# Guide to the TMTproC Module

## Step 1 – Run a MaxQuant search

This is not meant to be an all-encompassing guide to using MaxQuant. It is the barebones steps needed to get a viable data set. You may need to modify one or several steps depending on the experiment and/or sample preparation.

For more details, our lab previously published a more in-depth book chapter on proteomics data analysis using MaxQuant here: https://link.springer.com/protocol/10.1007/978-1-4939-8784-9_14

Max Quant and the full documentation can be downloaded from: https://www.maxquant.org/

This module was built using MaxQuant version 1.6.17.0

0. Add TMTpro to the peptide modification list
    a. Navigate to the Configurations tab
    b. Select Modifications and Add
    c. Name the modification TMTproNterm
    d. On the Composition row, delete the "H(2) O". Select Change and enter each atom and the appropriate number to get a final formula of C(8) Cx(7) H(25) N Nx(2) O(3). The mass should be 304.2071453
    e. Position should be any N-term
    f. Type is Standard
    g. New terminus is None.
    h. Next to Specificities select the K and click "–".
    i. Next to Specificities click the "+" and find the "– " in the drop-down menu and add it.
    j. Under the Actions tab in Configuration section select Modify table.
    k. Under the Table actions tab in Configuration section select Save changes.
    l. Select the TMTproNterm modification that you just created and then press "Duplicate" in the top panel.
    m. Re-name the new modification TMTproK
    n. Change the Position to "Anywhere"
    o. Next to Specificities select the – and click –.
    p. Next to Specificities select the + and add K from the drop-down menu.
1. Select the relevant peptide modification
    a. Navigate to the Group-specific parameters tab. Select the Modifications panel.
    b. Under variable modifications, select Acetyl (N-term) on the right and press <.
    c. Under static modifications, select Carbamidomethyl on the right and press <.
    d. Under static modifications, select TMTproNterm and press >.
    e. Under static modifications, select TMTproK and press >
    f. (Optional: Be sure to add any other sample prep or experiment-specific modifications as well, e.g. NEM alkylation of cysteine residues)
2. Direct the MaxQuant in-silico digestion
    a. Navigate to the Group-specific parameters tab. Select the Digestion panel.
    b. For a Trypsin+LysC digestion (recommended), leave the default Trypsin/P
    c. For a Trypsin only digestion, select Trypsin/P on the right and press <, then select Trypsin on the left and press >.

    d. For a LysC-only digestion, select LysC on the left and press >.
3. Load the FASTA file(s)
    a. Download the relevant files and add them to the MaxQuant folder
    b. Navigate to the Global Parameters tab. Select the Sequences panel.
    c. Click Add and select the relevant file
    d. Click Test to ensure that MaxQuant is parsing the FASTA file correctly
4. Uncheck protein quantification options
    a. Navigate to the Global Parameters tab. Select Tables.
    b. Uncheck all boxes (we'll do all quantification outside of MaxQuant)
5. Load the raw file(s) (Note that you may wish to save the parameter settings before continuing)
    a. Navigate to the Raw data tab.
    b. Click "Load" and select your raw file(s)
    c. Optional: Use the "Set Experiment" button to name the run
6. Run the search
    a. In the bottom menu, change the number of processors to the # of physical CPU cores on your PC (ignoring hyperthreading) If you want to continue using your computer during the analysis, set the number to core count – 1.
    b. Select Start in the bottom left. The search may take anywhere from 30 minutes to several hours depending on the number of processors. (Note: you may need to set <useDotNetCore> to FALSE in the mqpar.xml file)
    c. The progress of the search can be monitored under the Performance table

## Step 2 – Prepare Software

This module is built to run on Windows. We've also provided the scripts if you wish to modify it for a Mac or Linux system.

There are two programs needed to run the TMTproC module: Python and Matlab Runtime. Both are freely available.

**Python**

- This module was built using Python version 3.9.1
- Anaconda is a freely available compilation of Python and several scientific packages. The only package this module makes use of is NumPy. https://anaconda.org/anaconda/python
- You can also type "python" into the Windows Command Prompt to download it from the Windows Store. You will also need to install NumPy with the command "pip3 install numpy".

**Matlab Runtime**

- Matlab Runtime V2018b (9.5) is required.
- Matlab Runtime can be freely downloaded from https://www.mathworks.com/products/compiler/matlab-runtime.html
- If you have access to a Matlab license, you can also run the Matlab scripts directly from TMTproC_MaxQuant_compatible.m (after running the raw file converter and python scripts)

## Step 3 – Run the Module

1. After the completion of the MaxQuant search, the program will save a file called "evidence.txt" within the folder "combined/txt".

2. Open this file in Excel or similar program and export it as a .csv file with a new name. We also recommend deleting some of the unnecessary columns to speed up the read step for Matlab. Below are the minimum columns needed for the module. You may want to keep others depending on the experiment.
    a. Sequence
    b. Protein names
    c. Raw file
    d. Charge
    e. Mass
    f. Retention time
    g. MS/MS Scan Number
    h. Delta score
    i. Peptide ID
3. Move the csv file from part 2 into the "data" folder of the TMTproC folder along with the .raw file used in your MaxQuant search. Examples of what the MaxQuant output should look like and a raw file are provided in the folder "example_data". This folder also contains the expected output of TMTproC from these two files.
4. Edit the TMTproC parameters. There are three parameter files included in the TMTproC "parameters" folder
    a. The first, "iso_window.txt", is a text file with the measured isolation window shape for a 0.4 Da isolation window on our lab's Orbitrap Fusion Lumos Tribrid Mass Spectrometer. If you use a different isolation window or have a different mass spectrometer, we recommend re-measuring the shape on your instrument.
    b. The second, "quant.ini", contains the parameters for the complementary ion peak picking and the mass shifts of each of the TMTpro tags. The default parameters should work for most TMTproC analyses (See 4.c.iv. below for one example).
    c. The third, "tmtproc_input_parameters.txt", contains several general parameters for the module.
        i. which_channels_to_use: the channels that were labelled in the experiment. The first value is for TMTpro0, second position is TMTPro126/127C, 9th position is TMTPro133N/134N, 10th position is currently not usable
        ii. noiseband: This is the conversion factor of Signal to Fourier Transform Noise (S:N) to pseudocounts (See Peshkin et al. 2019). Leave at the default of 1 for most experiments. Can be applied later if needed.
        iii. sn_cutoff: The minimum total S:N in the complementary region to call a peptide quantified. For detecting fold changes less than four, we recommend 5 times the number of channels labelled. If detecting higher fold changes is important for your experiment, use a higher value.
        iv. use_ppm_filter: If there is a large amount of potentially interfering peptides, this option can help alleviate missing peaks in the complementary region. If set to a value of 1, flags any peptide where a complementary peak was more than 10 ppm from its expected value. If you are using this option, set the ppm tolerance in "quant.ini" to 40.
        v. use_precursor: Binary, whether to use the isotopic envelope of the remaining precursor in the MS2 spectra to estimate the isolation window shape. Set to 1 to use, 0 to ignore. If you've previously measured the isolation window, this is unnecessary.

5. Open a Windows Command Prompt
6. Navigate to the TMTproC directory using the command “cd filepath”
7. Run the module using the command “run_TMTproC.bat RawFile MaxQuantOutput”, where you’ve replaced RawFile with your raw file name and MaxQuantOutput is the name given to the MaxQuant output .csv from Step 3.2, both of which should be located in the “data” folder (no file extensions on either file name)
8. The script will take several minutes to several hours to run. The .csv output and several intermediate steps are written into the “data” folder. The most important variables in the output:
    a. ratio1-8: The deconvolved relative signal in each of the complementary channels
    b. sum_SN: The total S:N in the complementary ion envelope.
    c. passed_ppm_filter: See 4.c.iv. 1 indicates that the peptide passed the filter, 0 if it did not.
    d. indeces: Whether the peptide passed the chosen signal to noise cutoff. 1 if the signal to noise was high enough, 0 otherwise.