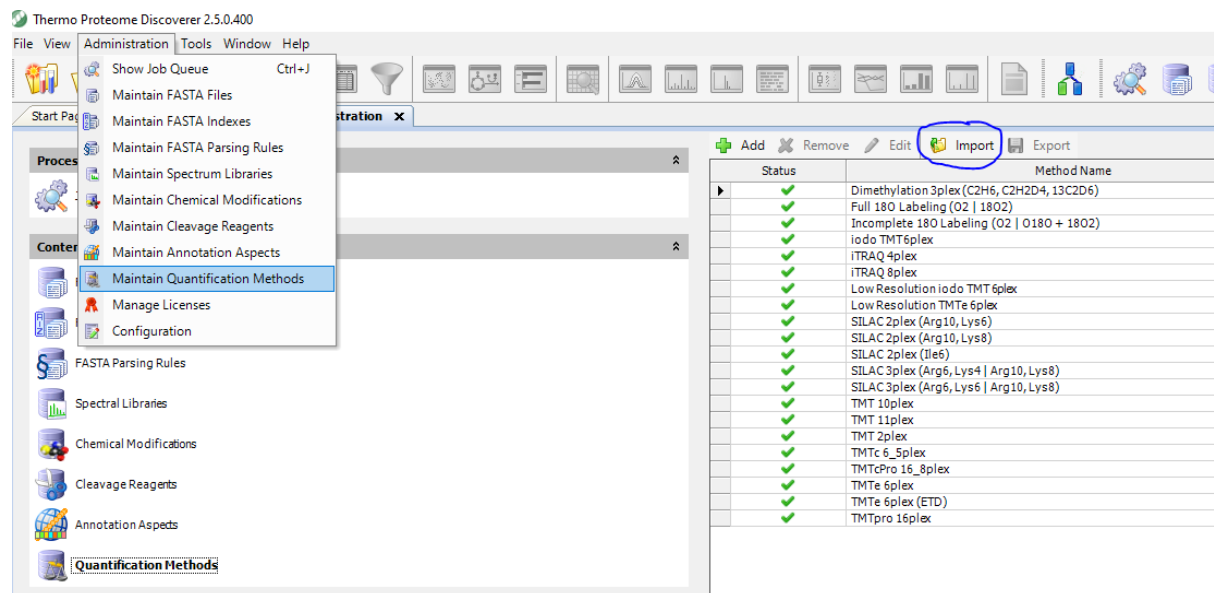


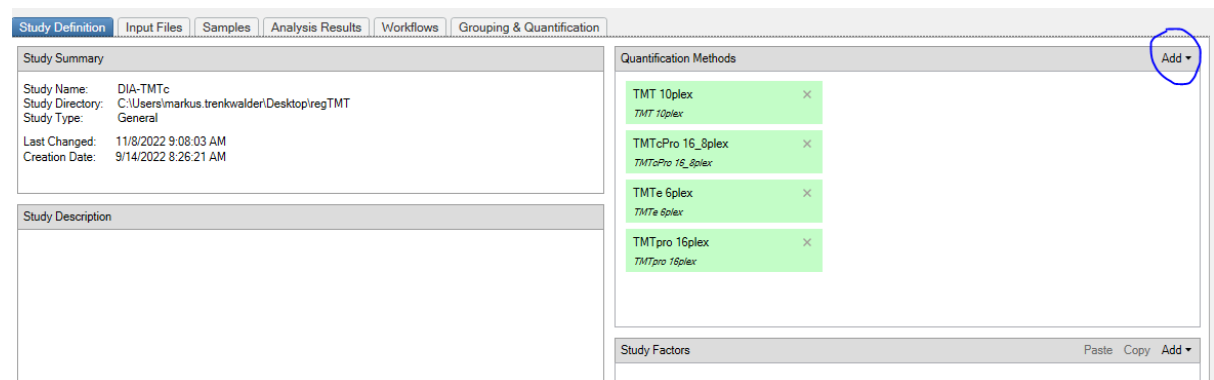
TMTc Tutorial

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

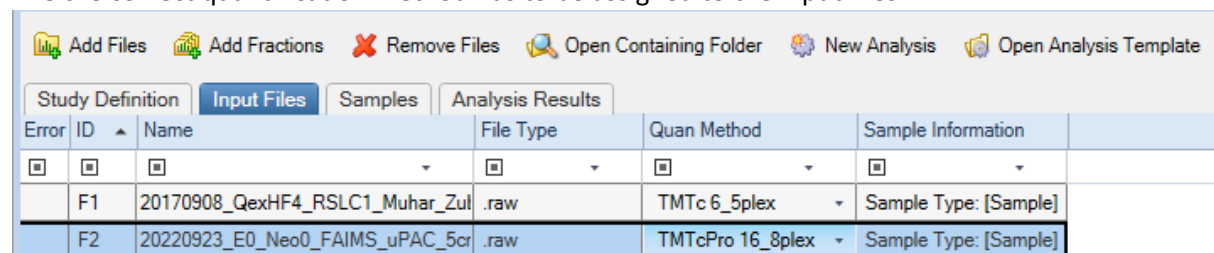
As a first step one has to import the quantification method files (TMTcPro 16_8plex.method and TMTc 6_5plex.method) provided with Hyperplex in the folder “HyperplexConfig”:



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 threshold 10
y-Fragment threshold 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