



# ANALYZING TMT PRO 16-PLEX DATA IN PEAKS SOFTWARE


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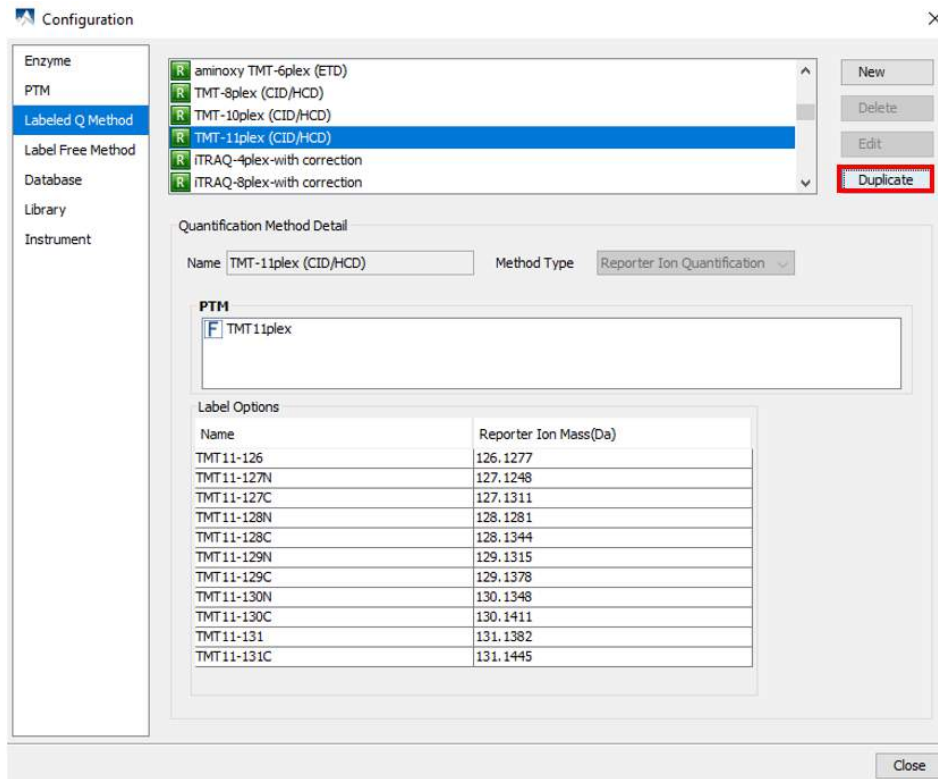
## Background

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.



Configuration

Enzyme  
 PTM  
**Labeled Q Method**  
 Label Free Method  
 Database  
 Library  
 Instrument

aminoxy TMT-6plex (ETD)  
 TMT-8plex (CID/HCD)  
 TMT-10plex (CID/HCD)  
 **TMT-11plex (CID/HCD)**  
 ITRAQ-4plex-with correction  
 ITRAQ-8plex-with correction

New  
 Delete  
 Edit  
**Duplicate**

Quantification Method Detail

Name: TMT-11plex (CID/HCD) Method Type: Reporter Ion Quantification

PTM

Label Options

Name	Reporter Ion Mass(Da)
TMT11-126	126.1277
TMT11-127N	127.1248
TMT11-127C	127.1311
TMT11-128N	128.1281
TMT11-128C	128.1344
TMT11-129N	129.1315
TMT11-129C	129.1378
TMT11-130N	130.1348
TMT11-130C	130.1411
TMT11-131	131.1382
TMT11-131C	131.1445

Close



- 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 includes the following fields:

- PTM name: TMT 16plex
- PTM abbreviation: (empty)
- Mass (Monoisotopic): 304.2071
- Residues that can be modified: K (Anywhere), X (N-term)
- Formula: (empty)
- Rule: (empty)

The 'New' button in the PTM Options dialog is highlighted with a red box.

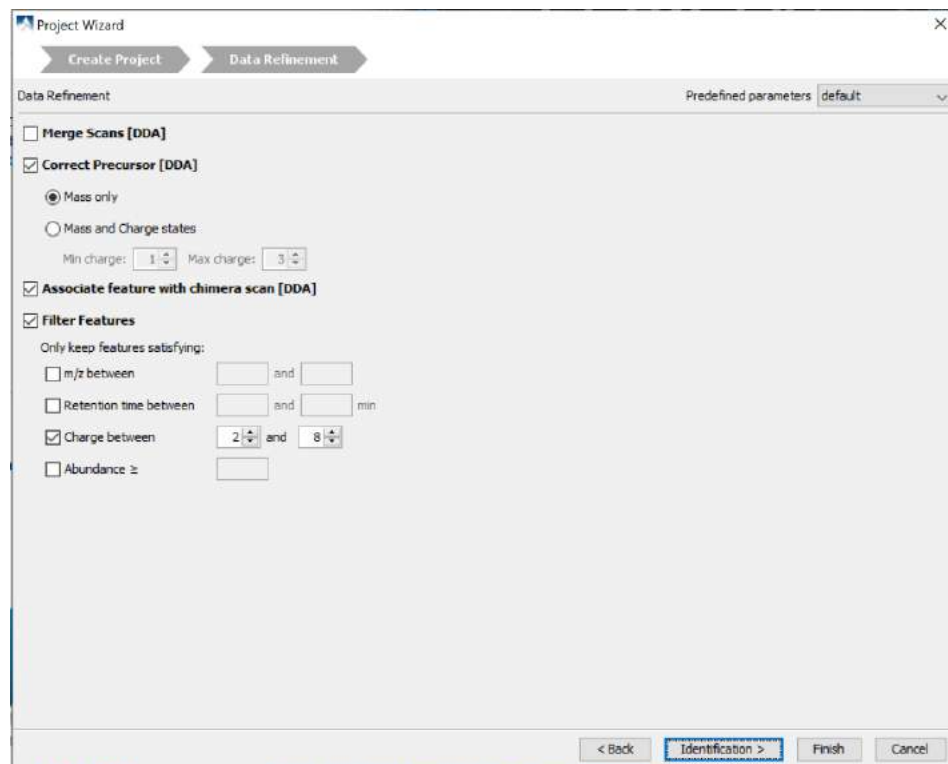
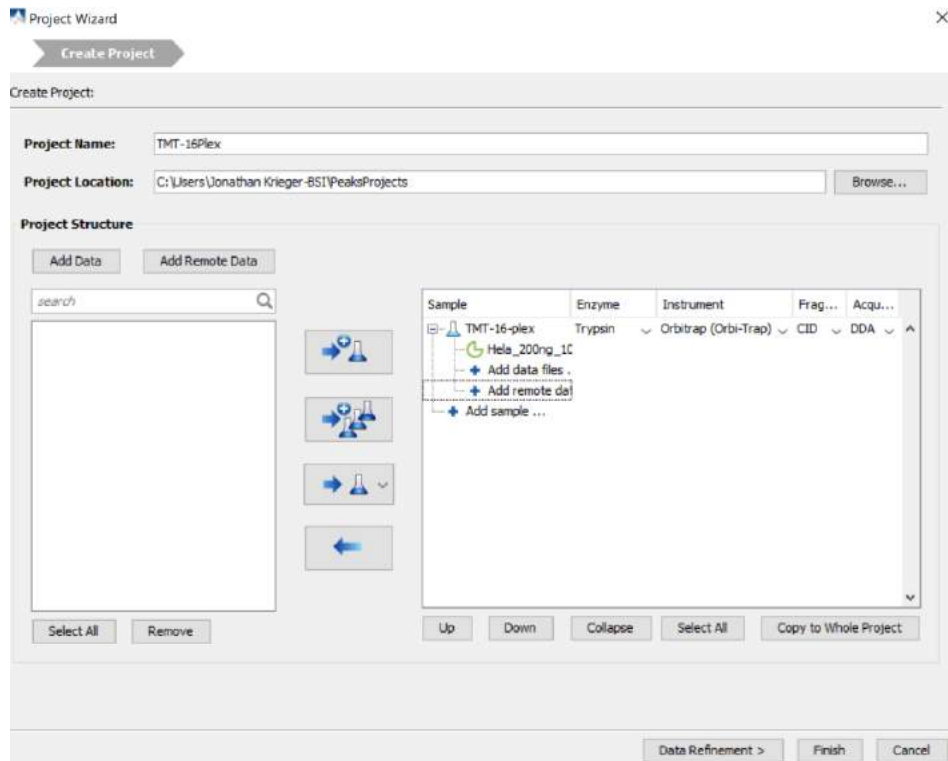
- Once the PTM is set, click **Add Label** and add the additional TMT16plex labels. Double-click on a line to edit the name or reporter ion mass values. Click **OK** to save.

The screenshot shows the 'New/Edit Quantification Method' dialog box. The 'PTM' field contains 'TMT 16plex'. The 'Label Options' table lists various TMT labels and their reporter ion masses. The 'Add Label' button is highlighted with a red box.

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



- 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: [v]

DB Search

Library Search

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

**Enzyme**  
 Trypsin [v]      [View]

Digest mode: Semispecific [v]  
 Maximum missed cleavages per peptide: 3 [v]

**PTM**

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

Maximum allowed variable PTM per peptide: 3 [v]

**Database**  
 Select database Database: uniprot\_sprot [v] [View]  
 Paste sequence Taxa: Homo sapiens (human) [Set/View taxa...]  
 Contaminant database: 20181123\_human\_upSp\_20408entries\_20181123111305\_UniProt\_contaminants [v] [View]

**General Options**  
 Estimate FDR with decoy-fusion. [v]  
 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: [v]

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

[Remove All]    [Collapse]

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

[OK]    [Cancel]

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

