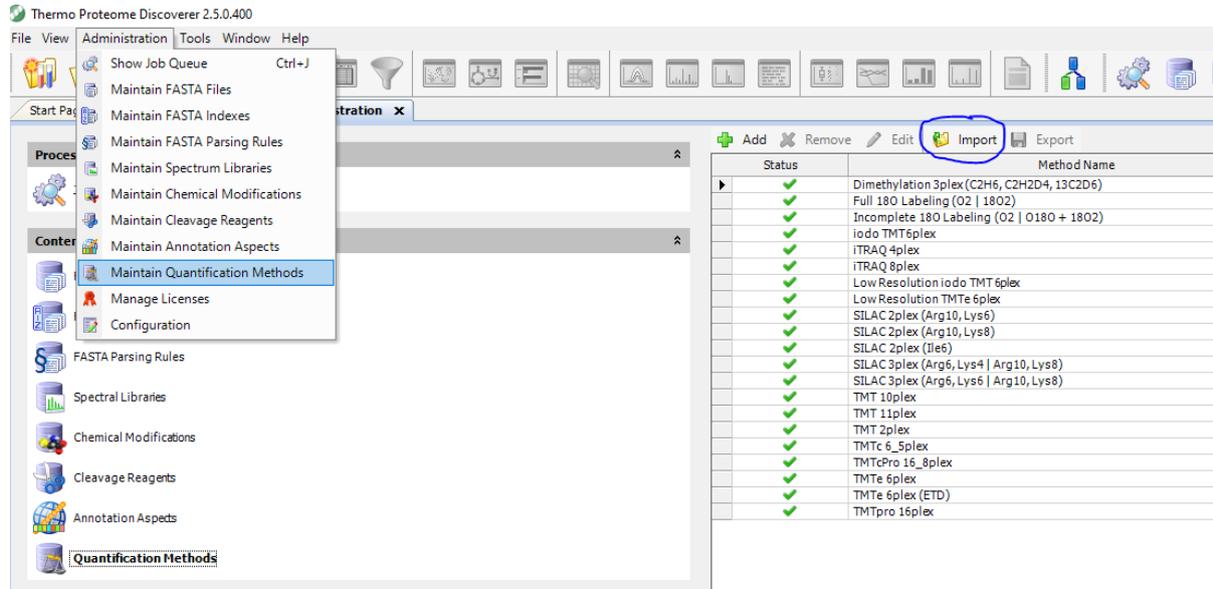


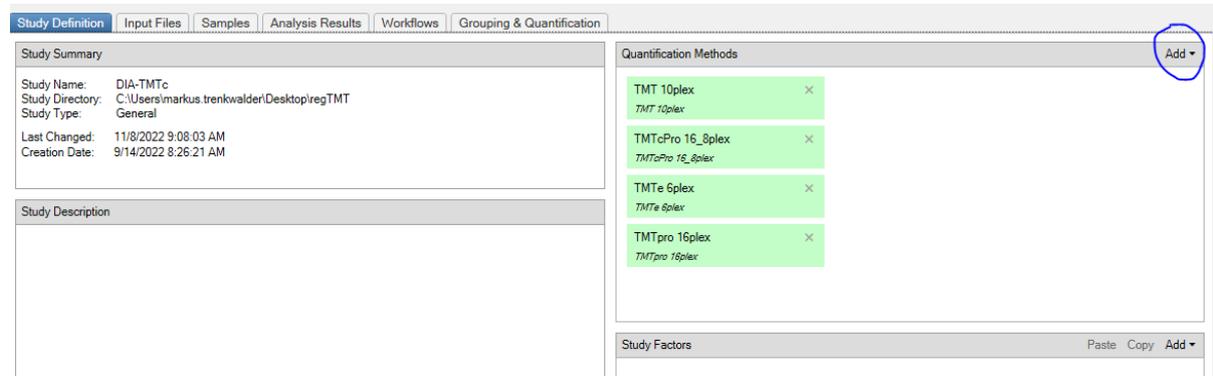
# TMTc Tutorial

In order to work with TMTc quantification in Hyperplex, a few things have to be set up correctly.

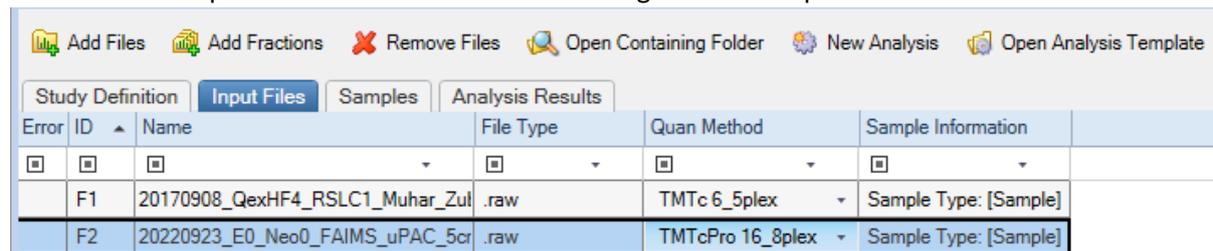
As a first step one has to import the quantification method files (TMTcPro 16\_8plex.method and TMTc 6\_5plex.method) provided with Hyperplex in the folder "HyperplexConfig":



The desired quantification methods have to be added to the study:



The the correct quantification method has to be assigned to the input files:



Then the Hyperplex node has to be configured:

Hide Advanced Parameters

- 1. General**
  - Reporter Mass Toler: 20 ppm
  - Quantification Methc: TMTc
  - Main Method: TMTc
  - Reporter Masses file: C:\Program Files\Thermo\Proteome
  - Indistinguishable Coi:
  - Fallback to TMT Re: False
  - Apply impurity correc: False
  - Write MZ table: False
  - Debug Mode: False
- 2. Algorithm Settings**
  - Interpret thresholds: False
  - b-Fragment thresholc: 10
  - y-Fragment thresholc: 10
  - MS2 Precursor thres: 10
  - Separate precursor: False

For TMTc quantification please select TMTc as the “Quantification Method” and as “Main Method”. Depending on whether a 6-plex TMT or a 16-plex TMTpro kit was used the correct Reporte Masses file needs to be selected for parameter “Reporte Masses File”.

Please note that for 6-plex TMTc only 5 channels can be used (126, 127, 128, 130 & 131) as the TMTc reporter ion for channel 129 are identical with the ones produced by the label of channel 130. The same is true for channels 126 & 127C of the TMT-pro kit. Furthermore N- and C- labels of the same reporter mass cannot be distinguished in the TMTc cluster which currently allows to only use 8 channels of the 16-plex kit (127C, 128C, 129C, 130C, 131C, 132C, 133C and 134N).

TMTc 6\_5plex (not used, readonly)

Quan Channels

Residue Modification: TMT6plex / +229.163 Da K

N-Terminal Modification: TMT6plex / +229.163 Da

TMT Reporter Ion Isotope Distributions

Mass Tag	Reporter Ion Mass	-2	-1	Main	+1	+2	Active
126	126.127726	0	0	100	0	0	Used
127	127.124761	0	0	100	0	0	Used
128	128.134436	0	0	100	0	0	Used
129	129.131471	0	0	100	0	0	Not Used
130	130.141145	0	0	100	0	0	Used
131	131.13818	0	0	100	0	0	Used

Peptide Groups Peptide Isoforms PSMs

Abundances

126\* 127 128 130 131

TMTcPro 16\_8plex (not used, readonly)

Quan Channels

Residue Modification: TMTpro / +304.207 Da K

N-Terminal Modification: TMTpro / +304.207 Da

TMT Reporter Ion Isotope Distributions

Mass Tag	Reporter Ion Mass	-2x13C	-13C-15N	-13C	-15N	Main	+15N	+13C	+15N+13C	+2x13C	Active
126	126.127726	0	0	0	0	100	0	0	0	0	Not Used
127N	127.124761	0	0	0	0	100	0	0	0	0	Not Used
127C	127.131081	0	0	0	0	100	0	0	0	0	Used
128N	128.128116	0	0	0	0	100	0	0	0	0	Not Used
128C	128.134436	0	0	0	0	100	0	0	0	0	Used
129N	129.131471	0	0	0	0	100	0	0	0	0	Not Used
129C	129.13779	0	0	0	0	100	0	0	0	0	Used
130N	130.134825	0	0	0	0	100	0	0	0	0	Not Used
130C	130.141145	0	0	0	0	100	0	0	0	0	Used
131N	131.13818	0	0	0	0	100	0	0	0	0	Not Used
131C	131.144499	0	0	0	0	100	0	0	0	0	Used
132N	132.141535	0	0	0	0	100	0	0	0	0	Not Used
132C	132.147855	0	0	0	0	100	0	0	0	0	Used
133N	133.14489	0	0	0	0	100	0	0	0	0	Not Used
133C	133.15121	0	0	0	0	100	0	0	0	0	Used
134N	134.148245	0	0	0	0	100	0	0	0	0	Used

Proteins Protein Groups Peptide Groups Peptide Isoforms PSMs

Abundances

127C 128C 129C 130C 131C 132C 133C 134N\*

If you want to correct for isotope impurities, please fill in isotope impurity information supplied with your batch of TMT reagent in the Quantification Method. This can be facilitated using the “Reporter Ions Quantifier” Node in the Consensus Workflow (Parameter “Apply Quan Value Corrections”).

2. Reporter Quantification

Reporter Abundance Based On	Intensity
Apply Quan Value Corrections	True