



Sep 17, 2020

# Preparing Annotated Spectra from MaxQuant Output in xiSpec

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Emmott Lab

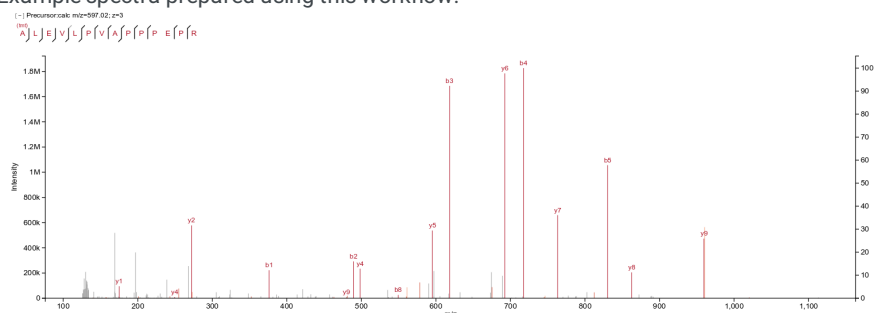
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## ABSTRACT

Annotated spectra can be required to highlight specific features of interest, or for providing in supplementary data in support of peptide and PTM identification/localisation.

This protocol provides a workflow for preparing annotated spectra from MaxQuant output files (evidence.txt, .apl files) in [xiSpec](#).

Example spectra prepared using this workflow:



## EXTERNAL LINK

<http://emmottlab.org>

## PROTOCOL CITATION

Ed Emmott 2020. Preparing Annotated Spectra from MaxQuant Output in xiSpec. **protocols.io**  
<https://protocols.io/view/preparing-annotated-spectra-from-maxquant-output-i-bi6ykhfw>

## EXTERNAL LINK

<http://emmottlab.org>

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## CREATED

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Sep 17, 2020

## PROTOCOL INTEGER ID

39864

- 1 Open the MaxQuant evidence.txt file for the spectra of interest. This is located within the folder of the maxquant output.
- 2 Find the relevant row. Key data are the peptide sequence, z, Rawfile name and MS\_MSScanNumber. Other columns may be useful depending on your need (e.g. Score, PIF, PEP).
- 3 Navigate to **/combined/andromeda** in windows explorer. Note the large number of individual .apl files.
- 4 To merge the individual .apl files to a single file suitable for searching for peak list data open the **/combined/andromeda/** folder in the command prompt.

```

Select C:\Windows\system32\cmd.exe
D:\SARS2Nterm\VeroE6\VeroE6_Enriched_bRP5Frac_Search\combined\andromeda>dir
Volume in drive D is Analysis
Volume Serial Number is 9206-36AC

Directory of D:\SARS2Nterm\VeroE6\VeroE6_Enriched_bRP5Frac_Search\combined\andromeda

21/05/2020  09:23    <DIR>          .
21/05/2020  09:23    <DIR>          ..
21/05/2020  09:23             1,798 allSpectra.CID.FTMS.iso.apar
21/05/2020  09:23             1,797 allSpectra.CID.ITMS.iso.apar
21/05/2020  09:23             1,797 allSpectra.CID.TOF.iso.apar
21/05/2020  09:23             1,801 allSpectra.CID.Unknown.iso.apar
21/05/2020  09:23             1,800 allSpectra.ETCID.FTMS.iso.apar
21/05/2020  09:23             1,799 allSpectra.ETCID.ITMS.iso.apar
21/05/2020  09:23             1,799 allSpectra.ETCID.TOF.iso.apar
21/05/2020  09:23             1,803 allSpectra.ETCID.Unknown.iso.apar
21/05/2020  09:23             1,798 allSpectra.ETD.FTMS.iso.apar
21/05/2020  09:23             1,797 allSpectra.ETD.ITMS.iso.apar
21/05/2020  09:23             1,797 allSpectra.ETD.TOF.iso.apar
21/05/2020  09:23             1,801 allSpectra.ETD.Unknown.iso.apar
21/05/2020  09:23             1,800 allSpectra.ETHCD.FTMS.iso.apar
21/05/2020  09:23             1,799 allSpectra.ETHCD.ITMS.iso.apar
21/05/2020  09:23             1,799 allSpectra.ETHCD.TOF.iso.apar
21/05/2020  09:23             1,803 allSpectra.ETHCD.Unknown.iso.apar
21/05/2020  09:23             1,798 allSpectra.HCD.FTMS.iso.apar
21/05/2020  09:23             4,422,533 allSpectra.HCD.FTMS.iso_0.apl
21/05/2020  12:16              95,377 allSpectra.HCD.FTMS.iso_0.res
21/05/2020  09:23             4,382,551 allSpectra.HCD.FTMS.iso_1.apl
21/05/2020  12:17             91,506 allSpectra.HCD.FTMS.iso_1.res
  
```

- 5 At the command prompt type:

**copy \*.apl merged.apl**

```

Select C:\Windows\system32\cmd.exe
2 Dir(s)  3,194,367,991,888 bytes free

D:\SARS2Nterm\VeroE6\VeroE6_Enriched_bRP5Frac_Search\combined\andromeda>copy *.apl merged.apl
allSpectra.HCD.FTMS.iso_0.apl
allSpectra.HCD.FTMS.iso_1.apl
allSpectra.HCD.FTMS.iso_10.apl
allSpectra.HCD.FTMS.iso_100.apl
allSpectra.HCD.FTMS.iso_101.apl
allSpectra.HCD.FTMS.iso_102.apl
allSpectra.HCD.FTMS.iso_103.apl
  
```

merged.apl is your output file and contains the data from all the individual files.

- 6 Open the merged.apl file in a text editor
- 7 Find the peak list for your spectra of interest. For example from rawfile 'A', MSMSscan number 666. Search for: 'A Index: 666'

- 8 Select and copy the two columns of numbers below the scan header. Upto but not including the 'peaklist end' text.

```
mergedEmmott.apl - Notepad
File Edit Format View Help
1171.6169 197621.629882813
1211.681 5601.97863769531
1225.7147 54706.9375
1269.7409 1599665.31005859
peaklist end

peaklist start
mz=635.374777885611
fragmentation=HCD
charge=2
header=RawFile: EE200515_VeroE6SARS2Enriched_bRP_F4 Index: 23608 Precursor: 0 _multi_
101.07214 25649.0885009766
102.05605 7376.99389648438
110.07242 31711.7833251953
112.05164 54622.1378173828
112.08806 26906.9470214844
115.08775 24613.1569824219
120.08188 195994.626708984
121.99715 5187.94067382813
126.12886 60036.2557373047
127.12585 90817.5603027344
127.13221 71453.86328125
128.1292 73434.9501953125
128.13563 71285.8146972656
129.1326 83419.1098632813
129.13892 89557.4313964844
130.13588 90696.8432617188
130.14222 205664.718261719
130.75663 5327.61096191406
131.13934 124541.315917969
131.14555 93663.1018066406
132.14263 121718.951416016
132.14891 124547.675048828
133.14594 109002.330322266
133.15223 56226.9050292969
134.14935 85722.8752441406
```

Select from here...

```
mergedEmmott.apl - Notepad
File Edit Format View Help
821.50418 22616.0053710938
839.51563 41061.8568115234
863.52591 8731.32482910156
864.51005 106757.957397461
871.76045 5204.859375
881.53748 497984.76159668
903.41039 8623.06567382813
971.56924 5163.88403320313
986.58468 5901.76525878906
1011.575 5681.31567382813
1016.496 6471.56909179688
1028.6066 165718.026123047
1110.6524 9511.52880859375
1127.6734 31184.7292480469
1214.3111 5384.43347167969
1224.6929 92481.955078125
1272.0934 4791.26135253906
1285.5089 4912.31079101563
peaklist end

peaklist start
mz=423.920854199479
fragmentation=HCD
charge=3
header=RawFile: EE200515_VeroE6SARS2Enriched_bRP_F4 Index: 10713 Precursor: 0 _multi_
90.056116 22299.3312988281
91.055379 14299.75390625
92.017537 7485.70385742188
98.061173 8101.80578613281
100.05168 24888.9306640625
101.01784 18661.0393066406
101.07215 5747.59411621094
102.05608 50718.9429931641
104.054 21122.1605224609
110.07232 91966.1206054688
112.08799 207809.727416992
113.07705 69053.5738525391
```

... to here.

9    Navigate to xiSpec: [spectrumviewer.org](https://spectrumviewer.org)

10   Click on 'upload' and then 'Data input - manually upload your spectrum data'

11   Add your peptide sequence. Any modifications should be indicated in brackets after the relevant amino acid. for example 'XXK(tmt)XX'

12   In the row below, select 'linear peptide', type the precursor charge, select the ions of interest (typically b, y), and choose the relevant ppm (20).

13   Type in the modification mass for any indicated peptide modifications. For example TMTpro:

tmt: 304.2071453354

Adjust the specificity as required. Though note for modifications the software takes this from the positions you have indicated in your peptide sequence.

The previous sections should appear as: e.g.

Data Input - Manually input your spectrum data

T(tmt)TSSSITLR

95.024928   4809.86157226563

97.029459   13143.6326904297

101.07199   11572.2783203125

102.95603   17467.5004882813

104.22321   4630.01818847656

110.07238   48900.4948730469

112.05155   112422.853515625

112.08793   24155.3826904297

115.08754   14215.9749755859

116.07163   9814.27465820313

120.08172   33054.931640625

Peptide Preview:

T(tmt)

T T S S S I T L R

Click on a modification to change its position.

none (linear peptide)

2

b, y


20

ppm

Modification	Mass	Specificity
(tmt)	304.2071453354	T

14   Add any neutral losses you wish to display. Typically the software will color the relevant ions, but not label these. Neutral losses or other modifications to include are:

CH3SOH	63.99828547	MOx
H2O	18.01056027	S, T, D, E, CTerm
NH3	17.02654493	R, K, N, Q, NTerm

 protocols.io

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09/17/2020

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Modification	Mass	Specificity
(tmt)	304.2071453354	T

Neutral Loss	Mass	Specificity
CH3SOH	63.99828547	Max
H2O	18.01056027	S, T, D, E, CTerm
NH3	17.02654493	R, K, N, Q, NTerm

VIEW SPECTRUM

CROSSLINK EXAMPLE

LINEAR EXAMPLE

RESET

15 Click 'View Spectrum'

16 If you wish to adjust label positions (where they are too closer or overlap), click on the 'move labels' options on the upper left.

17 Save the file as a .svg by clicking on the down arrow, top left corner.

