



ANALYZING TMT PRO 16-PLEX DATA IN PEAKS SOFTWARE

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 November 2019

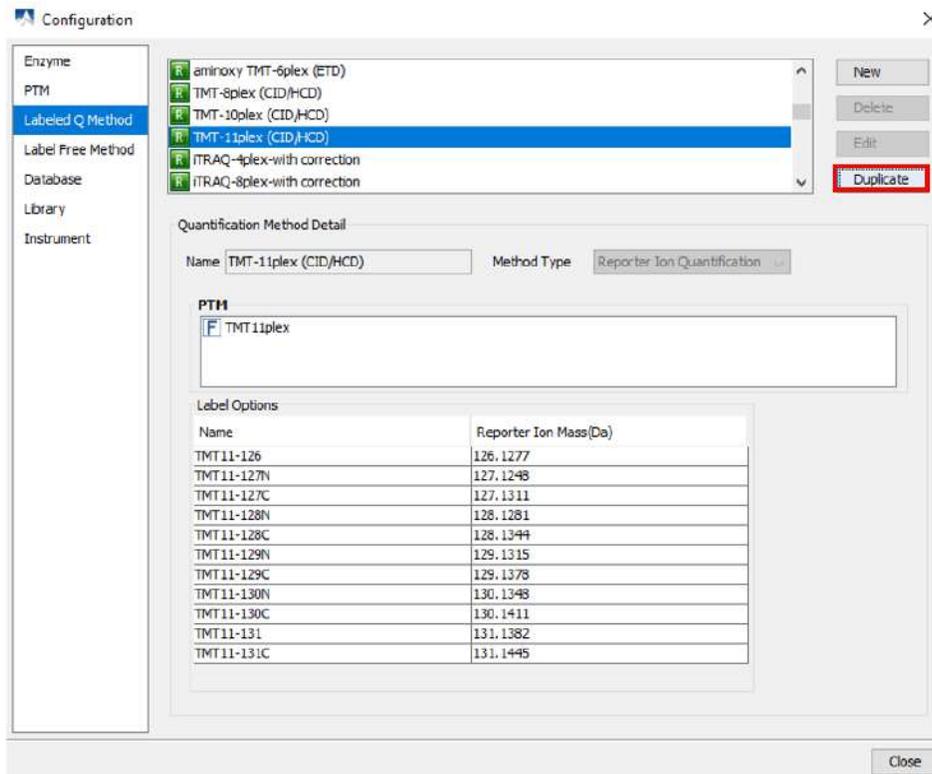
Background

Recently, Thermo Fisher Scientific and Pierce Scientific have released the isobaric TMT Pro 16-plex reagents to allow for the multiplexing of up to 16 samples in the same run. The release of these reagents came after the release of PEAKS X+ and thus we have created this technical note to assist those looking to use these reagents and search their data in one of the PEAKS software suites available.

TMT PRO 16-plex Labeled Q Method in PEAKS

To run a TMT16plex analysis in PEAKS, a custom Labeled Q Method and a custom PTM must first be created. To do this, follow the steps below:

1. Click the Configuration icon  and go to the *Labeled Q Method* page. To save time, the built-in TMT11-plex method can be duplicated, renamed, and edited as necessary.





- Edit the duplicated quantification method by first removing the fixed TMT11plex PTM. Click the **Set PTM** button then **New**, to create a new PTM. Enter the required information and click the **OK** button to save. Under the *Customized* tab, the new PTM will be listed and can be added as a fixed PTM.

The screenshot shows the 'PTM Options' dialog box. A 'New PTM' sub-dialog is open, allowing the user to define a new PTM. The 'New PTM' dialog has the following fields:

- PTM name: TMT16plex
- PTM abbreviation: (empty)
- Mass (Monoisotopic): 304.2071
- Residues that can be modified: K (Anywhere), X (N-term)
- Formula: (empty)
- Rule: (empty)

The 'New PTM' dialog has 'OK', 'Cancel', and 'Help' buttons. In the 'PTM Options' dialog, the 'New' button is highlighted with a red box. Other buttons include 'Remove', 'Remove All', 'Switch Type', 'OK', and 'Cancel'.

- Once the PTM is set, click **Add Label** and add the additional TMT16plex labels. Double-click on a line to edit the name or reporter ion mass values. Click **OK** to save.

The screenshot shows the 'New/Edit Quantification Method' dialog box. The 'Quantification Method Detail' section shows:

- Name: TMT-16plex (CID/HCD)
- Method Type: Reporter Ion Quantification

The 'PTM' section shows 'TMT16plex' with 'Set PTM', 'Remove', and 'Switch type' buttons.

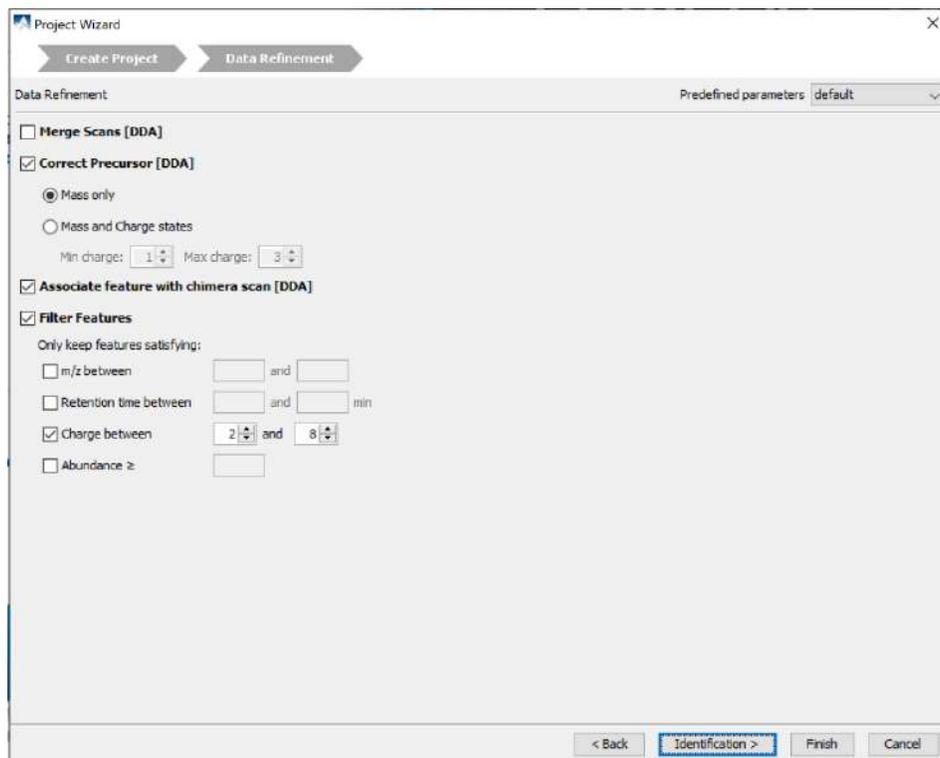
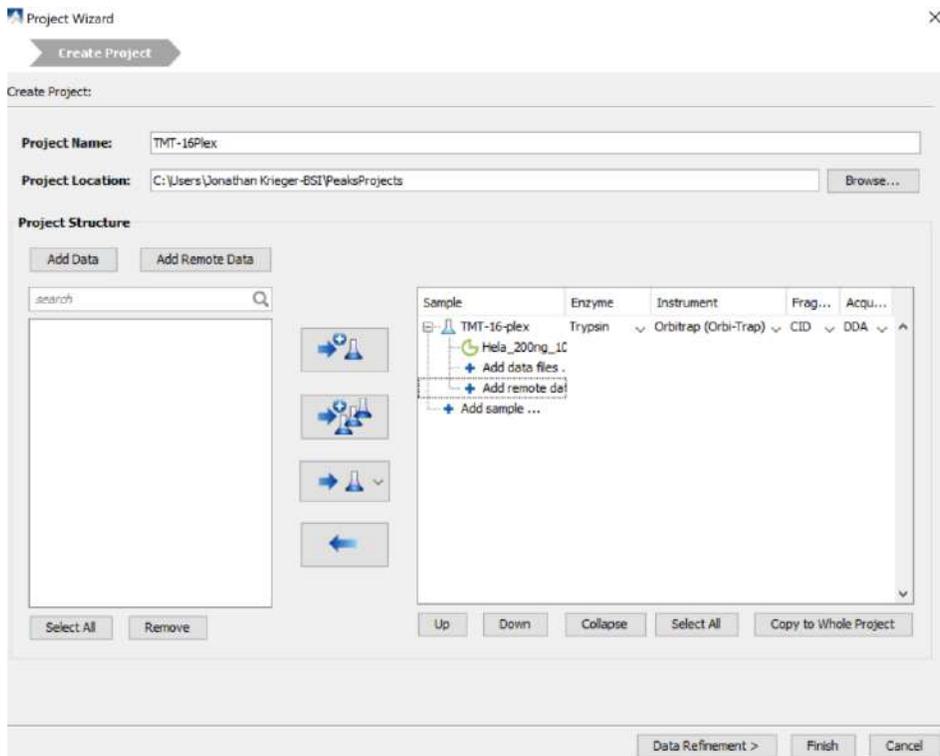
The 'Label Options' section contains a table with the following data:

Name	Reporter Ion Mass(Da)
TMT 11-126	126.1277
TMT 11-127N	127.1248
TMT 11-127C	127.1311
TMT 11-128N	128.1281
TMT 11-128C	128.1344
TMT 11-129N	129.1315
TMT 11-129C	129.1378
TMT 11-130N	130.1348
TMT 11-130C	130.1411
TMT 11-131N	131.1382
TMT 11-131C	131.1445
TMT 16-132N	132.1415
TMT 16-132C	132.1479
TMT 16-133N	133.1449
TMT 16-133C	133.1512
TMT 16-134	134.1482

The 'Add Label' button is highlighted with a red box. Other buttons include 'Delete Current Line', 'OK', and 'Cancel'.



- For a sample run on an Orbitrap Tribrid mass spectrometer in MS3 mode, the following would be the default settings:





Project Wizard

Create Project → Data Refinement → Identification

Methods: PEAKS Search Predefined parameters: [dropdown]

DB Search

Library Search

Error Tolerance
 Precursor mass: 10 ppm using monoisotopic mass Fragment ion: 0.6 Da

Enzyme
 Trypsin [View]
 Digest mode: Semispecific
 Maximum missed cleavages per peptide: 3

PTM

- Carbamidomethylation [Set PTM]
- TMT 16plex [Remove]
- Deamidation (NQ) [Switch type]
- Oxidation (M) [Switch type]

Maximum allowed variable PTM per peptide: 3

Database
 Select database Database: uniprot_sprot [View]
 Paste sequence Taxa: Homo sapiens (human) [Set/View taxa...]
 Contaminant database: 20181123_human_upsp_20408entries_20181123111305_UniProt_contaminants [View]

General Options
 Estimate FDR with decoy-fusion.
 Find unspecified PTMs with PEAKS PTM [Advanced Settings]
 Find more mutations with SPIDER

[Skip Identification] < Back Quantification > Finish Cancel

Quantification

Quantifications

Label Free

Reporter Ion Quantification eg. iTRAQ/TMT

Precursor Ion Quantification eg. SILAC

Reporter Ion Quantification Predefined parameters: [dropdown]

Select Methods: TMT-16plex (CID/HCD) [View]

Basic Options
 Mass Error Tolerance: 0.01 Da -10logP Threshold: 15.0
 Reporter Ion Type: MS2 MS3 FDR Threshold(%) 1.0

Purity Correction
 Perform Purity Correction [Edit Factors ...]

Experiment Groups (Optional)

search [input] [Q]

[Remove All] [Collapse]

Group	Color
Group 1 Sample 1: TMT11-...	[Red]
Group 2 Sample 1: TMT11-...	[Yellow]
Group 3 Sample 1: TMT11-...	[Blue]
Group 4 Sample 1: TMT11-...	[Purple]
Group 5 Sample 1: TMT11-...	[Grey]
Group 6 Sample 1: TMT11-...	[Pink]

[OK] [Cancel]

For any questions or inquires related to this technical note, please contact BSI Support.

