | 722.350701 | 114.091889 | 242.186852 | 357.213795 | ... | NaN | NaN | |
| 20 | 835.434765 | 176.103517 | 307.144002 | 378.181116 | 479.228795 | 594.255738 | 722.350701 | 114.091889 | 242.186852 | 357.213795 | ... | NaN | NaN | |
| 21 | 1285.606441 | 176.103517 | 277.151196 | 348.188310 | 477.230903 | 574.283667 | 737.346987 | 794.368451 | 923.411044 | 994.448158 | ... | NaN | NaN | |
| 22 | 1325.627201 | 175.119501 | 290.146444 | 403.230508 | 502.298922 | 649.334322 | 777.392900 | 940.456220 | 1054.499147 | 1168.542074 | ... | NaN | NaN | |
| 23 | 1325.627201 | 176.103517 | 291.130460 | 404.214524 | 503.282938 | 650.318338 | 778.376916 | 941.440236 | 1055.483163 | 1169.526090 | ... | NaN | NaN | |
| 24 | 655.320055 | 176.103517 | 291.130460 | 404.214524 | 503.282938 | 634.323423 | 762.382001 | 925.445321 | 1039.488248 | 1153.531175 | ... | NaN | NaN | |

25 rows × 1015 columns

In [127]: `df_theo_label`

Out[127]:

| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | ... | 1005 | 1006 | 1007 | 1008 | 1009 | 1010 | 1011 | 1012 | 1013 | 1014 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 1 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 2 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | IM(V)-NH3 | IM(A)-NH3 | IM(D)-NH3 | IM(N)-NH3 | IM(S)-NH3 | IM(T)-NH3 | IM(V)-NH3 | IM(A)-NH3 | IM(G)-NH3 | IM(r)-NH3 |
| 3 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 4 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 5 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 6 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 7 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 8 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 9 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | b1 | b2 | ... | None | None | None | None | None | None | None | None | None | None |
| 10 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | b1 | b2 | ... | None | None | None | None | None | None | None | None | None | None |
| 11 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 12 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 13 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 14 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 15 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 16 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 17 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 18 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | b1 | ... | None | None | None | None | None | None | None | None | None | None |
| 19 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | b1 | b2 | b3 | ... | None | None | None | None | None | None | None | None | None | None |
| 20 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | b1 | b2 | b3 | ... | None | None | None | None | None | None | None | None | None | None |
| 21 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 22 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 23 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |
| 24 | Precursor | y1 | y2 | y3 | y4 | y5 | y6 | y7 | y8 | y9 | ... | None | None | None | None | None | None | None | None | None | None |

25 rows × 1015 columns

A snapshot of output data (matched ions):

In [121]: `df_mz_label`

Out[121]:

| | unID_Title | Peptide | mod_Peptide | Charge | Pep_length | mz_Precursor | Cit_Count | peak_1 |
|---|---|---|---|---|---|---|---|---|
| 0 | QEHF1_10100_DNL.2385.2385.3 File:"QEHF1_10100_... | GGTHDPLQSVR+0.984 | GGTHDPLQSVr | 1 | 11 | 1167.575827 | 1 | [110.0720135, 42529.13671875, IM(H)] |
| 1 | QEHF1_10102_DNL.3984.3984.2 File:"QEHF1_10102_... | GGTHDPLQSVR+0.984 | GGTHDPLQSVr | 2 | 11 | 584.291826 | 1 | [110.0718416, 39672.3046875, IM(H)] |
| 2 | QEHF1_10101_DNL.3353.3353.2 File:"QEHF1_10101_... | GSHEPVADNSTVAGR+0.984 | GSHEPVADNSTVAGr | 2 | 15 | 749.350600 | 1 | [110.071755, 149039.875, IM(H)] |
| 3 | QEHF1_10099_DNL.5220.5220.1 File:"QEHF1_10099_... | YTPVEEKQNGR+0.984 | YTPVEEKQNGr | 2 | 11 | 661.323318 | 1 | [197.1288641, 56989.97265625, PV] |
| 4 | QEHF1_10100_DNL.5261.5261.1 File:"QEHF1_10100_... | YTPVEEKQNGR+0.984 | YTPVEEKQNGr | 2 | 11 | 661.323318 | 1 | [169.1335899, 8058.0991210938, PV-CO] |

# Step 6: Cit diagnostic ion analysis

Annotations and occurrence numbers of citrullination diagnostic ions are reported for each Cit PSM.

A snapshot of input data:

```
In [124]: df_mz_label_uniq
```

Out[124]:

| | unID_Title | Peptide | mod_Peptide | Charge | Pep_length | mz_Precursor | Cit_Count | peak_1 | |
|---|---|---|---|---|---|---|---|---|---|
| 0 | QEHF1_10100_DNL.2385.2385.3 File:"QEHF1_10100_... | GGTHDPLQSVR+0.984 | GGTHDPLQSVr | 1 | 11 | 1167.575827 | 1 | [110.0720135, 42529.13671875, IM(H)] | 4833 |
| 1 | QEHF1_10102_DNL.3984.3984.2 File:"QEHF1_10102_... | GGTHDPLQSVR+0.984 | GGTHDPLQSVr | 2 | 11 | 584.291826 | 1 | [110.0718416, 39672.3046875, IM(H)] | 6082 |
| 2 | QEHF1_10101_DNL.3353.3353.2 File:"QEHF1_10101_... | GSHEPVADNSTVAGR+0.984 | GSHEPVADNSTVAGr | 2 | 15 | 749.350600 | 1 | [110.071755, 149039.875, IM(H)] | 94 |
| 3 | QEHF1_10099_DNL.5220.5220.1 File:"QEHF1_10099_... | YTPVEEKQNGR+0.984 | YTPVEEKQNGr | 2 | 11 | 661.323318 | 1 | [197.1288641, 56989.97265625, PV] | 71 |
| 4 | QEHF1_10100_DNL.5261.5261.1 File:"QEHF1_10100_... | YTPVEEKQNGR+0.984 | YTPVEEKQNGr | 2 | 11 | 661.323318 | 1 | [169.1335899, 8058.0991210938, PV-COI | 506 |

A snapshot of output data (binary occurrence for the immonium ion and occurrence numbers for the other diagnostic ions):

```
In [130]: Final_result_filter
```

Out[130]:

| el | precNL_label | seqNL_label | ... | Total_NL_count | precNL_count | seqNL_count | intNL_count | Total_INT_count | Dipeptide_count | Tripeptide_count | IM_NH3_count |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-0-43 | | y1-43,y2-43,y3-43,y4-43,y10-43-NH3 | ... | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 |
| 3-4-43 | | y1-43,y2-43-H2O,y2-43,y4-43-H2O,y4-43 | ... | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 |
| 2O | | y2-43-H2O | ... | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 3-3-3-... | | y1-43,y2-43-H2O,y2-43,y3-43-H2O,y3-43,y7-43-H2... | ... | 13 | 0 | 13 | 0 | 0 | 0 | 0 | 1 |
| 3-3-3-... | | y1-43,y2-43-H2O,y2-43,y3-43-H2O,y3-43,y4-43-NH... | ... | 9 | 0 | 9 | 0 | 0 | 0 | 0 | 1 |

# Step 7: Evaluation of Cit PSMs by EN model

The EN model developed in the study is applied to evaluate the validity of each Cit PSM.

A snapshot of input data:

In [130]:
```
Final_result_filter
```

Out[130]:

| el | precNL_label | seqNL_label | ... | Total_NL_count | precNL_count | seqNL_count | intNL_count | Total_INT_count | Dipeptide_count | Tripeptide_count | IM_NH3_count |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-<br>0-<br>43 | | y1-43,y2-43,y3-43,y4-43,y10-43-NH3 | ... | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 |
| 3-<br>4-<br>43 | | y1-43,y2-43-H2O,y2-43,y4-43-H2O,y4-43 | ... | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 |
| 2O | | y2-43-H2O | ... | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 3-<br>3-<br>3-<br>... | | y1-43,y2-43-H2O,y2-43,y3-43-H2O,y3-43,y7-43-H2... | ... | 13 | 0 | 13 | 0 | 0 | 0 | 0 | 1 |
| 3-<br>3-<br>3-<br>... | | y1-43,y2-43-H2O,y2-43,y3-43-H2O,y3-43,y4-43-NH... | ... | 9 | 0 | 9 | 0 | 0 | 0 | 0 | 1 |

A snapshot of output data (Cit_probability, probability that a PSM contains Cit; and Cit_prediction = 1 when Cit_probability > 0.5):

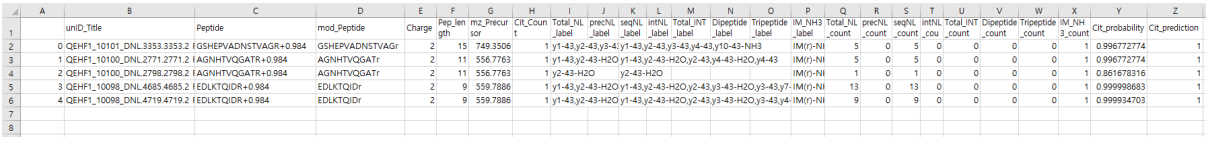
In [131]:
```
Final_result_filter
```

Out[131]:

| ... | Total_NL_count | precNL_count | seqNL_count | intNL_count | Total_INT_count | Dipeptide_count | Tripeptide_count | IM_NH3_count | Cit_probability | Cit_prediction |
|---|---|---|---|---|---|---|---|---|---|---|
| ... | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 | 0.996773 | 1 |
| ... | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 | 0.996773 | 1 |
| ... | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0.861678 | 1 |
| ... | 13 | 0 | 13 | 0 | 0 | 0 | 0 | 1 | 0.999999 | 1 |
| ... | 9 | 0 | 9 | 0 | 0 | 0 | 0 | 1 | 0.999935 | 1 |

# Step 7: Evaluation of Cit PSMs by EN model

Example output CSV file including the prediction results (Cit_probabilty and Cit_prediction):



| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | unID_Title | Peptide | mod_Peptide | Charge | Pep_length | mz_Precursor | Cit_Count | Total_NL_label | precNL_label | seqNL_label | intNL_label | Total_INT_label | Dipeptide_label | Tripeptide_label | IM_NH3_label | Total_NL_count | precNL_count | seqNL_count | intNL_cou | Total_INT_count | Dipeptide_count | Tripeptide_count | IM_NH3_count | Cit_probability | Cit_prediction |
| 2 | 0 | QEHF1_10101_DNL.3353.3353.2 | GSHEPVADNSTVAGR+0.984 | GSHEPVADNSTVAGr | 2 | 15 | 749.3506 | 1 | y1-43,y2-43,y3-43 | | y1-43,y2-43,y3-43,y4-43,y10-43-NH3 | | | | | IM(r)-NH | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 | 0.996772774 | 1 |
| 3 | 1 | QEHF1_10100_DNL.2771.2771.2 | AGNHTVQGATR+0.984 | AGNHTVQGATr | 2 | 11 | 556.7763 | 1 | y1-43,y2-43-H2O | | y1-43,y2-43-H2O,y2-43,y4-43-H2O,y4-43 | | | | | IM(r)-NH | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 | 0.996772774 | 1 |
| 4 | 2 | QEHF1_10100_DNL.2798.2798.2 | AGNHTVQGATR+0.984 | AGNHTVQGATr | 2 | 11 | 556.7763 | 1 | y2-43-H2O | | y2-43-H2O | | | | | IM(r)-NH | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0.861678316 | 1 |
| 5 | 3 | QEHF1_10098_DNL.4685.4685.2 | EDLKTQIDR+0.984 | EDLKTQIDr | 2 | 9 | 559.7886 | 1 | y1-43,y2-43-H2O | | y1-43,y2-43-H2O,y2-43,y3-43-H2O,y3-43,y7- | | | | | IM(r)-NH | 13 | 0 | 13 | 0 | 0 | 0 | 0 | 1 | 0.999998683 | 1 |
| 6 | 4 | QEHF1_10098_DNL.4719.4719.2 | EDLKTQIDR+0.984 | EDLKTQIDr | 2 | 9 | 559.7886 | 1 | y1-43,y2-43-H2O | | y1-43,y2-43-H2O,y2-43,y3-43-H2O,y3-43,y4- | | | | | IM(r)-NH | 9 | 0 | 9 | 0 | 0 | 0 | 0 | 1 | 0.999934703 | 1 |
| 7 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | | | | | | | | | | | | | | | |

Descriptions of the columns in the output CSV file:

| Column | Description |
|---|---|
| unID_Title | Unassigned MS2 spectrum title |
| Peptide | Original peptide sequence |
| mod_peptide | Simplified peptide with a predefined set of symbols for modifications |
| Charge | Charge state of precursor ion |
| Pep_length | Peptide length |
| mz_Precursor | Theoretical precursor *m/z* |
| Cit_Count | Number of citrullinated sites |
| Total_NL_label | Annotations of all diagnostic neutral loss ions |
| precNL_label | Annotations of precursor neutral losses |
| seqNL_label | Annotations of sequence ion neutral losses |
| intNL_label | Annotations of internal ion neutral losses |
| Total_INT_label | Annotations of all diagnostic internal ions |
| Dipeptide_label | Annotations of diagnostic dipeptides |
| Tripeptide_label | Annotations of diagnostic tripeptides |
| IM_NH3_label | Annotation of IM(Cit)-$NH_3$ |
| Total_NL_count | Number of all diagnostic neutral loss ions |
| precNL_count | Number of precursor neutral losses |
| seqNL_count | Number of sequence ion neutral losses |
| intNL_count | Number of internal ion neutral losses |
| Total_INT_count | Number of all diagnostic internal ions |
| Dipeptide_count | Number of diagnostic dipeptides |
| Tripeptide_count | Number of diagnostic tripeptides |
| IM_NH3_count | Number of IM(Cit)-$NH_3$ |
| Cit_probability | Probability (P) of citrullination status calculated by the EN model (HCD data only) |
| Cit_prediction | Classification of citrullination status using a P cutoff >0.5 (HCD data only) |