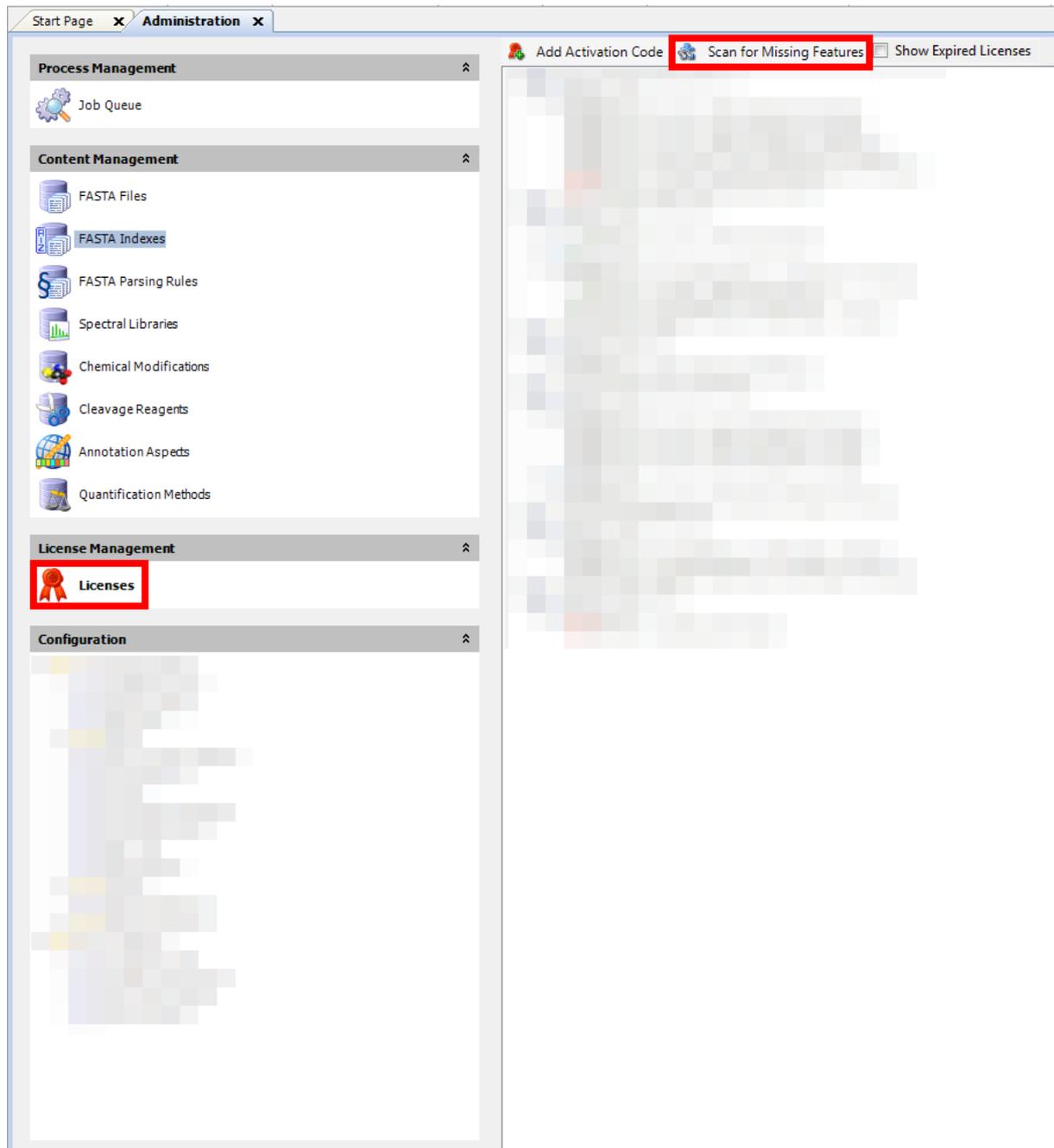## How to use Hyperplex

Installation

1) Extract all the contents of the zip to your Proteome Discoverer installation folder (usually located at C:\Program Files\Thermo\Proteome Discoverer x.x\System\Release).

2)
   a. Open the Administration Tab in Proteome Discoverer and Click on Licenses. From there click the Button "Scan for Missing Features" and afterwards restart Proteome Discoverer.


   b. Make sure Proteome Discoverer is closed. Open the command line in your Proteome Discoverer installation folder and run "Thermo.Magellan.Server.exe -install".

Usage

First, you need to set a Quantification Method in the Study Definition Tab and assign the Quantification Method to your Raw files.
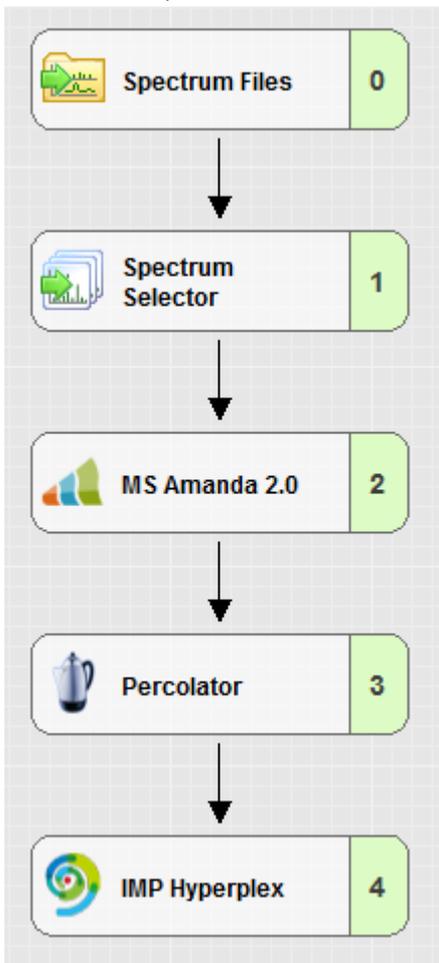
Then there's different Processing Workflows to use the Hyperplex.

1) The first one is, when it's only connected to the Spectrum Files Node:
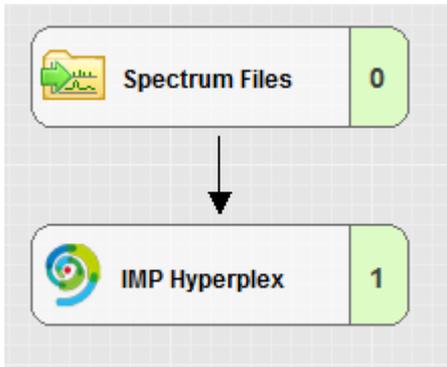
No other node is needed, although you probably want to connect other nodes to search the raw file. It will give you the Reporter Ions for every MS2 Spectrum in your raw file.

2) The second is, when it's connected to a PSM Validation Node:

Now only the spectra with matching PSMs will be quantified. Beware: It will report all the PSMs, even the low confident ones which will be filtered out in the subsequent Consensus Step.

To have all the Quantified Reporter Ions accessible, you need to use the Thermo's Reporter Ions Quantifier in the Consensus Step. For the Reporter Ions Quantifier to work properly, it needs to have a Protein Grouping node in the workflow as well, even if no Proteins are found or no search node is used:
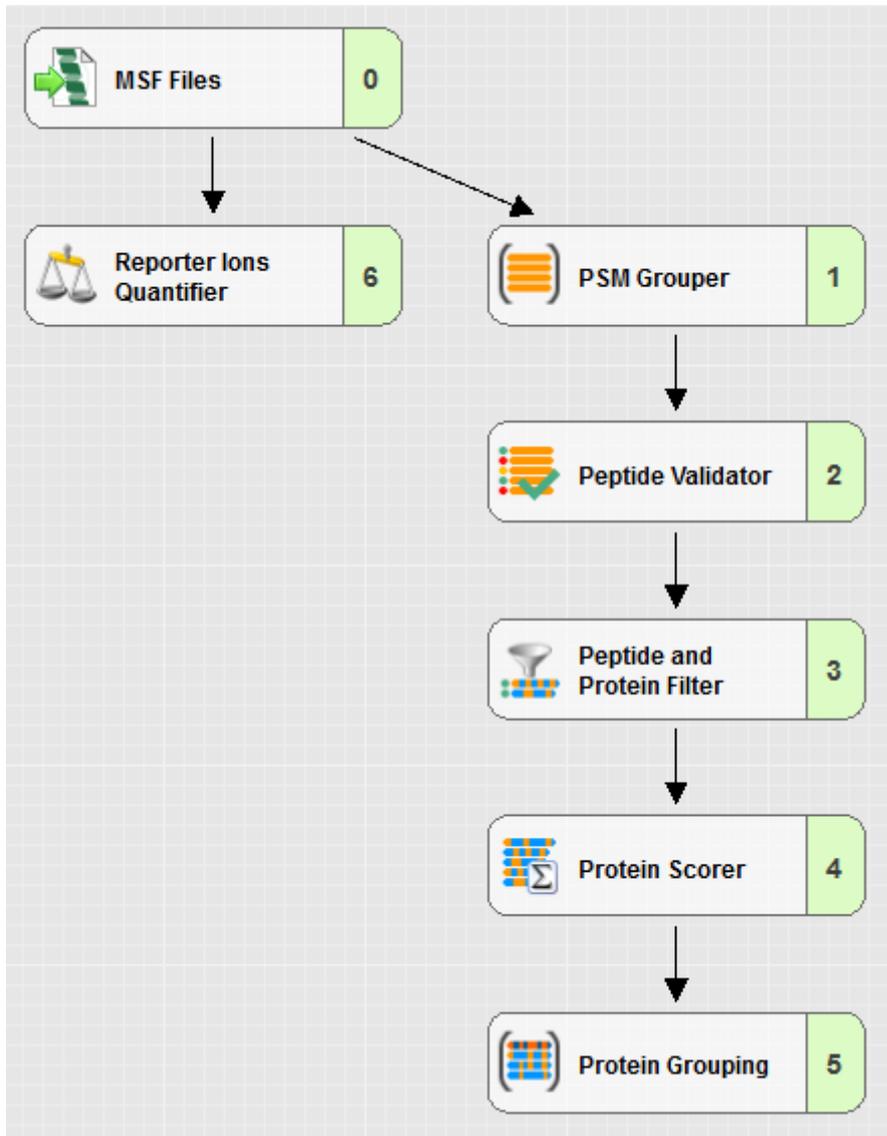
Here, all of the parameters are explained inside Proteome Discoverer really well, there's just one thing to notice: per default, the Reporter Ions Quantifier reports S/N values most of the times. To change this, the Parameter "Reporter Abundance Based On" should be changed to Intensity.

Parameters

Hyperplex has a lot of parameters, which will be discussed in more detail below:

Parameter	Default Value	Description
Reporter Mass Tolerance	10 ppm	The Mass Tolerance to look for the different Reporter Ions Peaks
Quantification Method		The Quantification Method(s), which should be used. A selection or a mixture of: Standard, EASI-Tag, TMTc and TMTc+. They will be discussed in more detail below.
Main Method	Standard	The values of the method which should be reported to the peptides and protein intensities. The other methods will be calculated as well and will be accessible in the Quan Spectra tab, but only this method will be used for peptide and protein intensity quantification.
Reporter Masses file		An optional Text File you can assign if you have different reporter masses than the ones provided with the Quantification Methods in the Study Definition Tab. Some methods are already defined in the Reporter Ion Corrector config folder in the zip file downloaded from the website.
Indistinguishable Complimentary Reporter Channels	3-4	The channels, which cannot be distinguished by your Mass spec, because they are to close together. Multiple values should be split by a comma, e.g. "3-4,5-6"
Fallback to TMT Reporters	True	If the custom quantification method (TMTc, EASI-Tag or TMTc+) fails, should the TMT Reporters be used instead?
Apply impurity correction	False	If an impurity correction of the Reporters should be applied, to further enhance the intensity search
Write MZ table	False	If an additional table for each used method should be generated reporting the m/z value of the found reporter
Debug Mode	False	If the debug mode should be used, reporting additional messages
Interpret thresholds as intensities	False	If the thresholds for the fragments and the precursor should be interpreted as intensities. Otherwise, they are interpreted as S/N ratios.
b-Fragment threshold	10	The average S/N (or intensity) of the b-Fragment isotope cluster must be greater than or equal to this value.
y-Fragment threshold	10	The average S/N (or intensity) of the y-Fragment isotope cluster must be greater than or equal to this value.
MS2 Precursor threshold	10	The average S/N (or intensity) of the MS2 precursor isotope cluster must be greater than or equal to this value.
Separate precursor scan	False	If a separate SIM-scan was used with the precursor information

Quantification Methods

Hyperplex has different Quantification Methods, which can be set:

Standard:

> The Intensities of the reporters will be reported "as is", so the algorithm will look at the specified m/z value for a peak and report the intensity for this peak. Very useful as well, when custom peaks need to be detected/specified.

TMTc:

> The intensities of the complimentary reporters will be reported. The algorithm tries to detect the complimentary reporters automatically. Refer to https://www.ncbi.nlm.nih.gov/pubmed/29522331 for more information

TMTc+:

> A more sensitive measure than TMTc, which tries to improve data loss with deconvolution. Refer to https://www.ncbi.nlm.nih.gov/pubmed/29522331 for more information.

EASI-Tag:

> A new algorithm, which tries to further enhance the problem with data loss because of the need to deconvolute. More information can be found here: https://www.nature.com/articles/s41592-018-0037-8