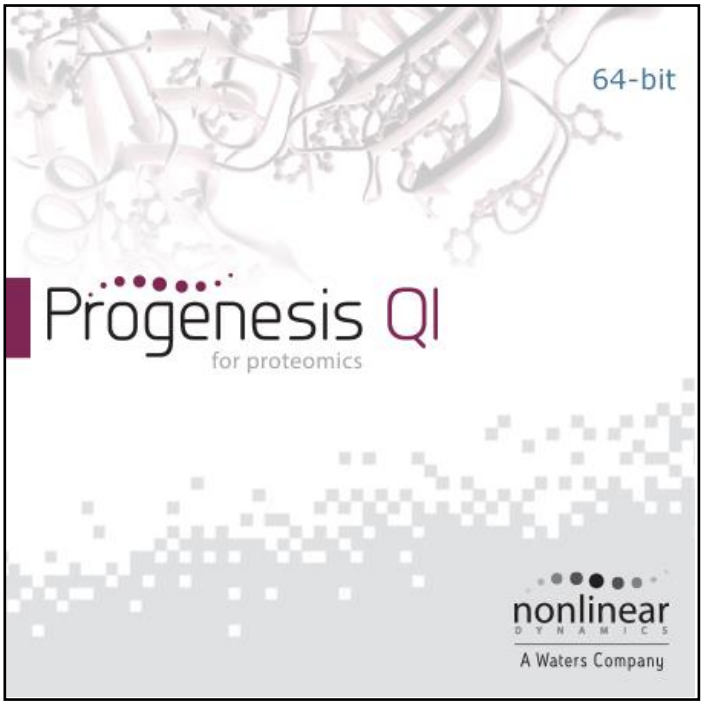

Progenesis QI for proteomics
HCP Spectral Library
User Guide

Analysis workflow guidelines for MS^E data

Waters

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Contents

Introduction

This user guide takes you through how to construct a Spectral Library from the analysis of a set of 12 LC-MS runs with 4 groups (3 replicate runs per group) using the unique Progenesis QI for Proteomics workflow. It describes the initial analysis of the data followed by the creation and use of a spectral library to identify proteins in 5 Batches of additional samples. More detailed descriptions of each step in the analysis workflow is described in the DDA and HDMSe User guides.

To allow ease of use the tutorial is designed to start with the restoration of Archived experiments where the data files have already been loaded. The document covers all the stages in the workflow, initially focusing on the Automatic Processing of the data then the use of the individual analysis stages. More details of the samples and the proteins present in them are available on page 4.

If you are using your own data files please refer to Appendix 1 (page 38) then start at page 6.

Note: the images used in Appendices 1 and 4 refer to the HDMSe User guide data set.

How to use this document

You can print this user guide to help you work hands-on with the software. The complete user guide takes about 60 to 90 minutes. This means you can perform the first half focused on LC-MS run alignment and analysis then complete the second half of analysis exploring comparative differences and Protein identity at a convenient time.

If you experience any problems or require assistance, please contact us at support@nonlinear.com

How can I analyse my own runs using Progenesis QI for proteomics?

You can freely explore the quality of your LC-MS data using Data Import and then licence your own LC-MS runs using this evaluation copy of Progenesis QI for proteomics. Instructions on how to do this are included in a section at the end of the user guide document.

LC-MS Data used in this user guide

For the purposes of this data set the MS^E parameters were set to 250:150 the default settings as defined in Appendix 1 (page 38). This was to done to reduce the time taken to demo the data analysis.

Tutorial Data

The samples were originally generated to examine the use of Spectral Libraries to monitor the presence of Host Cell Proteins (HCPs) in batches of the NIST mAb product. The idea being to simulate contamination of the mAb preparation with Chinese Hamster Ovary (CHO) cell proteins and therefore to monitor Host Cell Proteins with high accuracy using mass spectrometry.

Label free LC-MS was performed using a 30min gradient on a CSH C18 (M-class ACQUITY UPLC) attached to a Xevo-G2XS

There are 2 data sets: HCP_Spectral Library_**Created** and HCP_mAb_**Batches** containing the samples as indicated below, run in triplicate.

CHO proteins ppm	**Create**	**Batches**
50		Batch_5
100	CreateA	
500		Batch_4
1000	CreateB	
5000		Batch_3
10000	CreateC	
25000		Batch_2
50000		Batch_1
100000	CreateD	

The idea of the data sets is to allow you to explore the processes involved in making spectral libraries and then their use in the identification and quanification of Host Cell Proteins using different Batches where the HCPs have been simulated by introducing different levels of CHO proteins.

The raw data has already been loaded into Progenesis QI for proteomics 4.2 in the form of 2 experiments and licenced archives of these experiments have been created:

HCP_Spectral Library_Created.ProgenesisQIPArchive

HCP mAb_Batches.ProgenesisQIPArchive.

Note: these will open at the Identify Peptides screen when restored in Progenesis QI for proteomics

A fasta file, **NIST AB_chaperone_CHO.fasta,** is provided for identification of the proteins in the HCP_Spectral Library Created experiment. The FASTA contains sequences for CHO cell proteins, the NIST mAb product and the internal spiked E.coli Chaperone protein ClpB which was added to all the samples (200 fmol/sample).

All samples contain 41 pmol of NIST mAb product protein.

Note: there are also 2 experiment design setup (Create.spl and mAb_Batches.spl) files and 6 example Spectral Libraries .msp available.

The files described above are all available in the 2 Spectral Library Tutorial.zip files downloaded from http://www.nonlinear.com/progenesis/qi-for-proteomics/v4.2/user-guide/.

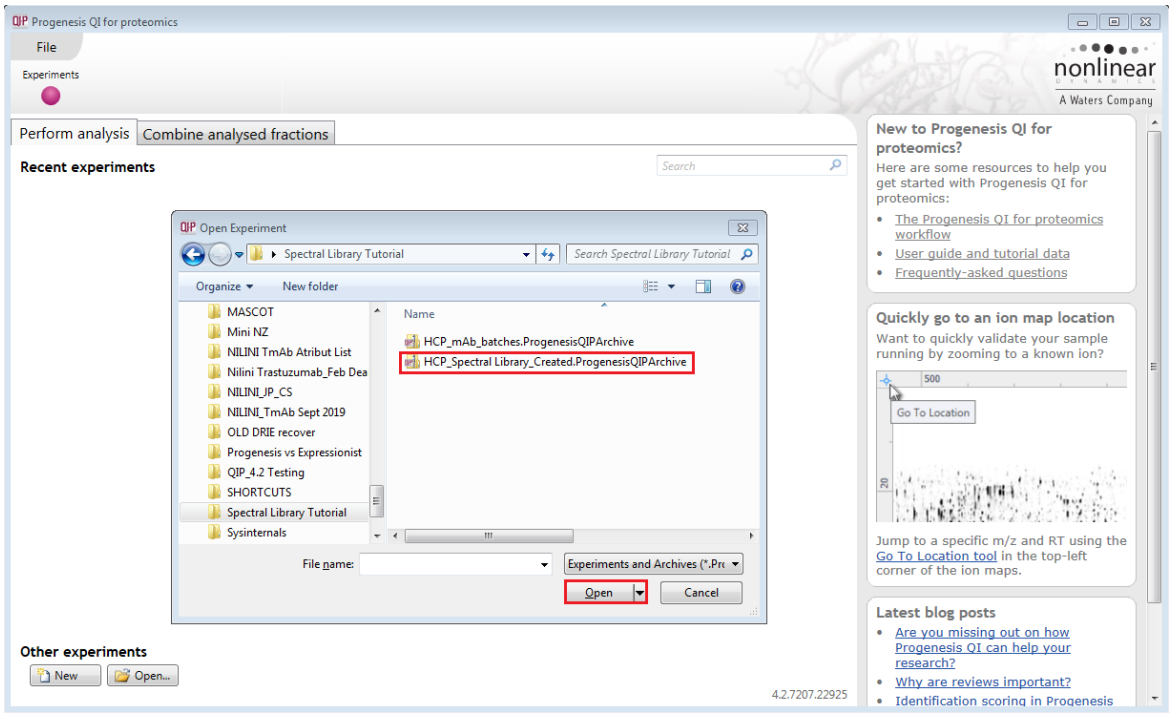
The following sections explain how to restore and process the data in Progenesis QI for proteomics v4.2.

Restoring the Tutorial

Open Progenesis QI for proteomics and download the Compressed files (.zip), **Library_creation_data_set.zip** and **Library_search_data_set.zip** from the 'User guide and tutorial' link shown below, placing them in a **new folder** on your desktop (i.e. Spectral Library Tutorial). Before restoring the tutorial in the software **you must** first right click on the (.zip) files and extract them to the same folder.

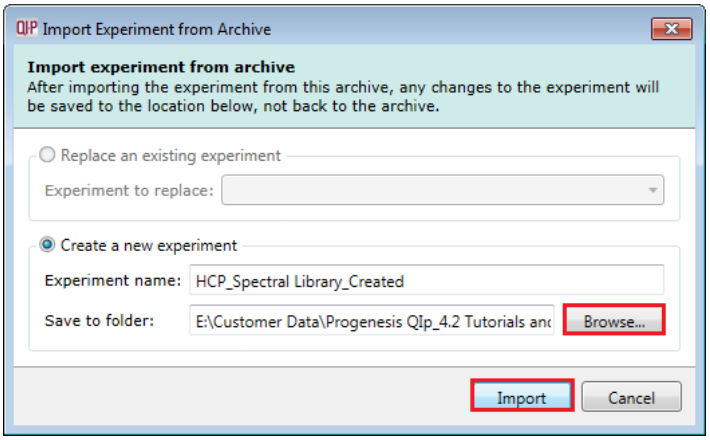
There are 2 archive files, 2 Grouping files, a fasta file and example Library files.

Now restore the 2 archive files (HCP_Spectral Library_Created and HCP_mAb_Batches). To do this, locate the '**name'.Progenesis QIP Archive** file using the **Open** button and press Open.



This opens the "Import Experiment from Archive" dialog.

Select the **Create a new experiment** option and select the folder in which you placed the archive, using Browse.



Then click **Import.**

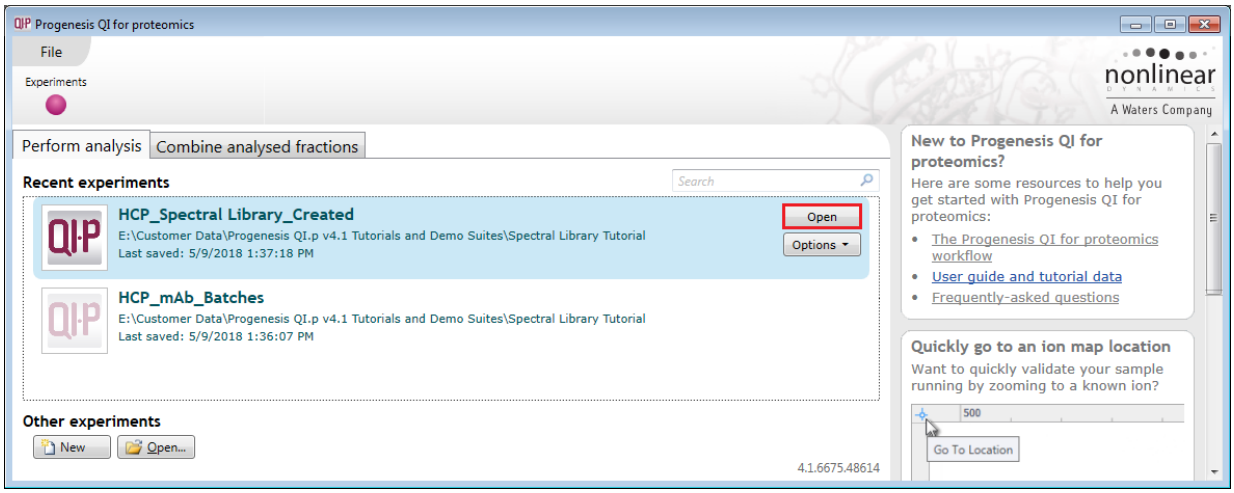
After the import is complete select **Close** from the **File menu** and then repeat the process for the other archive (Batches), restoring them into the same folder.

Note: use the **Replace an existing experiment** option if you want to over-write an existing version of the tutorial.

Tip: at each stage in the software there are links to more information and help on the website.

Stage 1: Perform automatic processing of the Create Library samples

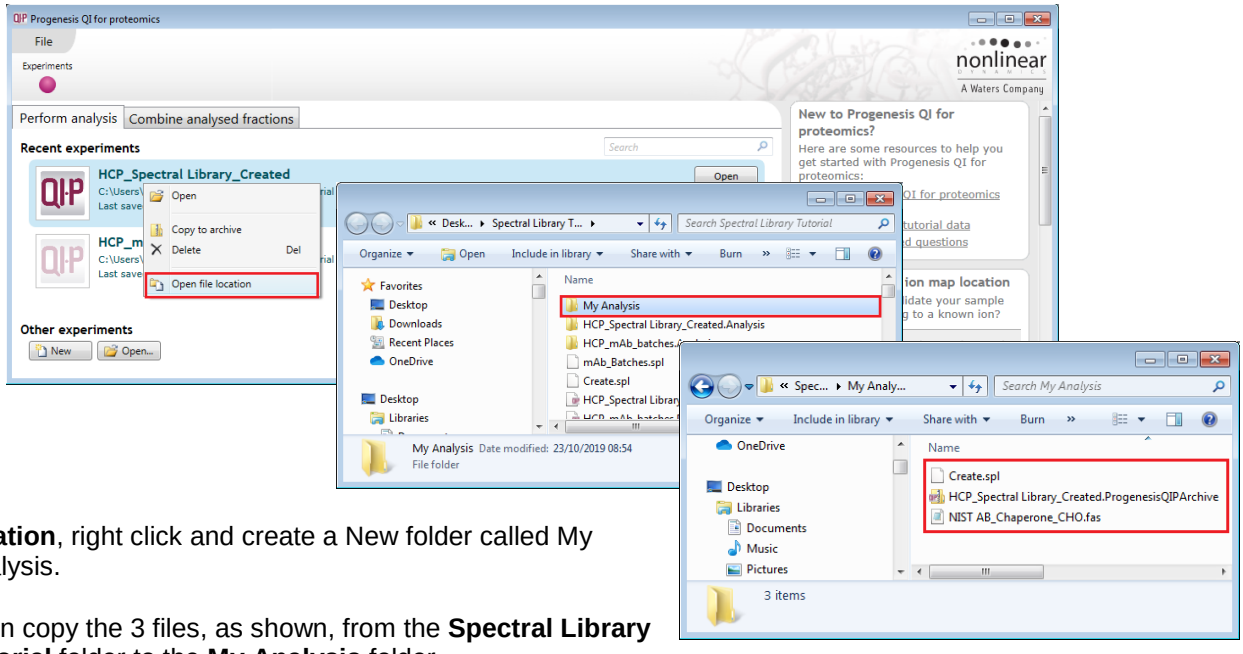
Having opened the Progenesis archive files the experiments will now be displayed when you open Progenesis QI for proteomics. Select the HCP_Spectral Library_Created experiment and click **Open**.



Note: having restored the **HCP_Spectral Library_Created** experiment, go to Stage 9 (page 26), where you can proceed with the creation of the various spectral libraries from the analysed experiment.

Alternatively, you can use the first 7 stages of this guide to perform the Automatic processing of the Created data set and then go through the processes involved in the selection and review of Peptide ions to add to the Spectral libraries. To do this you can generate a third experiment using the same **HCP_Spectral Library_Created** archive then remove the analysis by clicking **Restart automatic processing** as described below.

First you must create an additional folder called **My Analysis** in the same folder you restored the original archives, to do this right click on the HCP_Spectral Library_Created experiment and select **Open file**

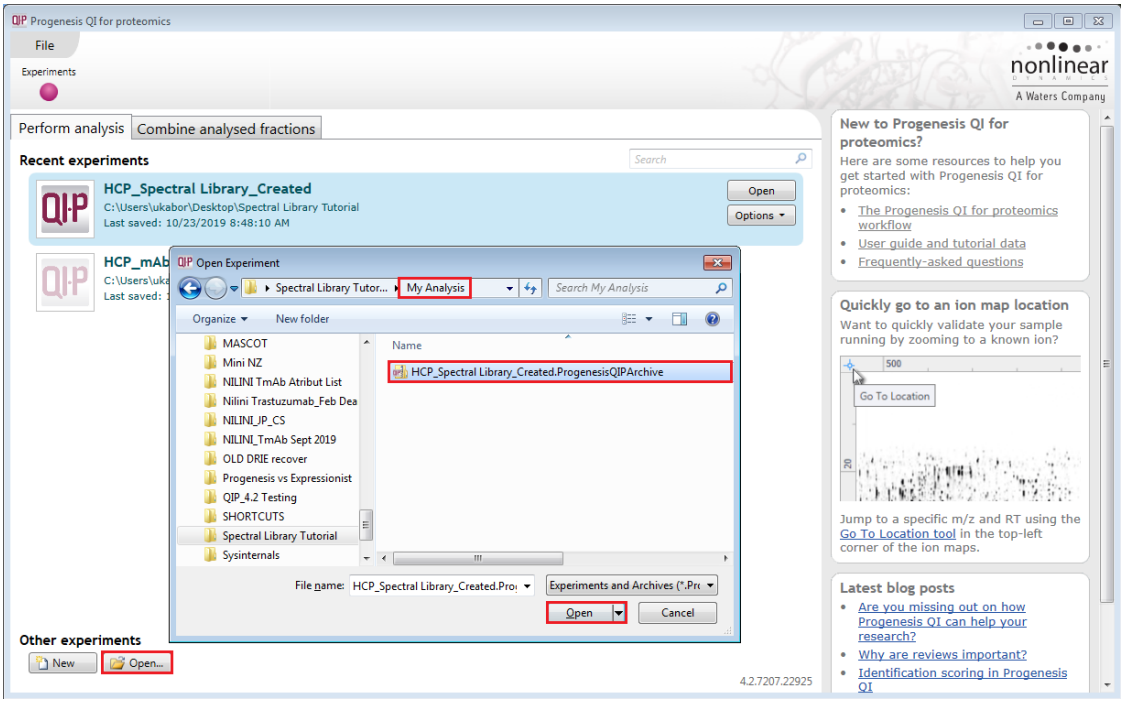


location, right click and create a New folder called My Analysis.

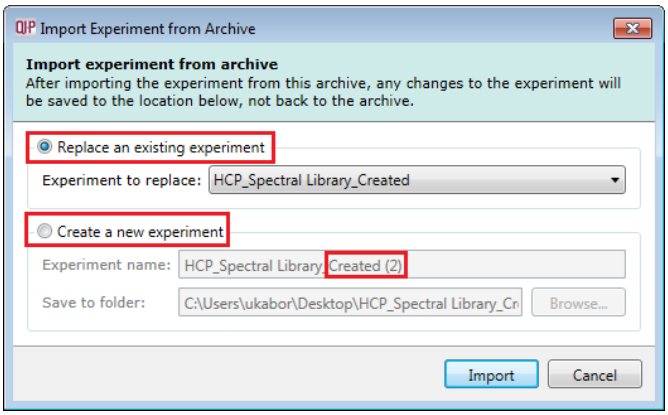
Then copy the 3 files, as shown, from the **Spectral Library Tutorial** folder to the **My Analysis** folder.

Now select **Open** on the Progenesis Experiments page and Navigate to the **My Analysis** folder.

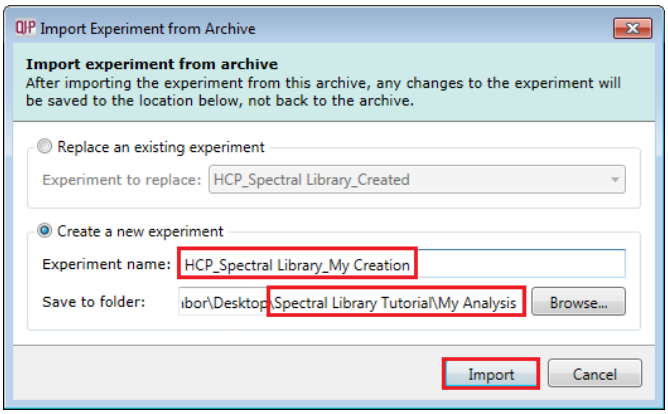
Select the **HCP_Spectral Library_Created.Progenesis QIPArchive** and click Open



The following dialog will open asking you to **Replace an existing experiment**, do **not** do this.



Select **Create a new experiment** and amend the experiment name to **HCP_Spectral Library_My Creation**



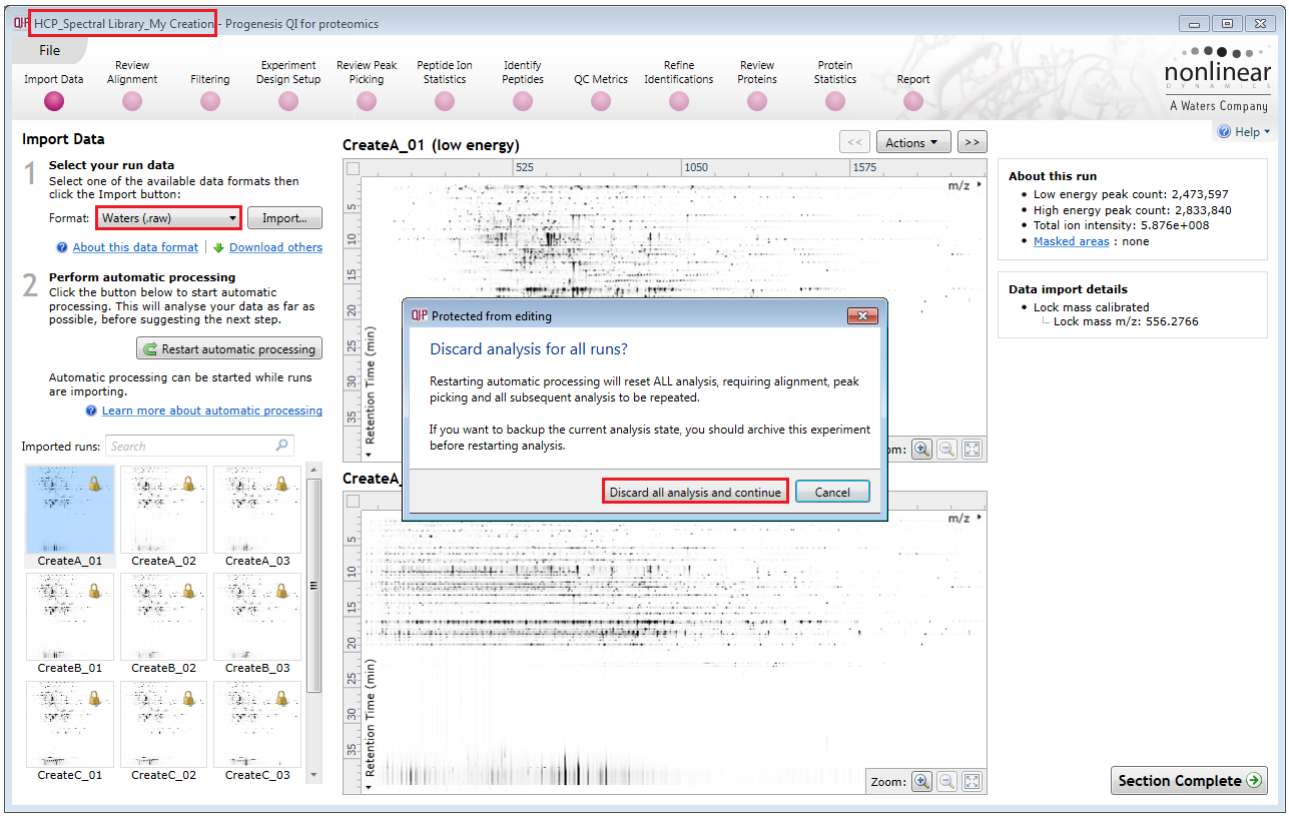
Then make sure that Save to folder is set to **My Analysis** using the **Browse** facility.

Then click **Import**

The newly created **HCP_Spectral Library_My Creation** experiment will open at the **Identify Peptides** stage on the workflow.

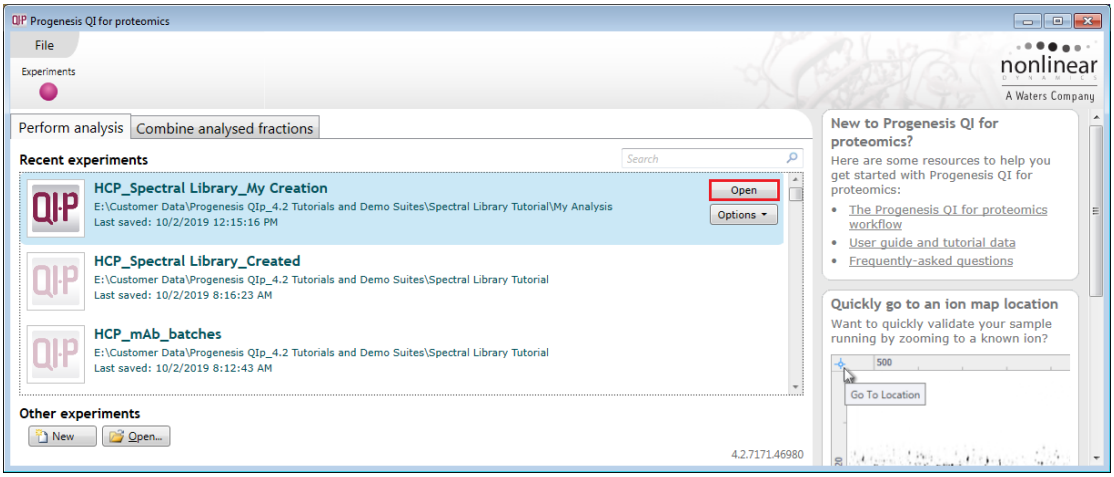
Click on **Import Data** and then click **Restart automatic processing.** This will bring up a warning dialog.

Select **Discard all analysis and continue** this will remove all existing analysis of this data set.



The Auto processing wizard opens and a series of dialogs allow you to select how you want the runs to be analysed in Progenesis.

Click **Cancel** and the Close from the File menu (Top left of the Screen)



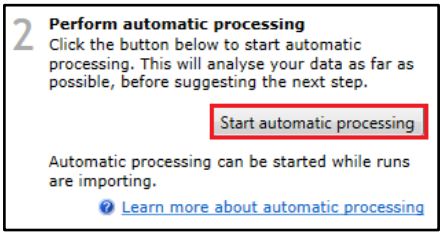
Note: the appearance of the new experiment in the list

The following pages describe how to proceed with the full analysis of this data.

Click **Open** and move to the next stage.

Stage 2: Automatic Processing of your data

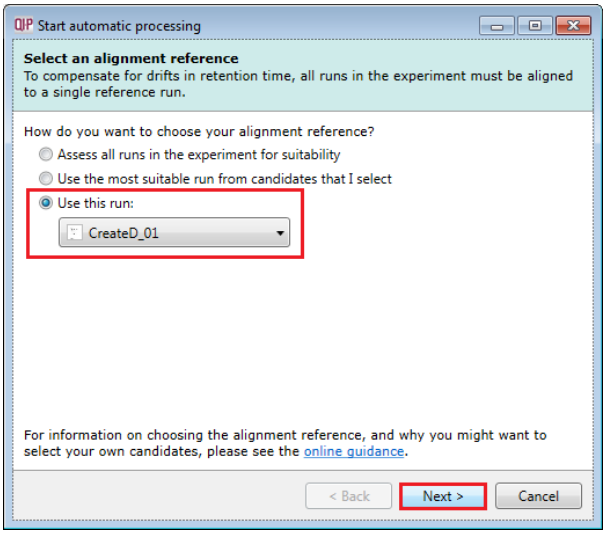
The Automatic Processing of your data can be set up and started before the import of your data has been completed by clicking on **Start automatic processing.**



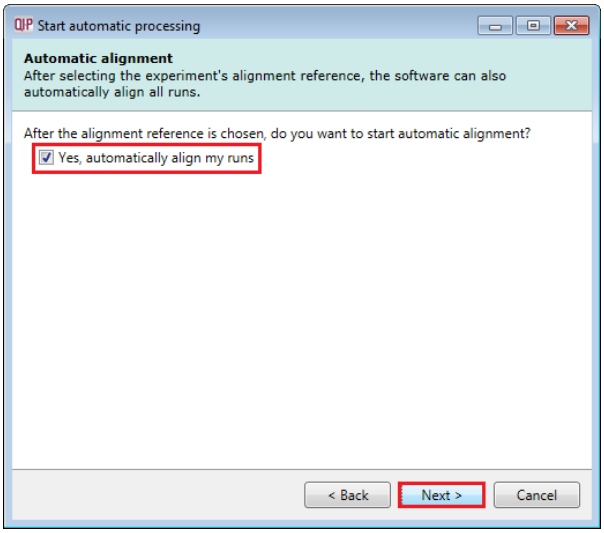
Note: for this tutorial the data has been imported already.

For the processing of the 12 files in this experiment make the following selections:

Step 1: Select the third option, **Use this run**. This tells QIp to set the Alignment reference for to your chosen image. (In this example CreateD_01)

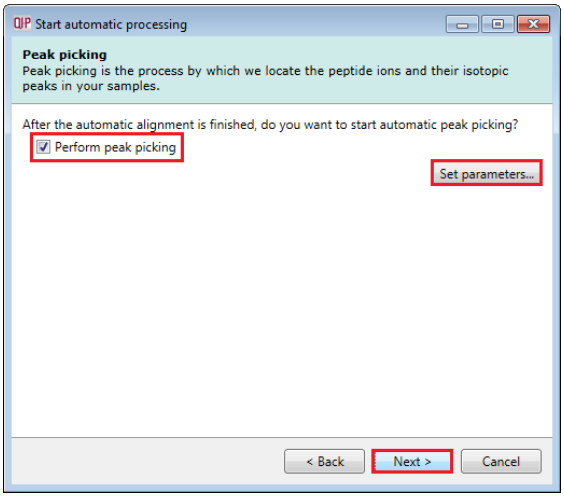


Click **Next**. The option to perform Automatic alignment will be selected by default.



The next page of the processing wizard will ask you if you want to **Perform peak picking** and allow you to set appropriate parameters.

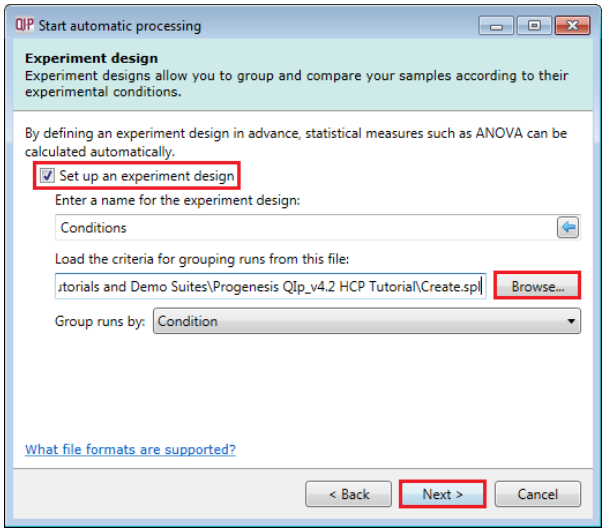
Step 2: For the purposes of this User guide we will use the default settings for peak picking which you can review by clicking on Set parameters.



Click **Next**.

Step 3: To apply the experiment design in advance Click **Set up an experiment design** then locate the appropriate file (**Create.spl** available in the folder you extracted the .zip file) using Browse.

Note: if you do not enter a name for the experiment design it will adopt the name of the first column in the Create.spl, in this case Conditions, this can be changed as required in the main workflow.



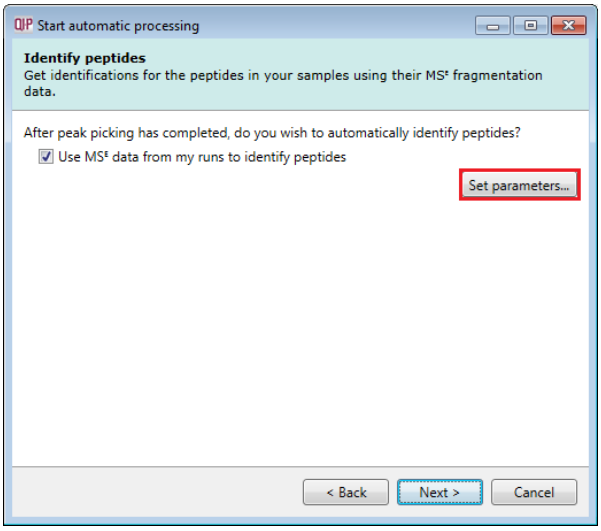
Note: you can also create additional experiment designs following the completion of automatic processing.

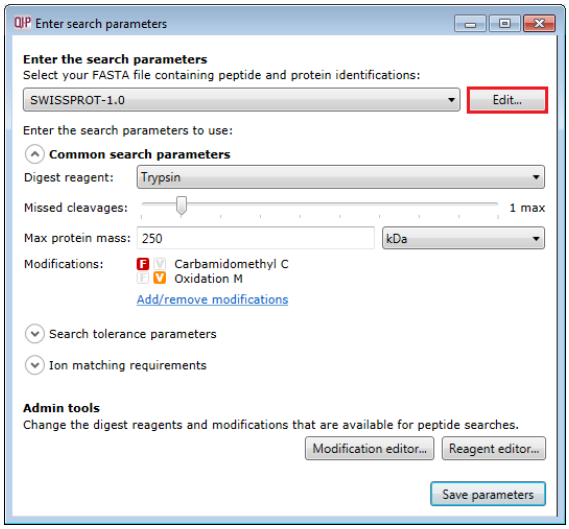
Select Conditions and then click **Next**.

For MS^E fragmentation data you can set up the peptide identification to be performed automatically.

Step 4: To automatically process the identification of peptides using there MS^E fragmentation data in the tutorial data click on **Set parameters**. The default Databank is for Swissprot-1.0 (which is a locked example).

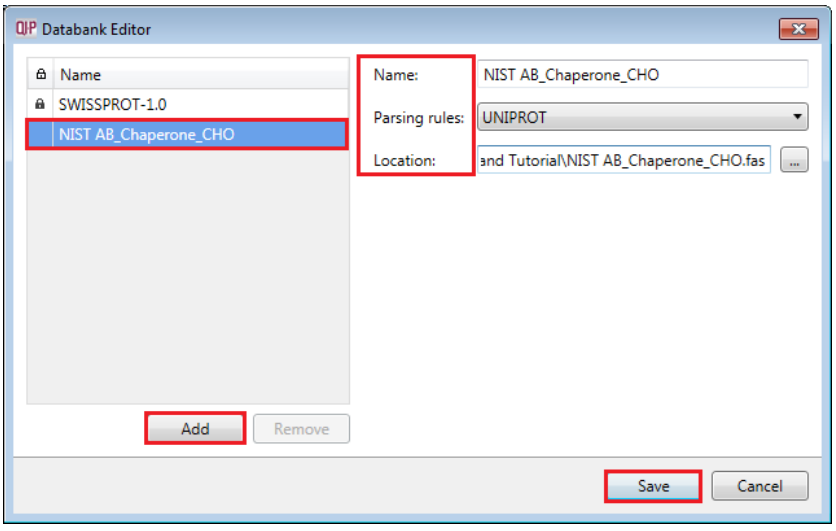
Note: if the software has detected the presence of MS^E data then this option will be ticked by default.





To create a new Databank from a Fasta file click on **Edit** and then create a new one using the example FASTA file that is available with the Experiment Archive you restored at the beginning of this tutorial exercise (**NIST AB_Chaperone_CHO.fas**).

For a new Databank click **Add.** Then give it name (i.e. NIST AB_Chaperone_CHO), select the parsing rules (UNIPROT) and specify the location of the FASTA file, see the example below.



The new Data bank will appear in the left panel now click **Save** to return to the Search parameters.

If your databank is not already displayed then select it from the drop down list.

Check the **Common search parameters**

The default settings are displayed:

Digest reagent: is set as Trypsin**.** Alternative Digest reagents are available from the list and additional ones can be added to the list using the **Reagent editor...**

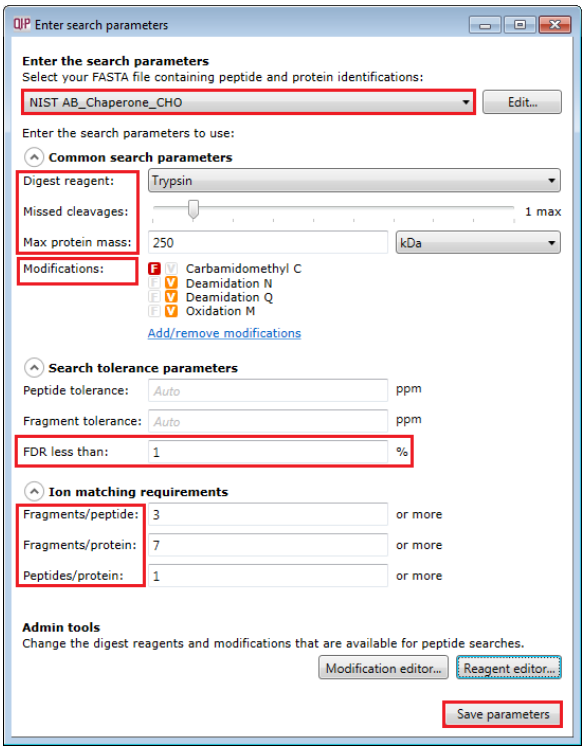
Missed cleavages: is set as 1.

Maximum protein mass: is set at 250kDa

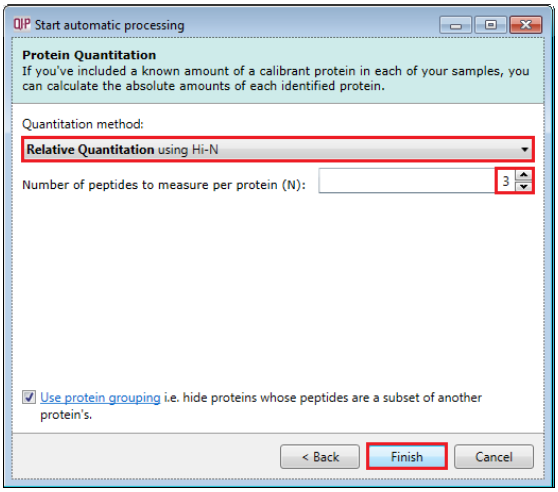
Modifications: are set Carbamidomethyl C (Fixed) and Oxidation M (Variable). More modifications are available from the list and additional ones can be added to the list using the **Modification editor…** In this example Deamidation on N and Q have been added

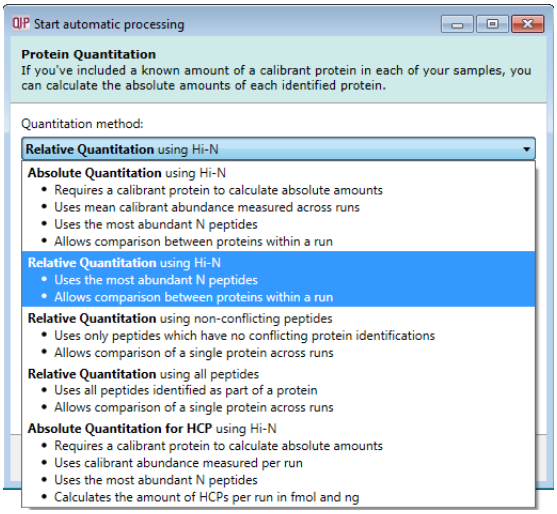
Search Tolerance parameters: The false discovery rate (FDR) has been set to 1%

Ion matching requirements: are set at Fragments/peptide: 3, Fragments/protein: 7 and Peptides/protein: 1 by default



Click **Save parameters** and then **Next.**

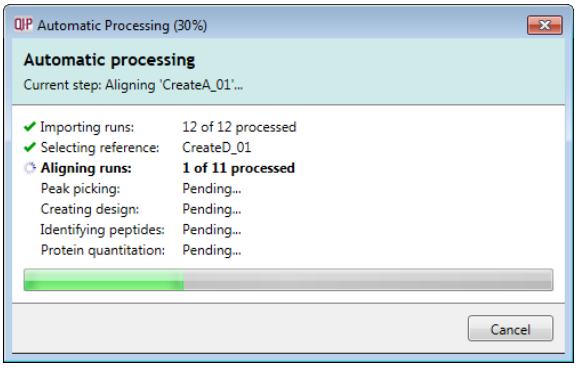


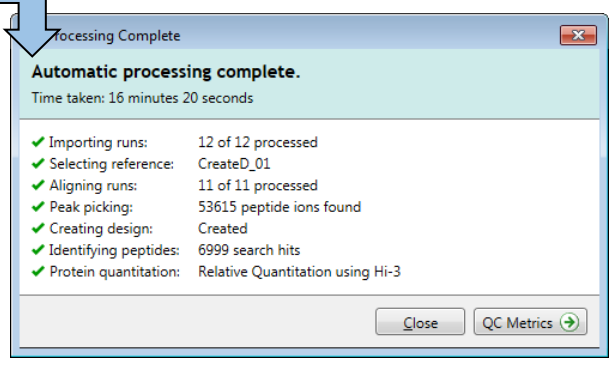


The **Protein Quantitation** dialog opens displaying the default method, **Relative Quantitation using Hi-N** which uses up to 3 peptides per protein to compute the relative amount of each 'identified' protein.
Use the drop down to reveal the alternative methods for protein quantitation

Select the Default option and click **Finish.**

The process starts with the selection of an alignment reference and completes with Protein quantitation.

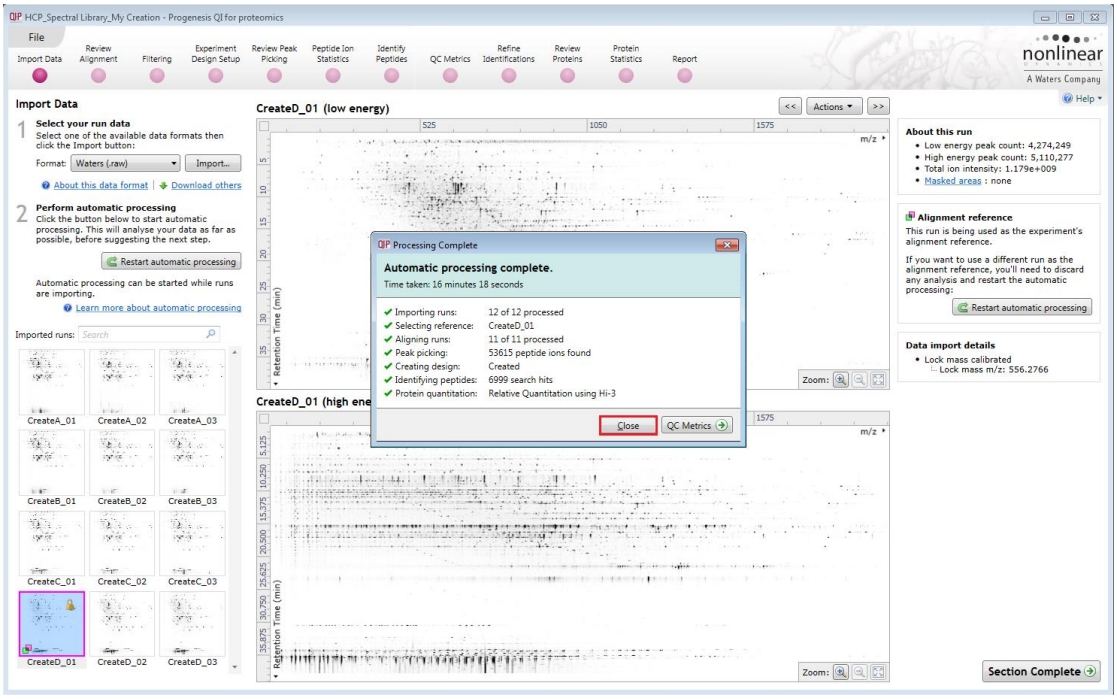




As the whole process continues you get information on what stage has been performed and also the % of the process that has been completed.

Note: if processing fails to complete successfully there are a number of suggested strategies you can use to proceed with your analysis. These are out lined in Appendix 2 (page 43).

Note: this does not include the time to load the data



When Processing completes click on **Close** and then on **Review Alignment** in the Workflow

Note: you can explore and re-perform the steps, sequentially and/or as part of the automatic processing as described in this guide

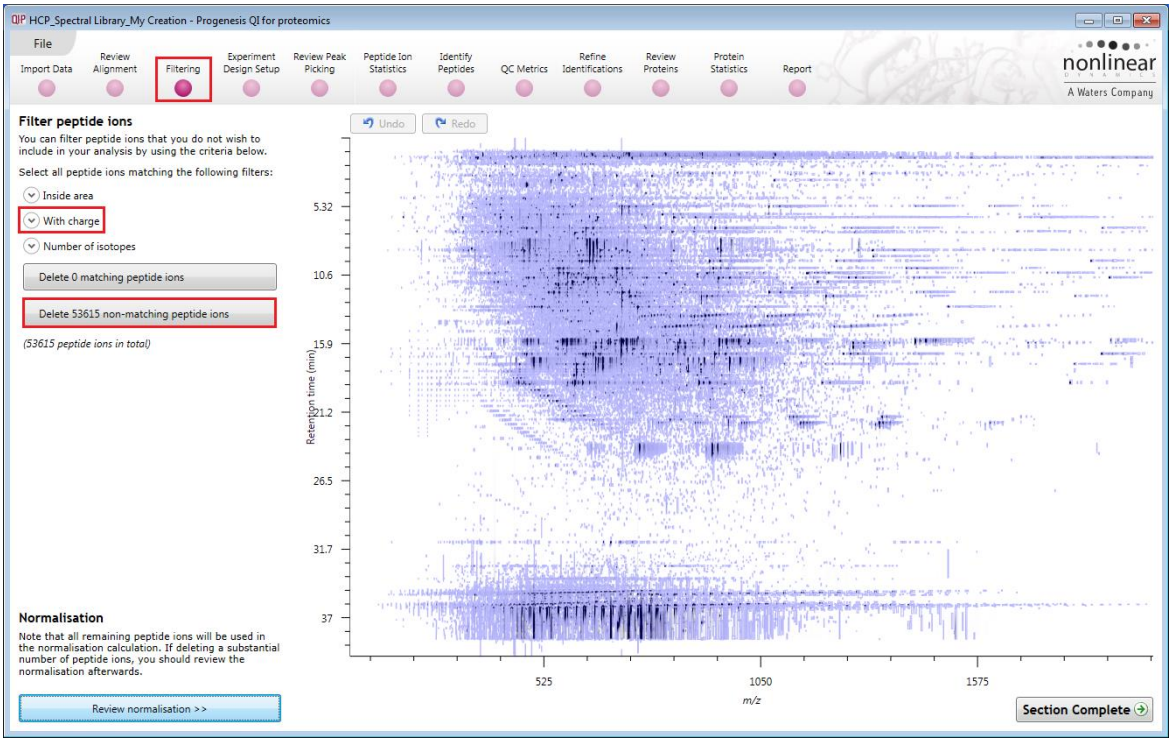
Note: additional details on alignment are available in Appendix 4 (page 46).

For this data set, the quality of the alignment (as indicated by the high scores) and detection following automatic processing does not require to be re-performed.

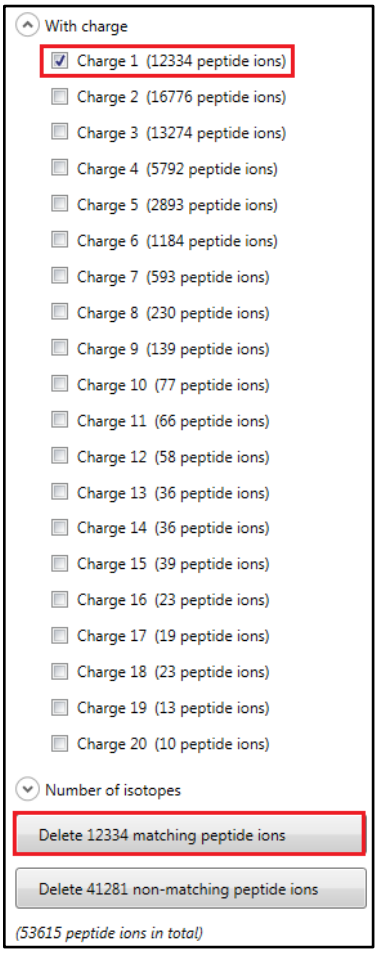
Click on **Filtering** on the workflow to move to the next section.

Stage 3A: Filtering

At Filtering you can review the total number of peptide ions detected (53,615) and choose to keep all or those which meet certain filtering criteria.



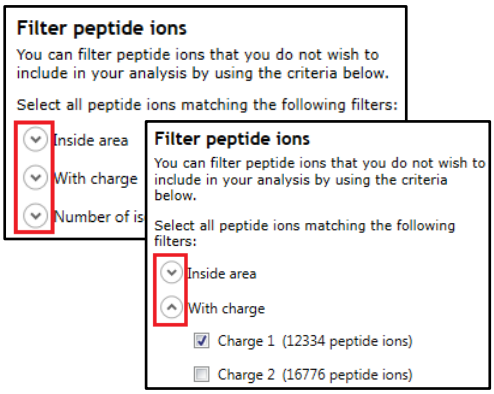
For this particular data set we will reduce the peptide ions to having a 'charge state' of 2 and above by filtering out the Charge state 1 peptide ions.



Select **With charge** and tick the Peptide ions you wish to remove from the analysis.

Following deletion of the peptide ions that do not meet the criteria this leaves 41,281 peptide ions with a range of charge states 2 to 20.

Tip: when filtering on one property of the peptide ions i.e. 'With charge', make sure you have 'collapsed' the other filters as expanded filters are applied concurrently.

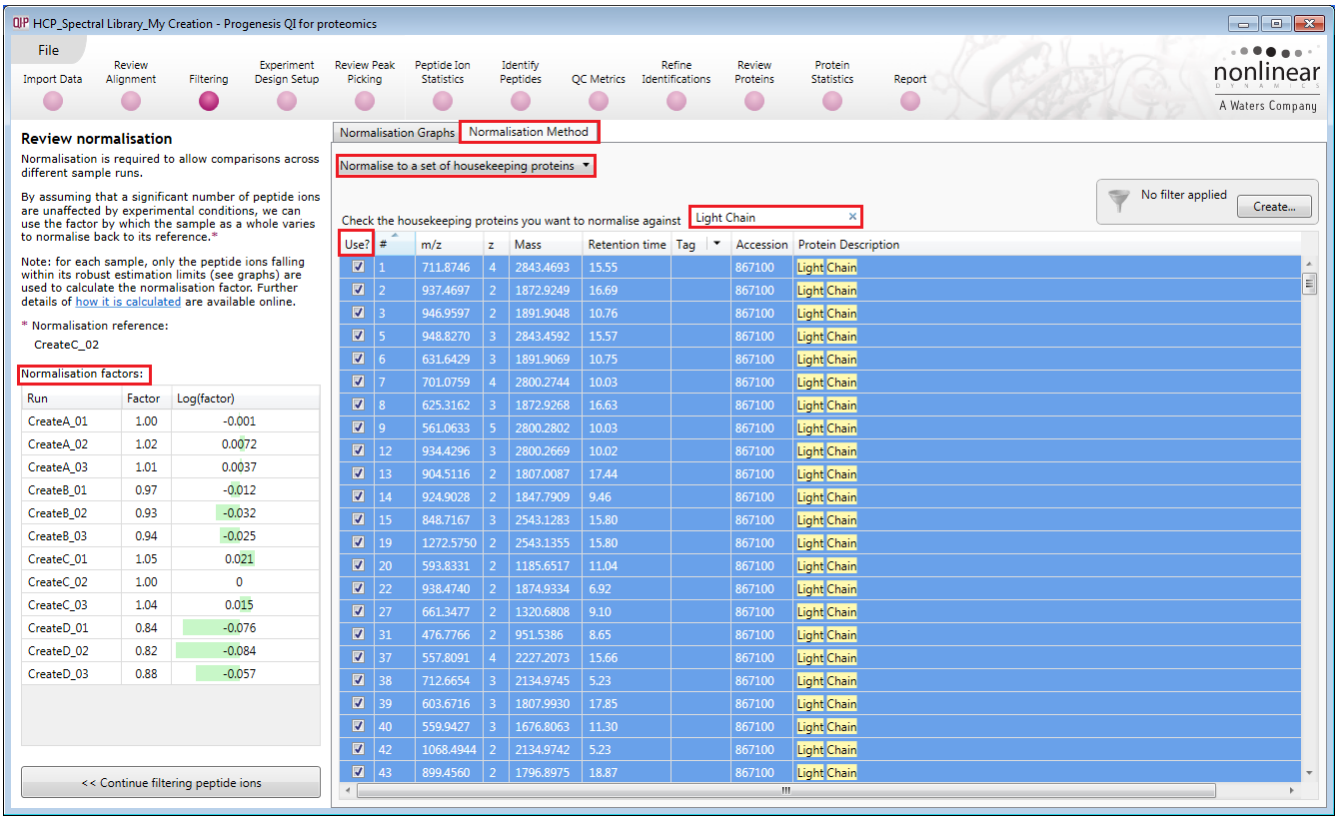


Now move on to **Normalisation** by clicking on 'Review normalisation', bottom left of the Filter page.

Stage 3B: Normalisation

At this stage in the workflow you can review and change the normalisation method; by default it will be set to normalise to all proteins.

To change it click on the Normalisation Method tab and select **Normalise to a set of housekeeping proteins** from the drop down. Enter 'Light chain' in the proteins you want to normalise against. Select all of the peptide ions for this protein by selecting all the rows in the table and ticking the **Use** box.



Note: the Normalisation factors will update, reflecting the use of the selected protein for normalisation.

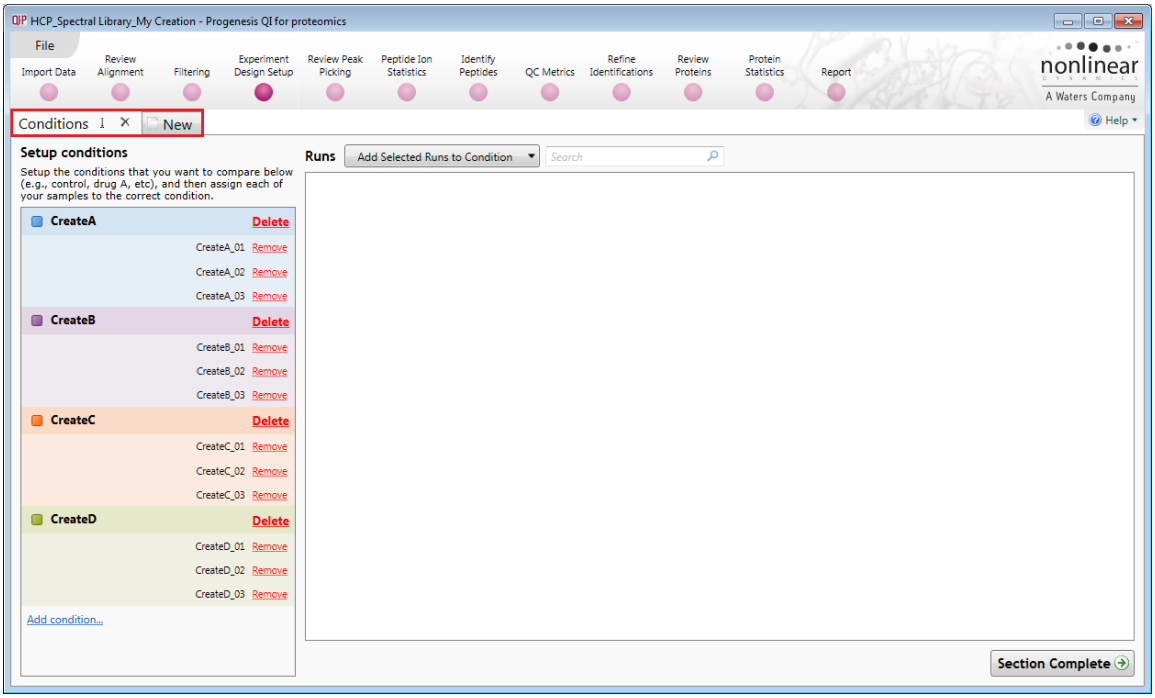
Note: As the same amount of mAb protein (41 pmol) is present in each sample the normalisation was performed against the peptide ions for this protein.

Now move on to **Experiment design setup** by clicking on the icon on the workflow.

Stage 4: Experiment Design Setup for Analysed Runs

At this stage in the workflow you can setup one or more experimental designs for your data.

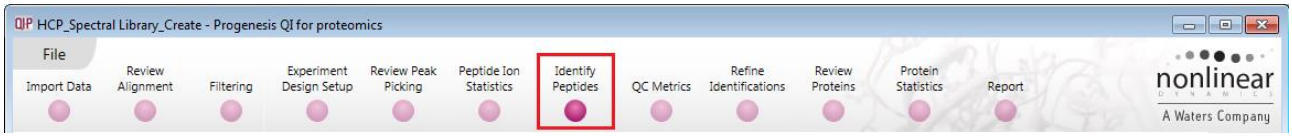
Currently the Between subject experiment design has been set during the Automatic processing of the data, with 4 conditions: CreateA to D (CHO proteins spiked @ 100 ppm to 100000ppm).



Note: you can use the New tab to create another Experiment design as required.

Details on the use of the next 2 steps in the Workflow: **Review Peak Picking** and **Peptide Ion Statistics** are available in the main HDMSe User guide.

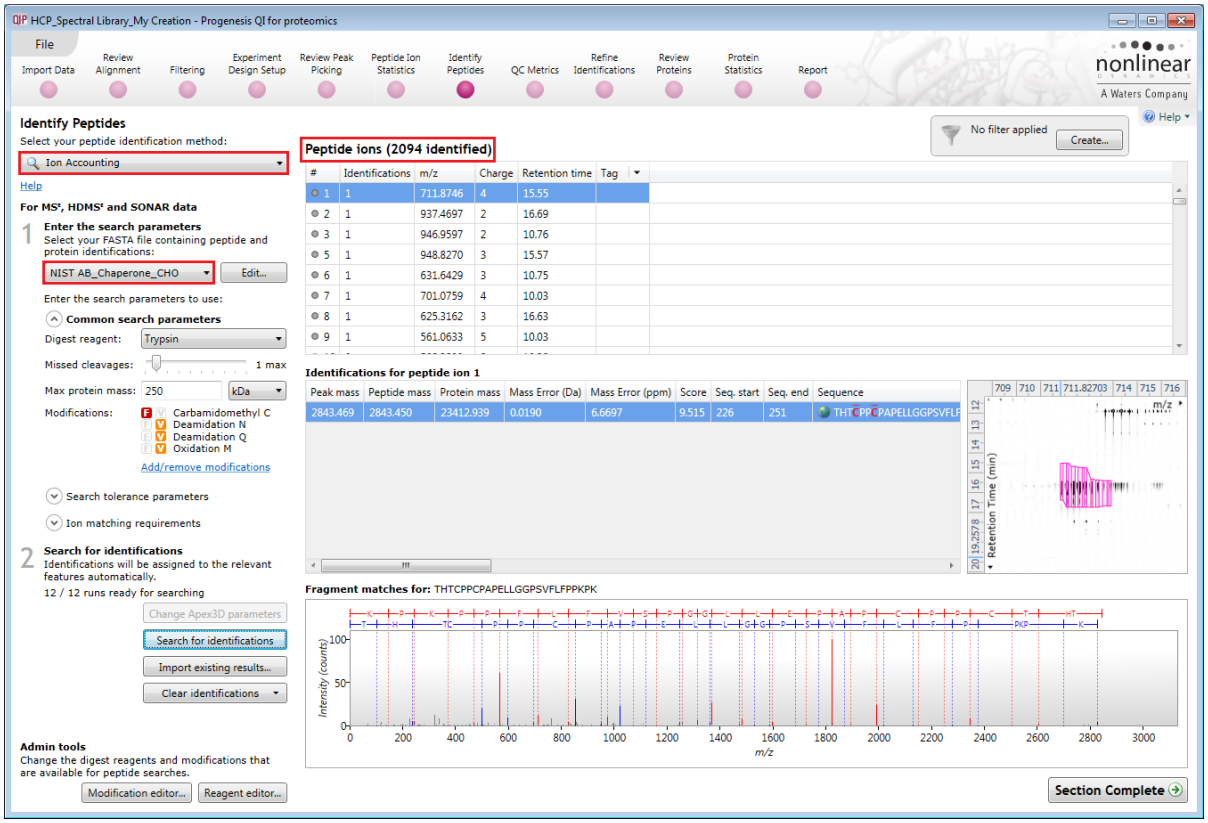
For the purposes of this Tutorial we will move directly to the **Identify Peptides** stage by clicking on the workflow.



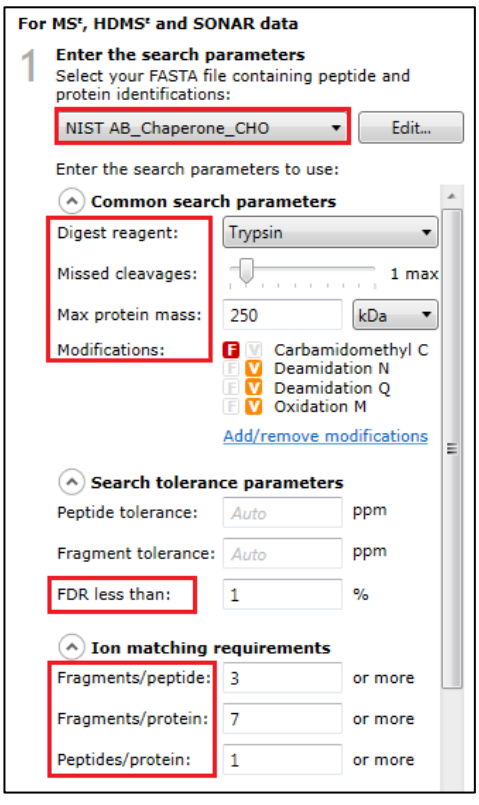
Stage 5: Identify peptides

Progenesis QI for proteomics is designed to perform peptide identifications either directly or by allowing you to export MS/MS spectra which can be used to perform peptide searches by various search engines. The resulting identifications can then be imported back into Progenesis QI for proteomics, using a number of different file types, and matched to your detected peptide ions.

The data described in this tutorial was acquired on a Waters Xevo G2XS it is MS^E and therefore the peptide identification method used was **Ion accounting** as shown below.



For this example we are using the direct method **Ion Accounting** as the peptide identification method.



Note: Following the automatic processing, described in Stage 2 (page 9) of this guide, the Identify Peptides page currently displays the full list of the detected peptide ions in your experiment.

Note: At the Filtering stage you will have removed the charge state 1 peptide ions, so you should re-perform the search using the parameters shown on the right.

There will be 2094 identified.

The left hand panel will display the Fasta File used (NIST AB_Chaperone_CHO) in the search and the parameters and settings used to control the search.

Stage 6: Refine Identifications

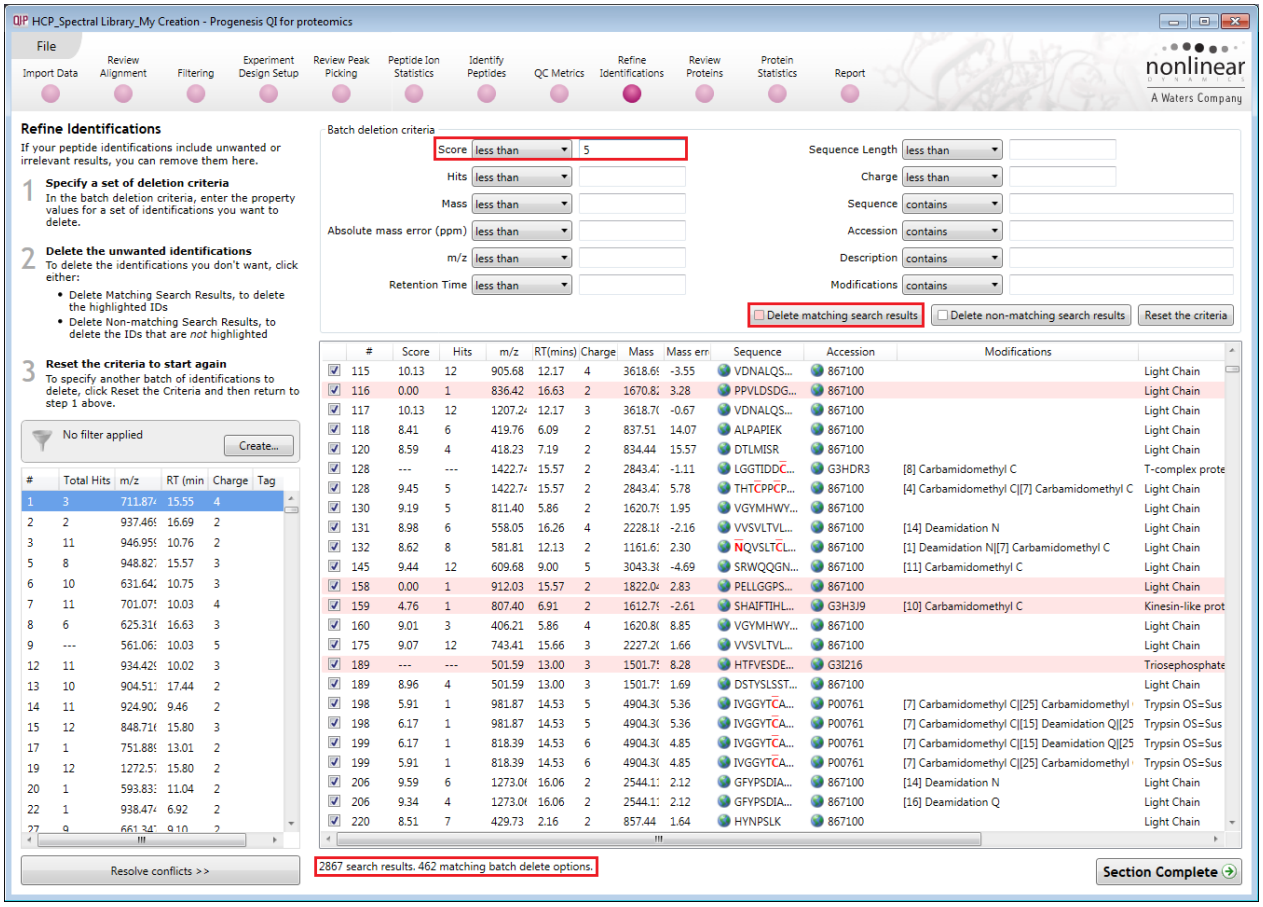
Before attempting to create a Spectral library from this data one should first refine the quality of the search results by filtering out peptide identifications base on the score, number of hits and absolute mass error.

Note: before removing any identifications, make sure there are **no** tag filters applied at the Identify peptides stage.

As an **example** the following section describes how sequential filtering of the Peptide results can be performed using the following thresholds described below:

- Remove identifications with a Score less than 5
- Hits less than 2
- Absolute mass error (ppm) greater than 10

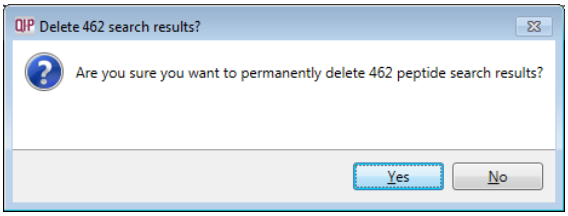
To perform these filters, on the Batch detection options panel, set the Score to less than 5, then **Delete matching search results**.



Note: the search results matching the filter criteria turn pink and the total is displayed at the bottom of the table (in this example: 462 matching out of 2867)

Note: a dialog warns you of what you are about to delete

Click **Yes**.



Then click **Reset the criteria** and enter the next criteria and repeat the process

Having applied the 3 filters there will be **1320** search results remaining

To validate the Peptide search results at the protein level select **Resolve Conflicts (bottom left)**.

Stage 7 Select Peptide ions for Spectral Library at Resolve Conflicts

This stage allows you to examine the behaviour of the identified peptides and choose to resolve any conflicts for the various peptide assignments at the protein level.

In addition to indicating the number of conflicting peptide identifications other tools have been introduced at this stage in the workflow that will facilitate the review and selection of peptide ions (Spectra) to be entered into the spectral library.

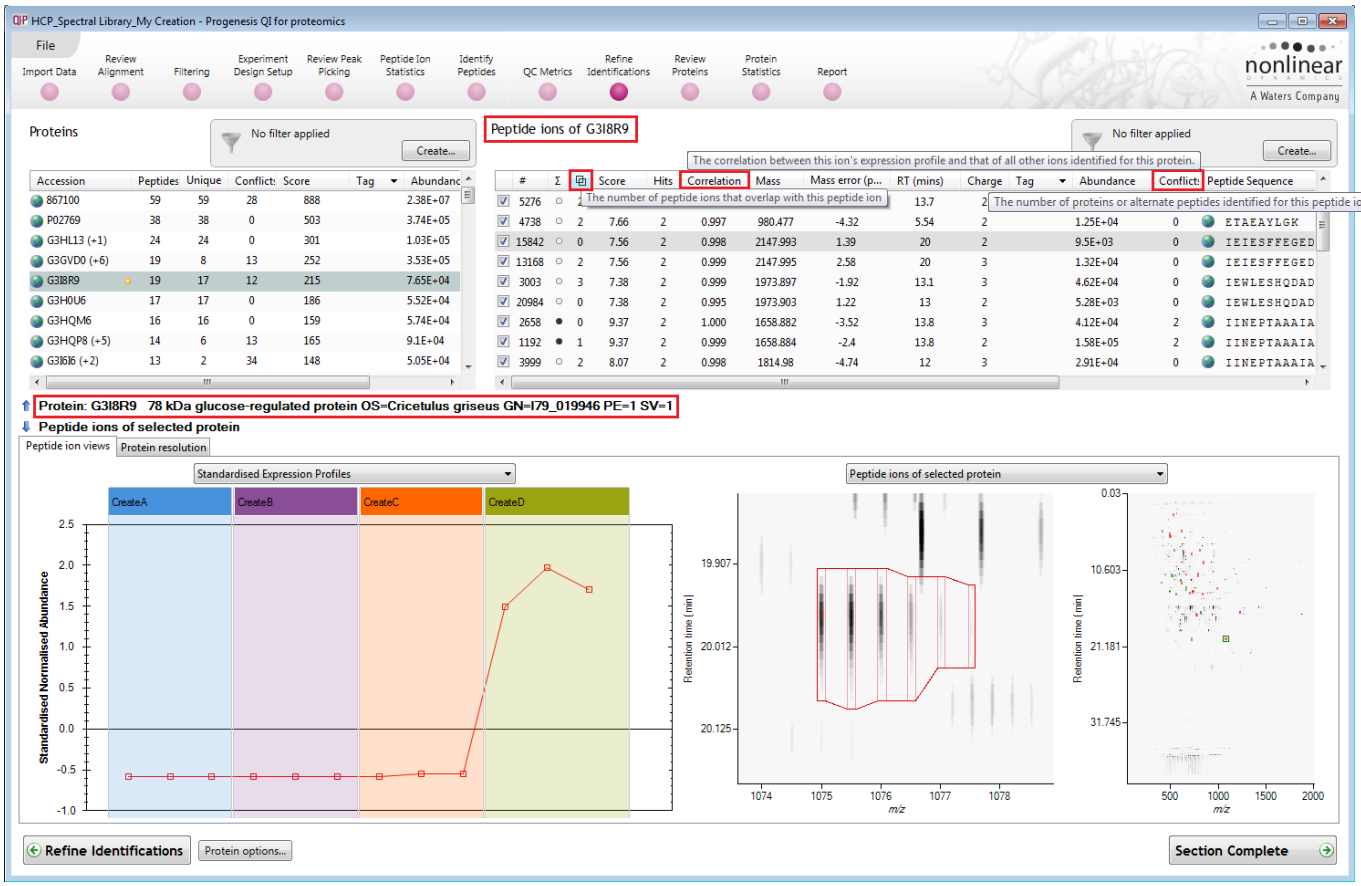
This allows you to choose spectra on the basis of:

a) Unique peptide ions, no alternative conflicting peptide identifications

b) No overlapping peptide ions

c) Correlation of the peptide ions expression profile with other peptide ions identified for the same protein

The **Resolve Conflicts** stage can be accessed at the bottom left of the **Refine Identifications** stage.



Note: If you decide to resolve all the conflicts before applying the selection strategy described below then refer to Appendix 6 (page 53)

For this tutorial, Conflict resolution was **NOT** performed.

The current protein, G3I8R9 (left hand table), has a total number of 19 peptides, 17 of which are uniquely assigned to this protein, the remaining 2 have a total of 12 conflicting protein assignments.

A possible strategy for the selection of peptide ions for the current protein is to use the tools in the peptide ion table to order and select only those peptide ions that have:

a) No overlapping peptide ions

b) No conflicts

c) Good correlation (i.e >0.925) of the peptide ion's expression with all the other ions identified for this protein

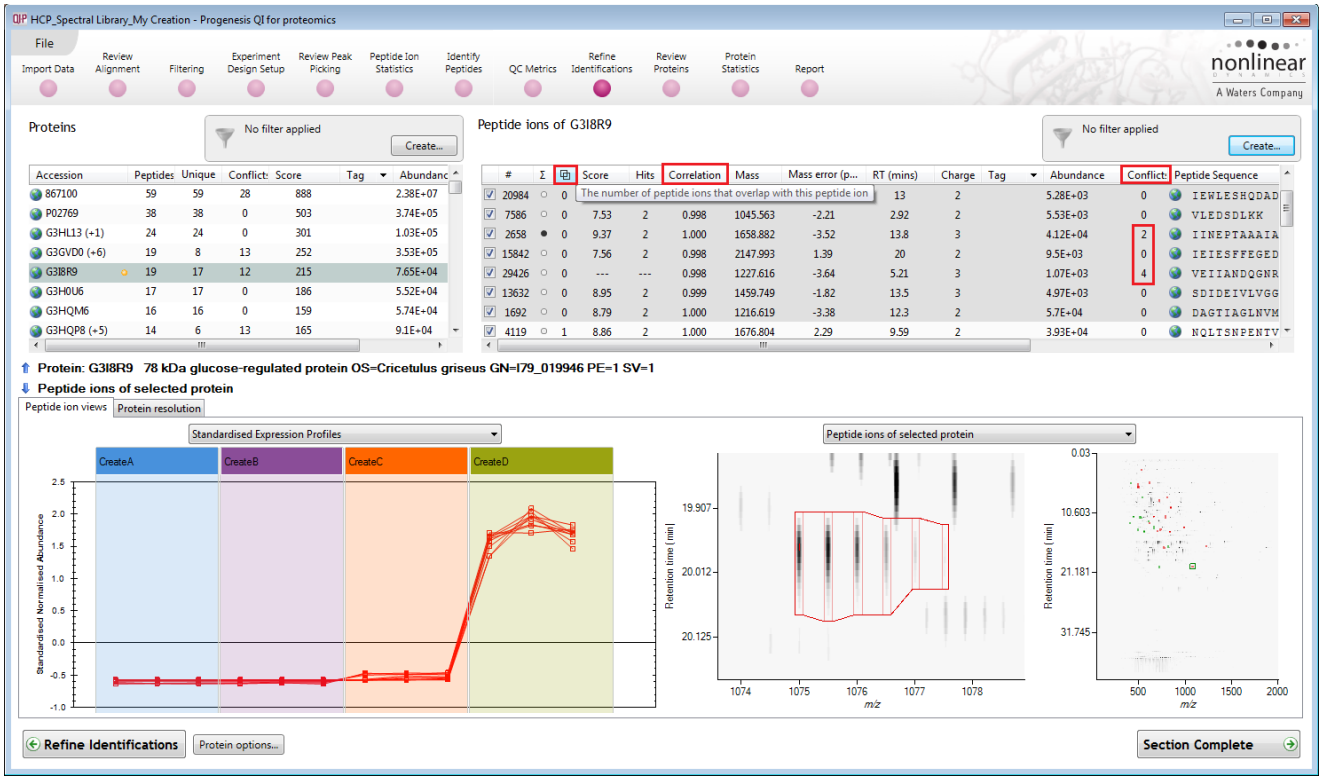
To achieve this you will make use of tagging the 'candidate' ions to be added to the library.

In this example data set we will create 2 spectral libraries:

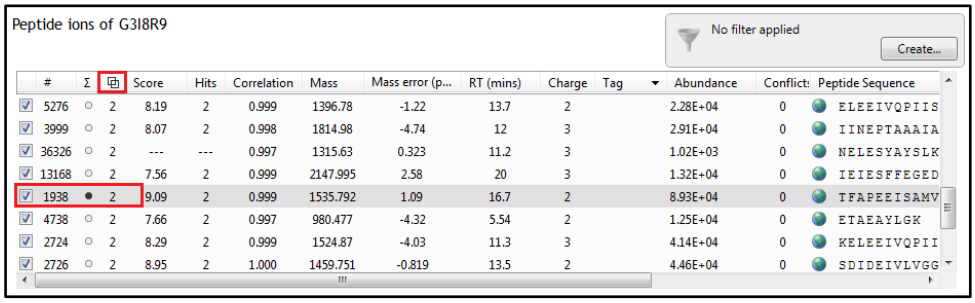
A) **HCP library_All** which will contain all the selected peptide ions for all the Host Cell proteins

B) **NIST_Product Library** which contains all the selected peptide ions for the mAb product

Starting with the HCP library_All we will continue with the G3I8R9 protein, as described above. First order the peptide ion table on Overlapping peptide ions and highlight all of the zero entries.

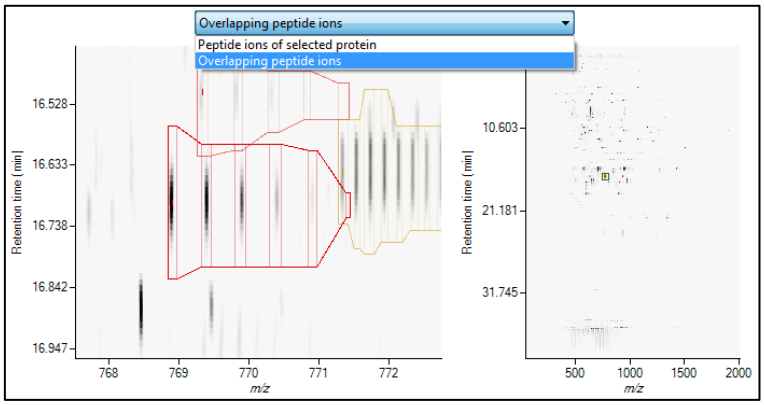


Note: the good correlation of the expression of the highlighted peptide ions as shown in the bottom left graph as well as the **Correlation** scores highlighted in the peptide ion table.

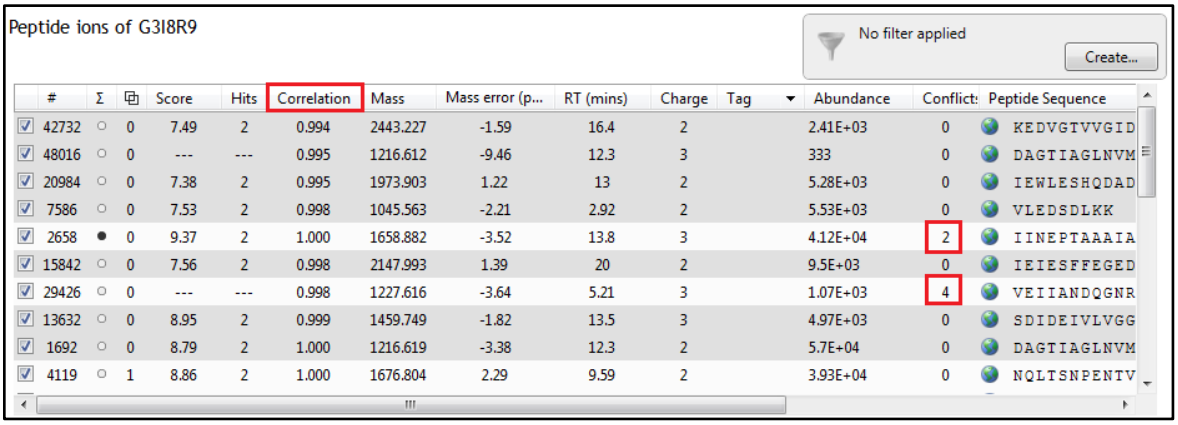


#	Σ	Score	Hits	Correlation	Mass	Mass error (p...	RT (mins)	Charge	Tag	Abundance	Conflict:	Peptide Sequence
5276	2	8.19	2	0.999	1396.78	-1.22	13.7	2		2.28E+04	0	ELEEIVQPIIS
3999	2	8.07	2	0.998	1814.98	-4.74	12	3		2.91E+04	0	IINEPTAAAIA
36326	2	---	---	0.997	1315.63	0.323	11.2	3		1.02E+03	0	NELESYAYSLK
13168	2	7.56	2	0.999	2147.995	2.58	20	3		1.32E+04	0	IEIESFFEGED
1938	2	9.09	2	0.999	1535.792	1.09	16.7	2		8.93E+04	0	TFAPEEISAMV
4738	2	7.66	2	0.997	980.477	-4.32	5.54	2		1.25E+04	0	ETAEAYLGK
2724	2	8.29	2	0.999	1524.87	-4.03	11.3	3		4.14E+04	0	KELEEIVQPII
2726	2	8.95	2	1.000	1459.751	-0.819	13.5	2		4.46E+04	0	SDIDEIVLVGG

Note: if you highlight a peptide ion with overlapping peptide ions, these can be displayed in the bottom right panel by selecting 'Overlapping peptide ions' from the drop down.



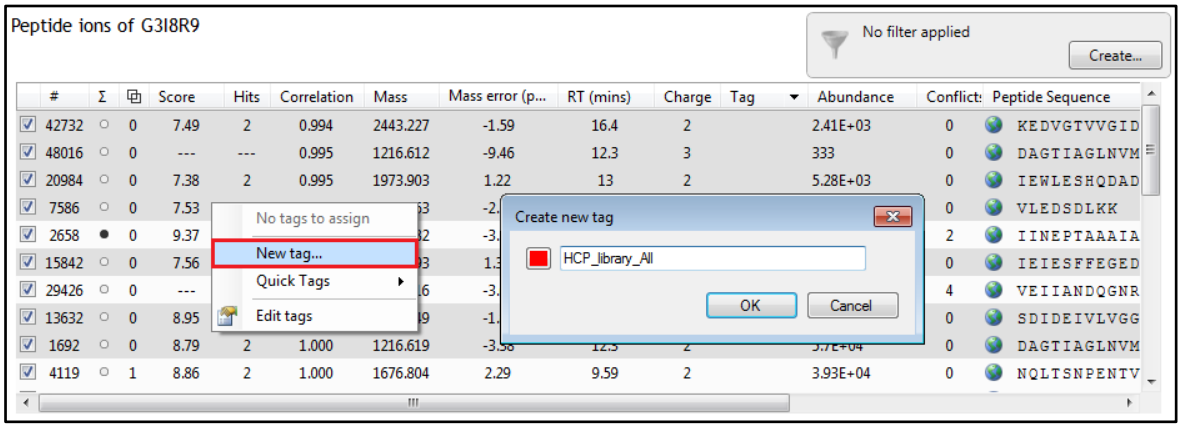
Returning to the highlighted peptide ions for protein with no overlapping peptide ions, now holding down the **Ctrl** key deselect the 2 ions showing 2 and 4 conflicts.



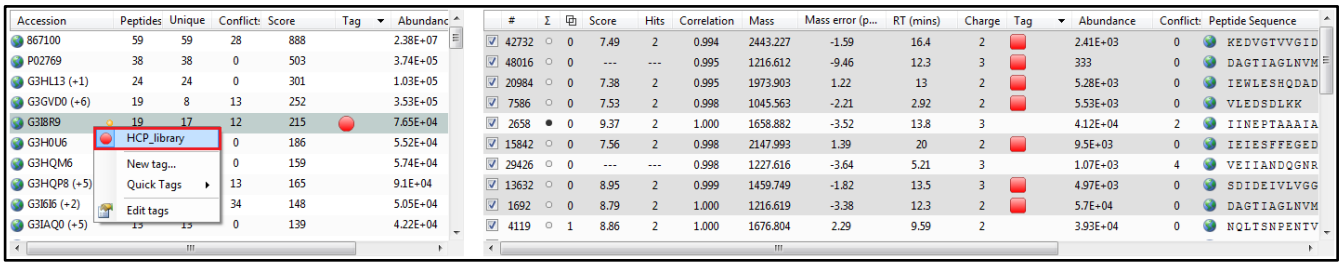
#	Σ		Score	Hits	Correlation	Mass	Mass error (p...	RT (mins)	Charge	Tag	Abundance	Conflict:	Peptide Sequence
42732	○	0	7.49	2	0.994	2443.227	-1.59	16.4	2		2.41E+03	0	KEDVGTVVGID
48016	○	0	---	---	0.995	1216.612	-9.46	12.3	3		333	0	DAGTIAGLNVM
20984	○	0	7.38	2	0.995	1973.903	1.22	13	2		5.28E+03	0	IEWLESHQDAD
7586	○	0	7.53	2	0.998	1045.563	-2.21	2.92	2		5.53E+03	0	VLEDSDLKK
2658	●	0	9.37	2	1.000	1658.882	-3.52	13.8	3		4.12E+04	2	IINEPTAAAIA
15842	○	0	7.56	2	0.998	2147.993	1.39	20	2		9.5E+03	0	IEIESFFEGED
29426	○	0	---	---	0.998	1227.616	-3.64	5.21	3		1.07E+03	4	VEIIANDQGNR
13632	○	0	8.95	2	0.999	1459.749	-1.82	13.5	3		4.97E+03	0	SDIDEIVLVGG
1692	○	0	8.79	2	1.000	1216.619	-3.38	12.3	2		5.7E+04	0	DAGTIAGLNVM
4119	○	1	8.86	2	1.000	1676.804	2.29	9.59	2		3.93E+04	0	NQLTSNPENTV

This leaves 7 remaining peptide ions, with no conflicts and no overlapping peptide ions all showing good correlation of their expression profiles.

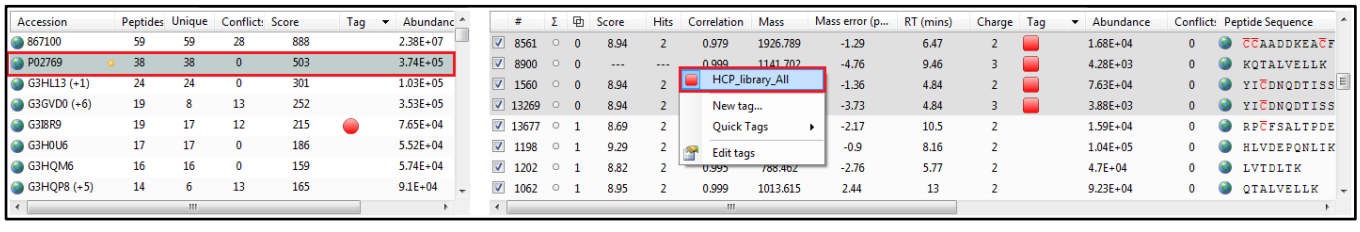
Now create a new tag for these peptide ions by right clicking on the highlighted ions, select **New tag** and call it HCP_library_All



To keep track of which proteins you have reviewed, create a protein tag (HCP_library) for the left hand table.

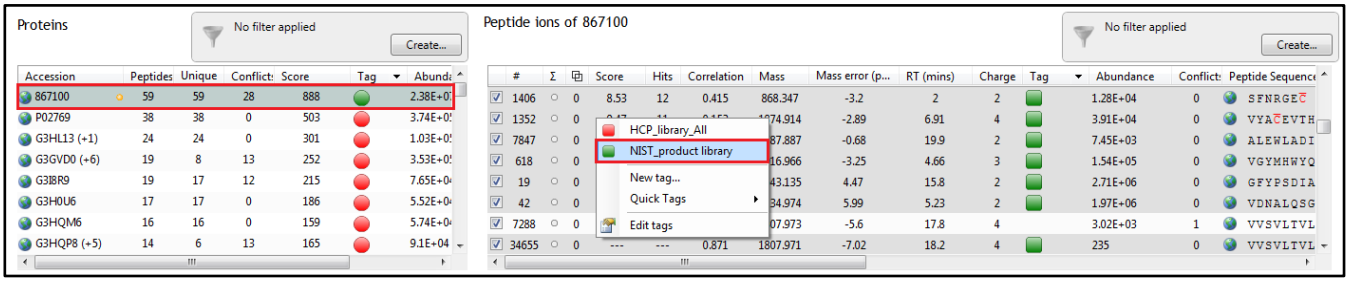


Now work down the list of proteins (ordered on Peptides) in the Left hand table, highlight in turn all the 'non overlapping' peptide ions (in the peptide ion table). Then while holding down the **Ctrl** key (to retain the selection) click on the peptide ions with conflicts to un-highlight them. Finally **right** click on the remaining highlighted peptide ions and select the **HCP_library_All** tag.

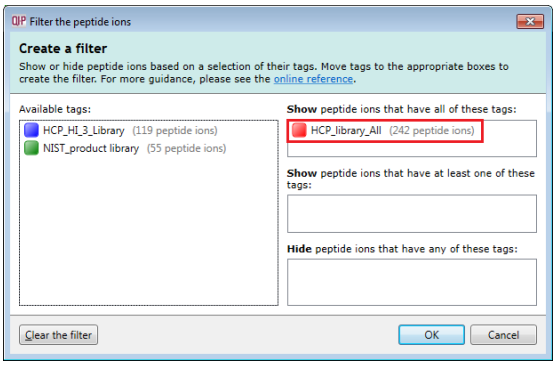


Repeat this process for all the HCP proteins with un-conflicted peptide ions.

Now highlight the NIST mAb protein, in this case the one with the greatest number of peptides, right click and tag it (NIST_Ab_Product). Then in the Peptide Ions table order on overlapping peptide ions and highlight all those with no overlaps. Un-highlight those with Conflicts. Then right click on the remaining highlighted peptide ions and create the tag **NIST_product library**.

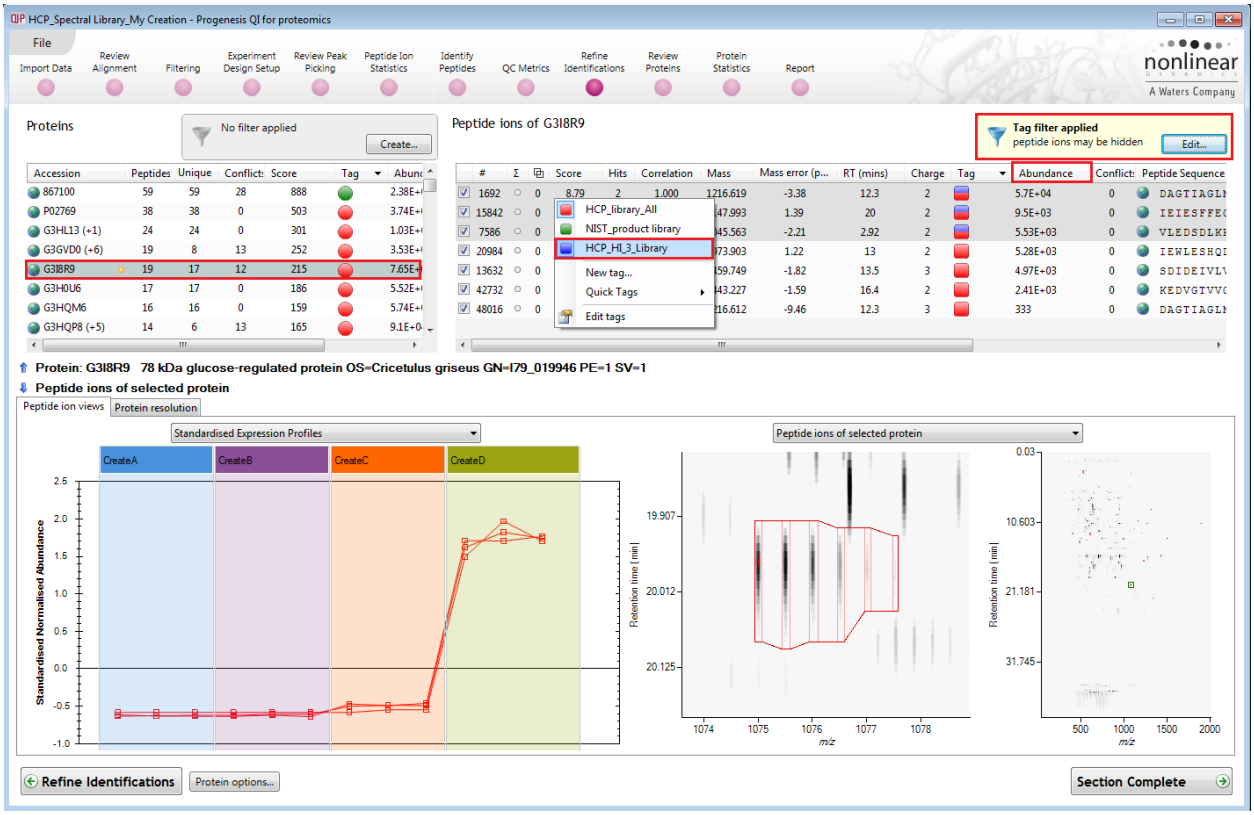


Note: at this stage, in addition to the tagged groups described above you **can** also create a very conservative library by only tagging the 3 most abundant peptide ions, assigned to different sequences that have no overlapping peptides, no conflicts and good correlation of their expression profiles.



To do this first apply a Filter so that only the Peptide ions that are tagged **HCP_library_All** are shown in the Peptide Ions table

For example returning to the G3I8R9 protein, order on Abundance and highlight the 3 most abundant peptide ions with different sequences



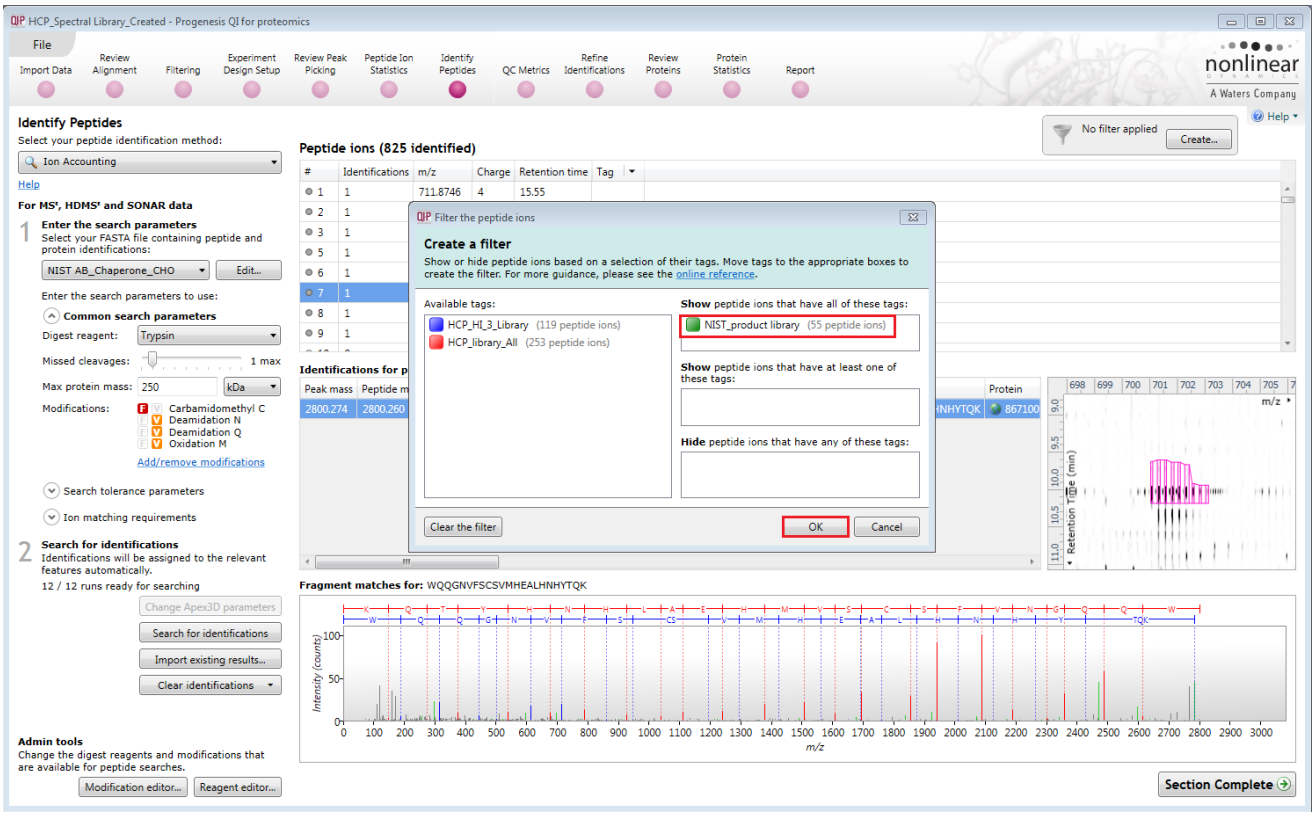
Now, before actually creating the Spectral Libraries from the tagged groups of peptide ions you can review the Quality of fragmentation for each 'candidate' spectra being entered into the library and add additional tags to indicate quality of fragmentation. This then allows to easily control the creation and addition of spectra to a library through application of tag filters.

Stage 8 Review fragmentation quality of candidate spectra

Once you have tagged your candidate spectra at the Resolve Conflicts stage you can now return to the Identify Peptides stage and review the quality of fragmentation for each peptide ion, before adding it to the spectral library.

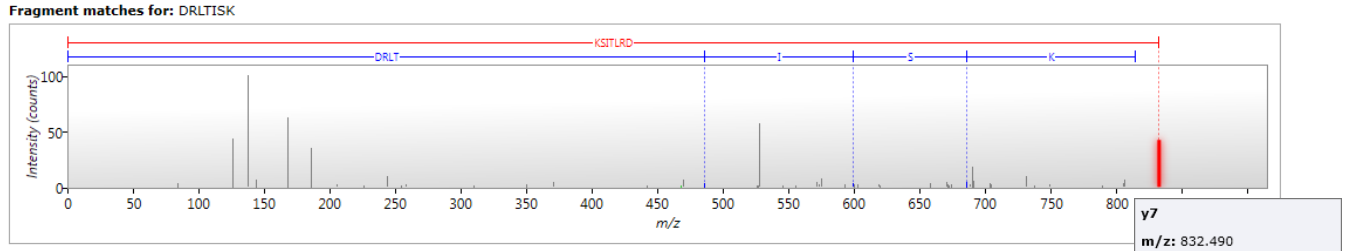
Note: the following section describes one 'possible strategy' on how to decide which spectra to add to the spectral library on the basis of the number and quality of fragment matches. It outlines the processes involved **but** it should be clear that it is by no means definitive as there will always be an element of subjectivity, quality is not an 'exact metric'.

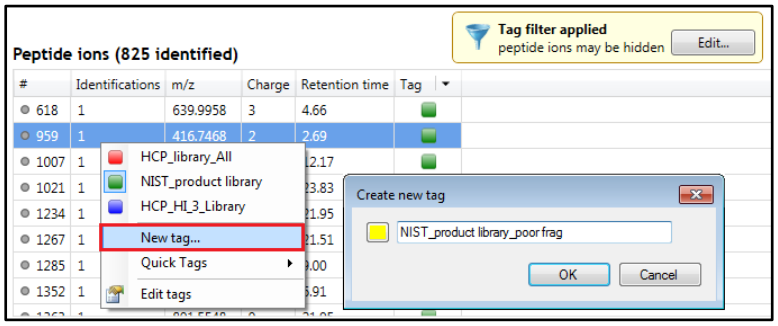
First select the group of candidate spectra to review i.e. start with the NIST_product library



Before going through the filtered list of tagged peptide ions for the NIST Ab product you should first create a new tag to attach to all the spectra that are judged poor in terms of fragmentation or no matches.

For example: for the fragmentation of DRLTISK, shown below, there is only a single match for the y (in this case y 7 representing the un-fragmented precursor) and only 4 b ions matched.



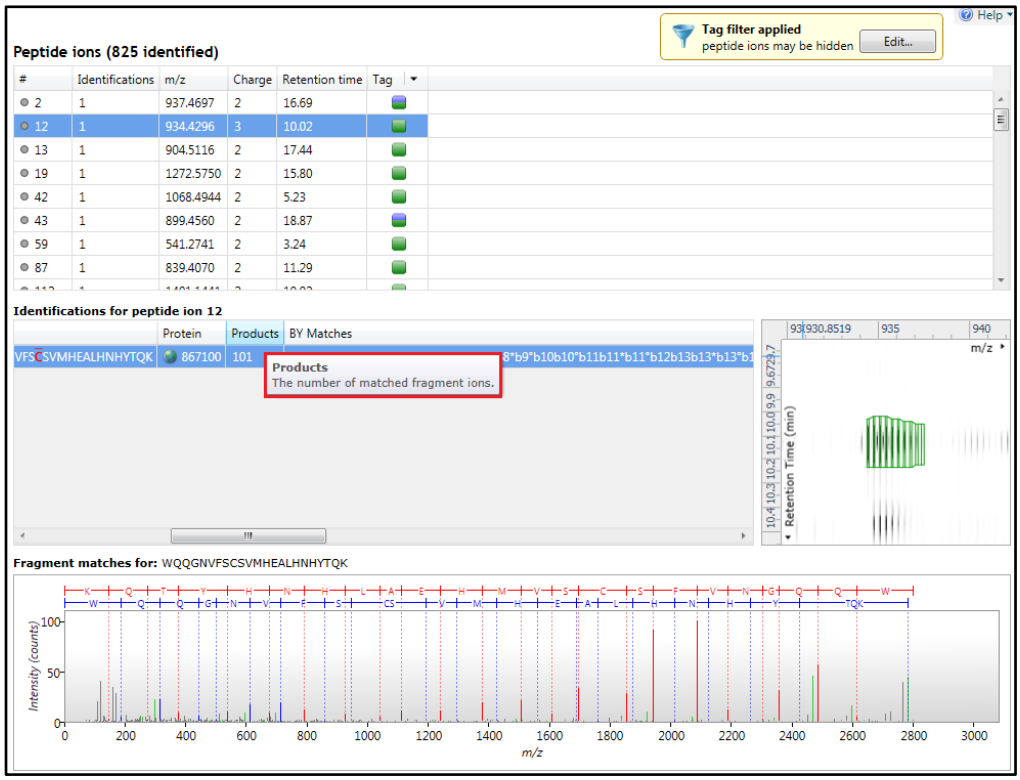


To tag this 'poorly fragmented peptide ion' right click on the ion in the table and create a new tag

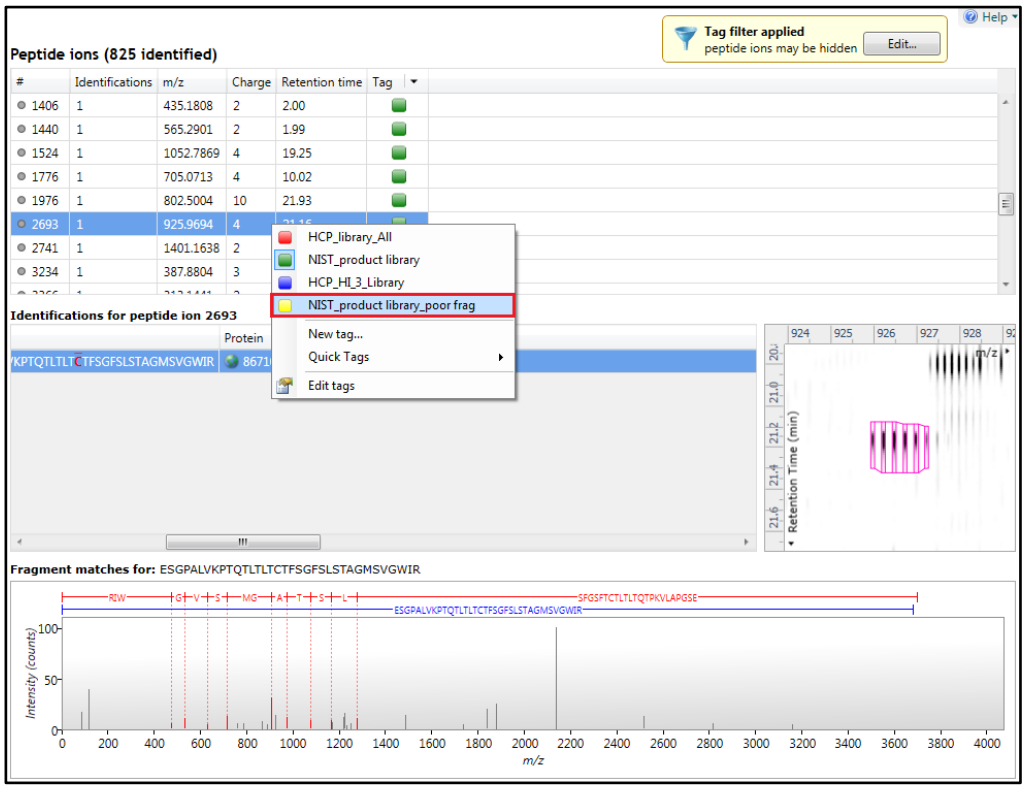
NIST_product library_poor frag

Return to the top of the filtered list of Peptide ions, then step through the list tagging the ‘poorly fragmented ions where there is a low number of Fragment matches, poor coverage of the peptide ion sequence or no matched fragments (due to inherited identification from another charge state).

Example of good fragmentation matches, leave untagged.

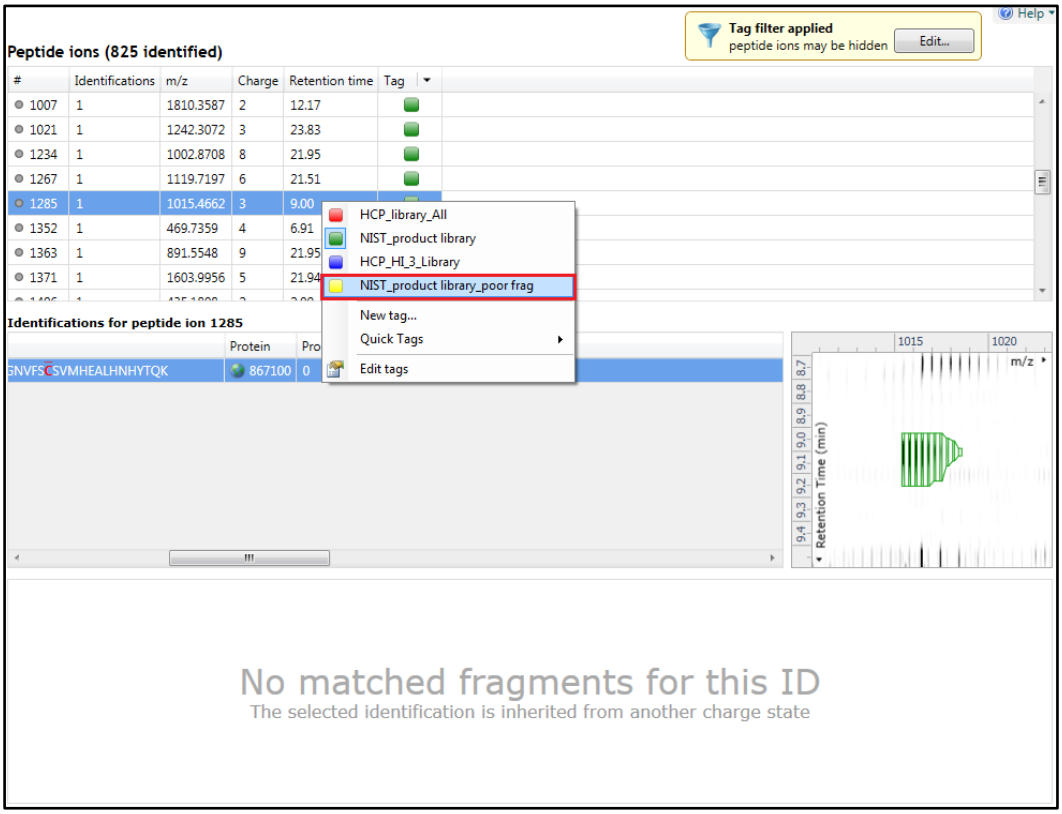


Example of poor fragmentation matches, right click on the peptide ion in the table and select the NIST_product library_poor frag



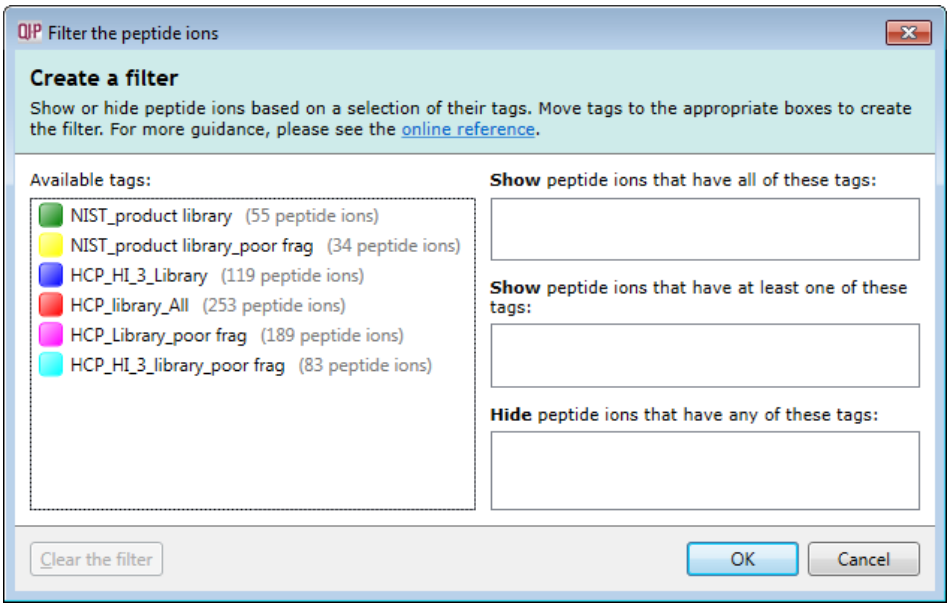
Here there is not only limited Fragment matches for y ions but also only the matching of the precursor ion.

Finally where you have initially tagged peptide ions, where the identity has been inherited from another charge state, this will show no Fragment matches. At this point, the option would be not to have tagged it in the first place, when you were creating the original tagged list as described in the previous section.



Once you have completed the review process of the NIST_product library then repeat the process for the remaining 2 candidate libraries (HCP_library_All and as required HCP_Hi_3_Library) creating additional tags for the corresponding poor fragmentation (i.e. HCP_library_poor frag).

Having done this you will have 6 tagged groups of peptide ions as shown below.



Note: the actual numbers will vary depending on how the spectra were reviewed.

Now move forward to the next section to see how these tags can be used to create Spectral Libraries.

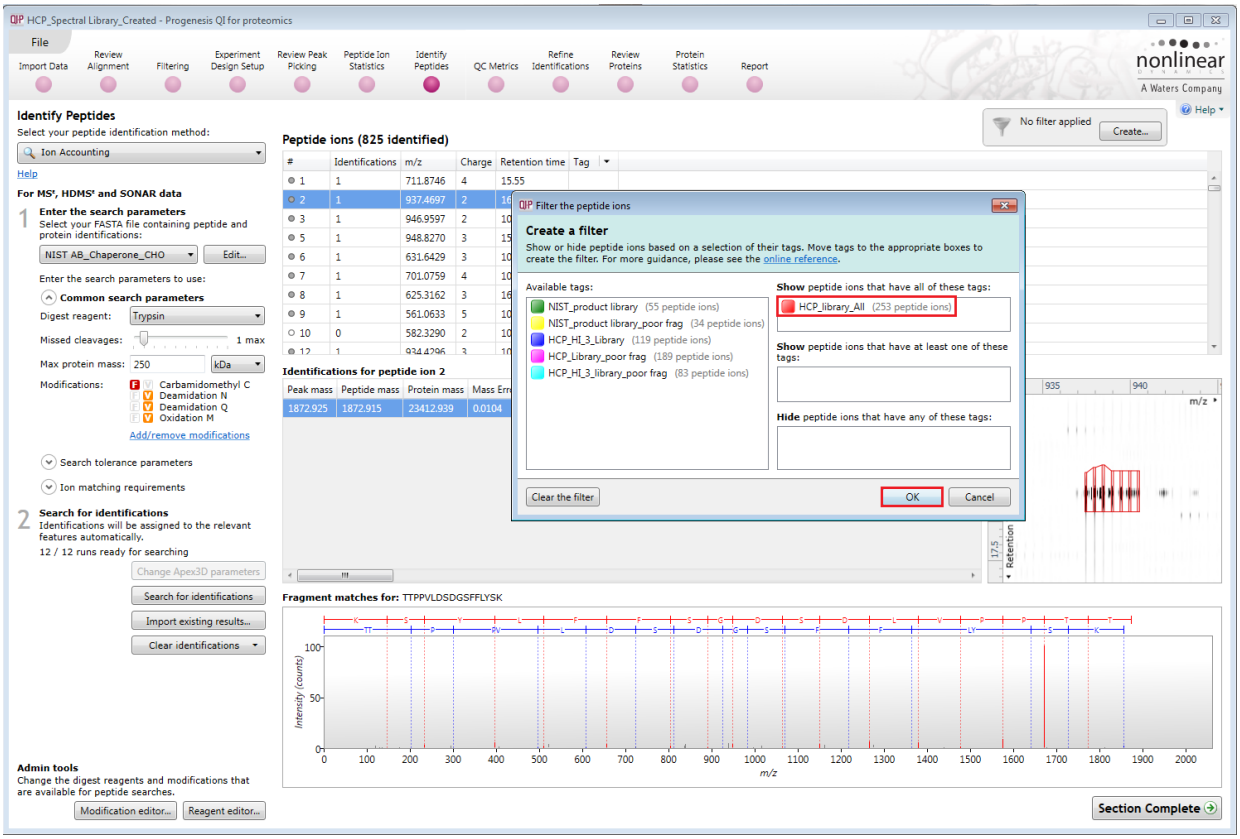
Stage 9: Creating a Spectral Library

Having tagged and reviewed the 'candidate' lists of peptide ions in the previous section you can now create the actual libraries by first filtering to the required list of peptide ions and then exporting them to a Spectral library.

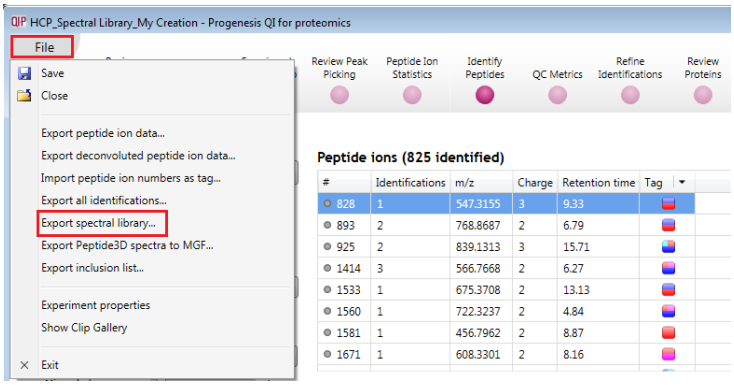
Note: if you have come straight to this point in the workflow and you have already unzipped the **Library_search_data_set.zip** into same folder then examples of the spectral libraries will already exist as described below

As an example, create two Spectral Libraries firstly a Spectral Library called **HCP_library_All_raw** which is based on all peptide ions tagged **HCP_library_All** and a second library **HCP_library_All_reviewed** is the same as the first library minus the spectra judged as having poor fragmentation.

Click on **Create** and drag the **HCP-library_All** tag on to the Show panel and click OK. This will reduce the table to showing only those peptide ions which have this tag.



To create the library from the tagged filter group, select **Export spectral library…** from the file menu

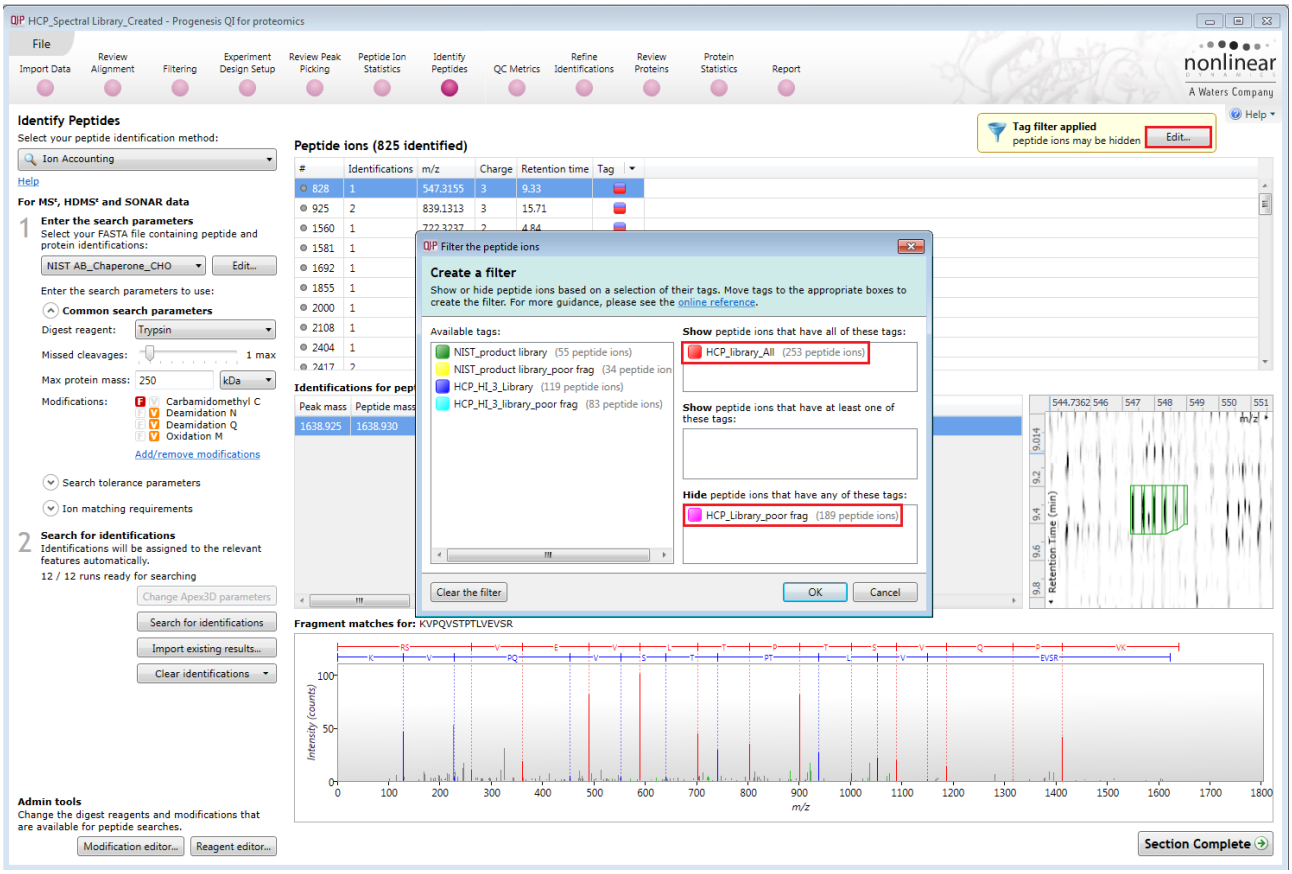


Then save the new spectral library as **HCP_library_All_raw.msp**

Now create the **HCP_library_All_raw_reviewed** spectral library.

First modify the applied tag filter to hide the spectra that have been judged as poorly fragmented.

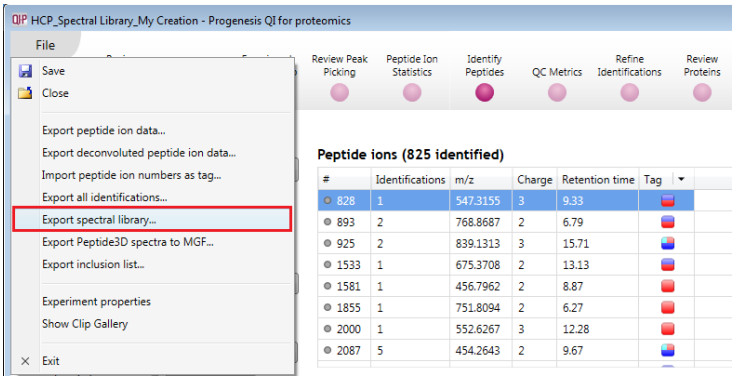
Click on **Edit** to open the existing Tag filter. Then drag the tag HCP_Library_poor frag on to the **Hide** panel while leaving the existing tag in the **Show** panel



This will further reduce the number of tagged peptide ions in the table.

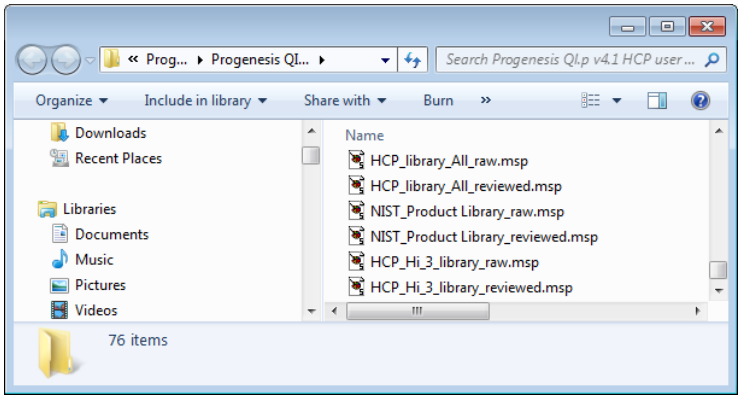
As before to create the library from the tagged filter group, select **Export spectral library…** from the file menu

Then name and save the library, **HCP_library_All_reviewed**.msp



Tip: if saving the library to the same folder as the example library files, use a different name if you wish to retain both libraries.

Now clear the filter by clicking on **Edit** followed by **Clear the filter**, then repeat the process for the **NIST_product library** and the **HCP Hi_3_Library.**

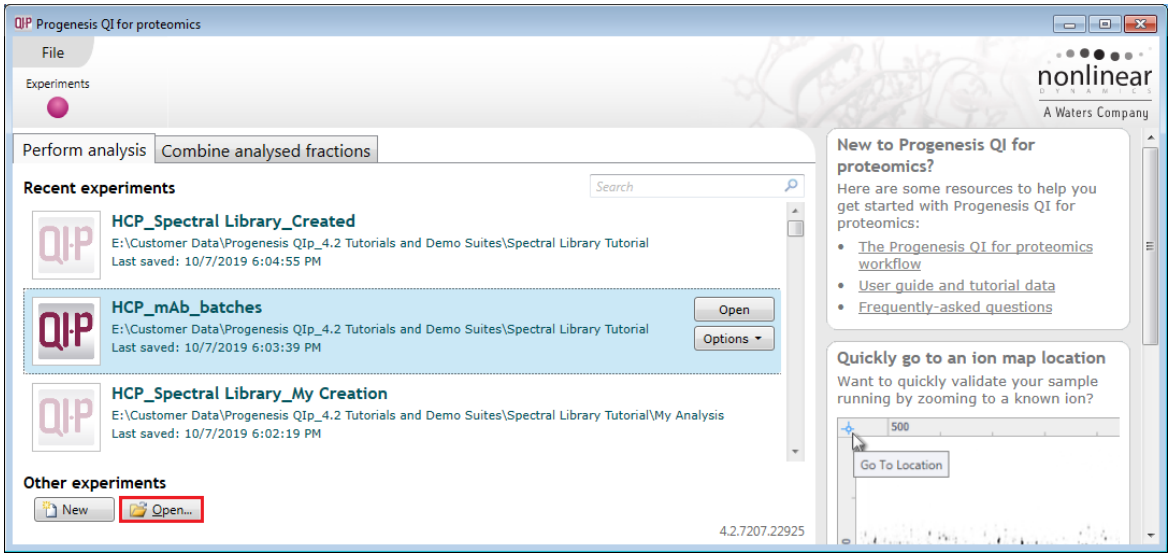


Once you have done this you will have 6 .msp files that you can use for searching the Batch samples described in the next section.

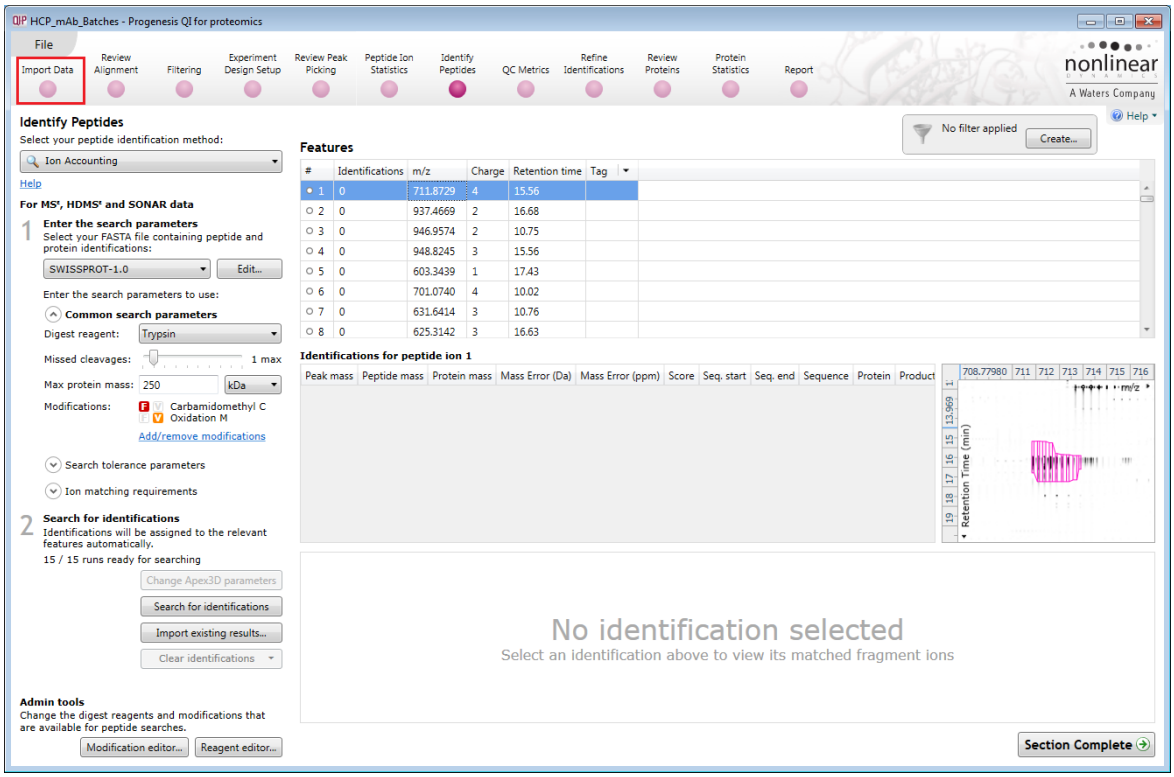
Note: Examples of these Spectral Libraries are provided with the Tutorial data that you downloaded to perform this tutorial.

Stage 10: Autoprocessing of the Batch Samples

As an example of using the spectal library you have just created use it to identify and measure the Host Cell Proteins (HCP) present in the **HCP_mAb_Batches** of NIST Ab product. To do this use the second tutorial archive that you restored at the beginning of this tutorial**.**



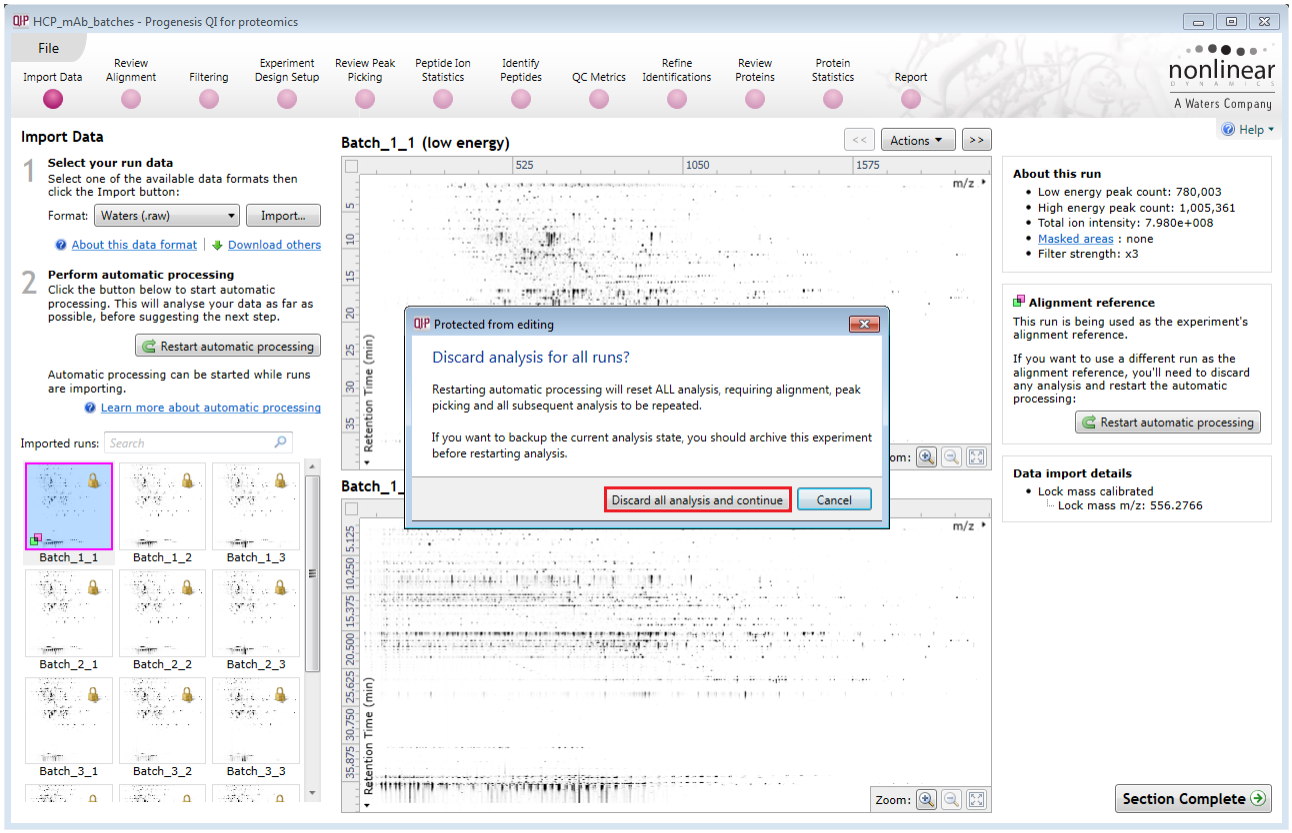
When you open the restored **HCP_mAb_Batches** experiment it will be already at the Identify Peptides stage but no identification has been performed.



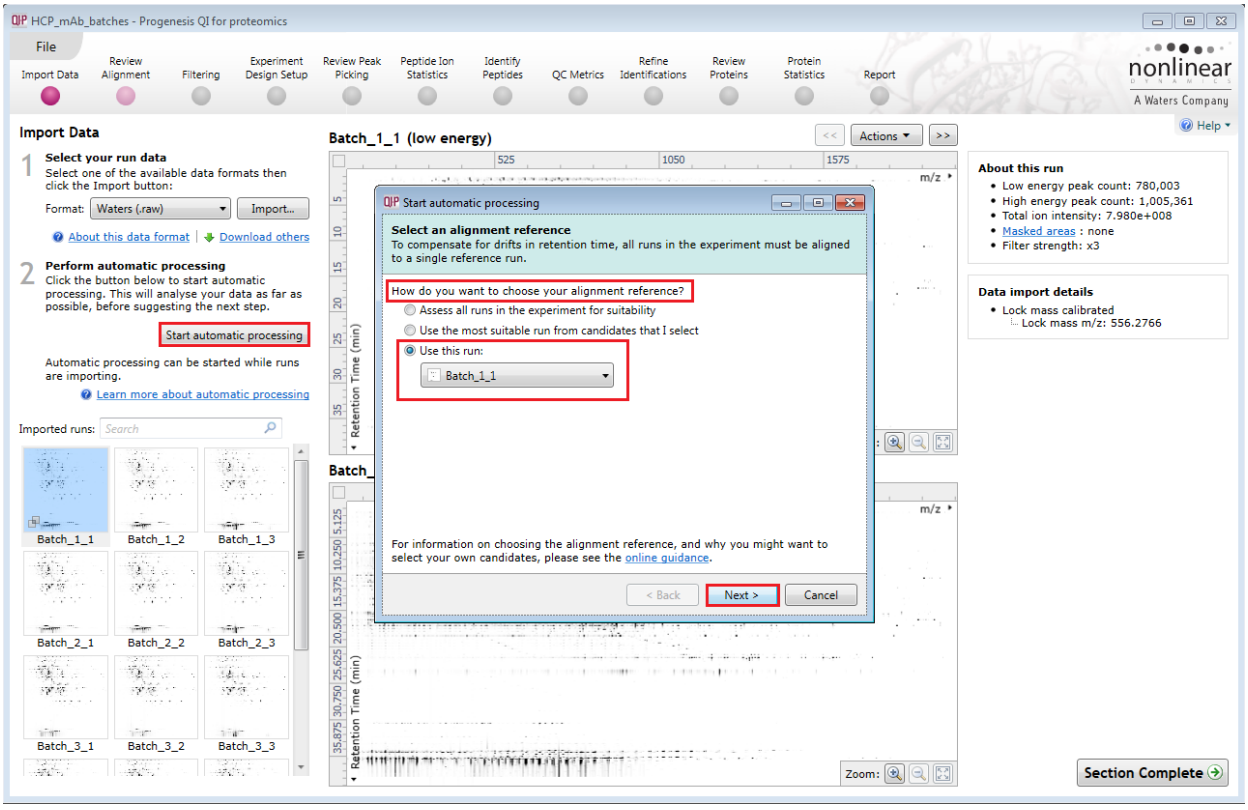
Note: the data has been autoanalysed and an Experiment Design applied as part of this automatic analysis.

To perform Identification using the Spectral Libraries that you made in the previous section go to Stage 11 (page 32)

The following pages describe the automatic analysis that has been performed on this data which you can repeat by first discarding the automatic analysis by clicking **Restart automatic processing.**



On clicking **Discard all analysis and continue** the first Automatic processing dialog will open asking how you want to choose the alignment reference to be determined

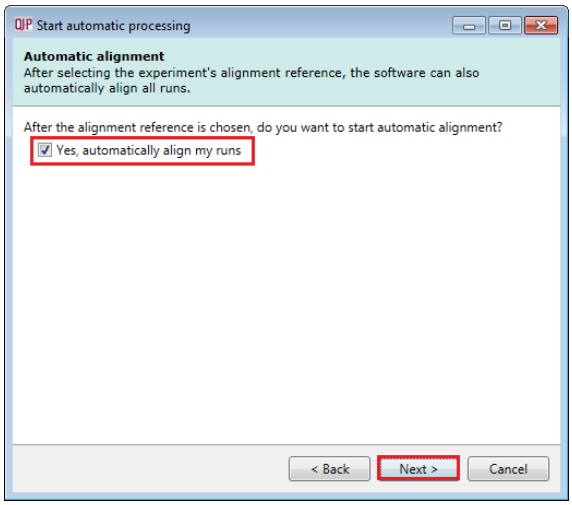


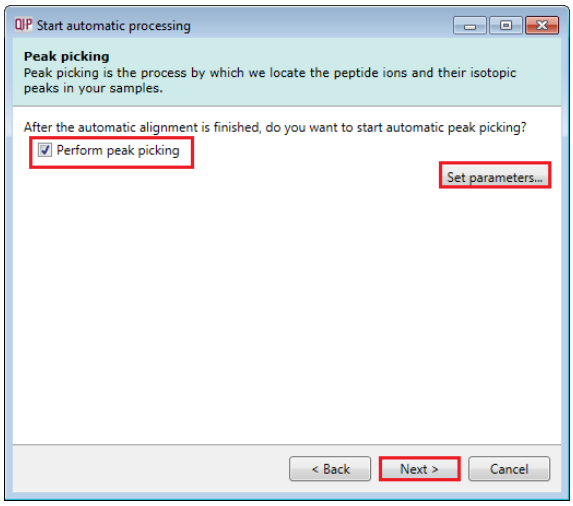
The list below describes what options were applied at each stage to automatically analyse the Batches data set.

Setting up the steps for the auto processing:

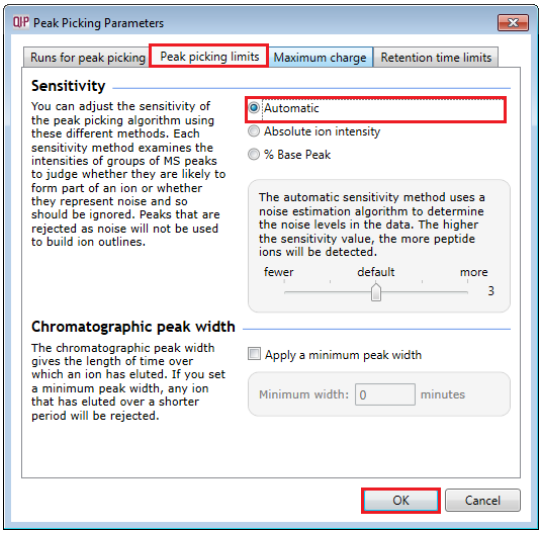
The Alignment Reference was set as ‘Batch 1_1’ (as shown above).

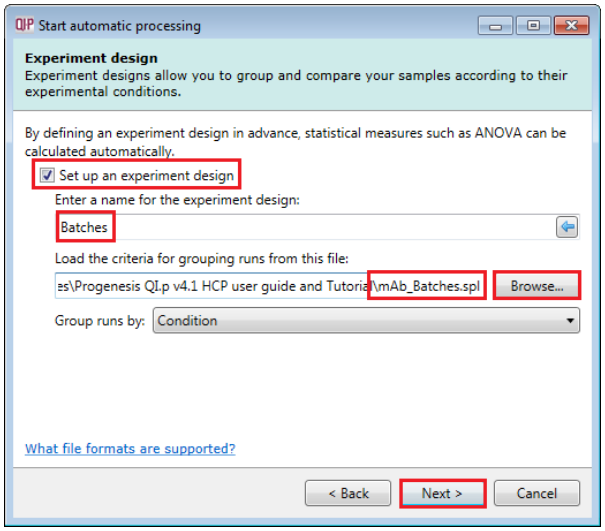
Automatic alignment was performed and the default settings for peak picking.



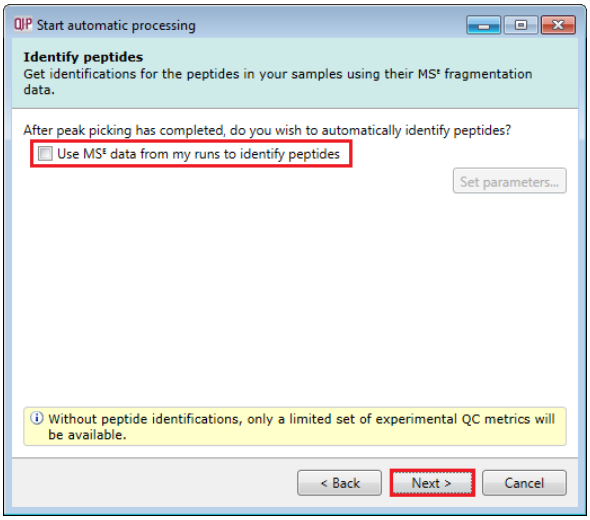


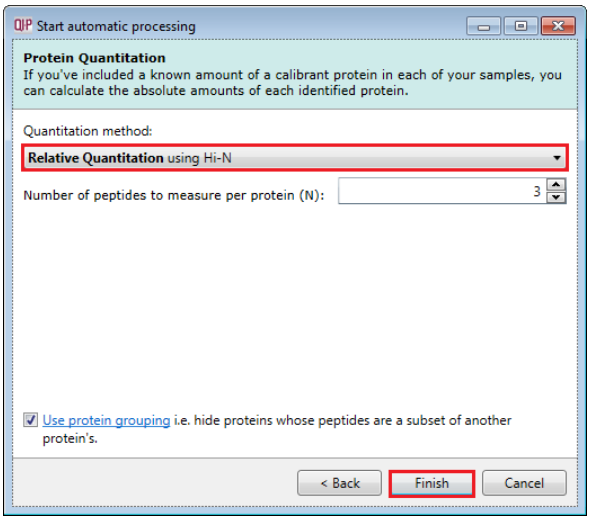
The default Peak picking limits are set as Automatic (as shown below)





For Experiment design setup: use file mAb_Batches.spl (available in same folder that the **Library_search_data_set.zip** was extracted.



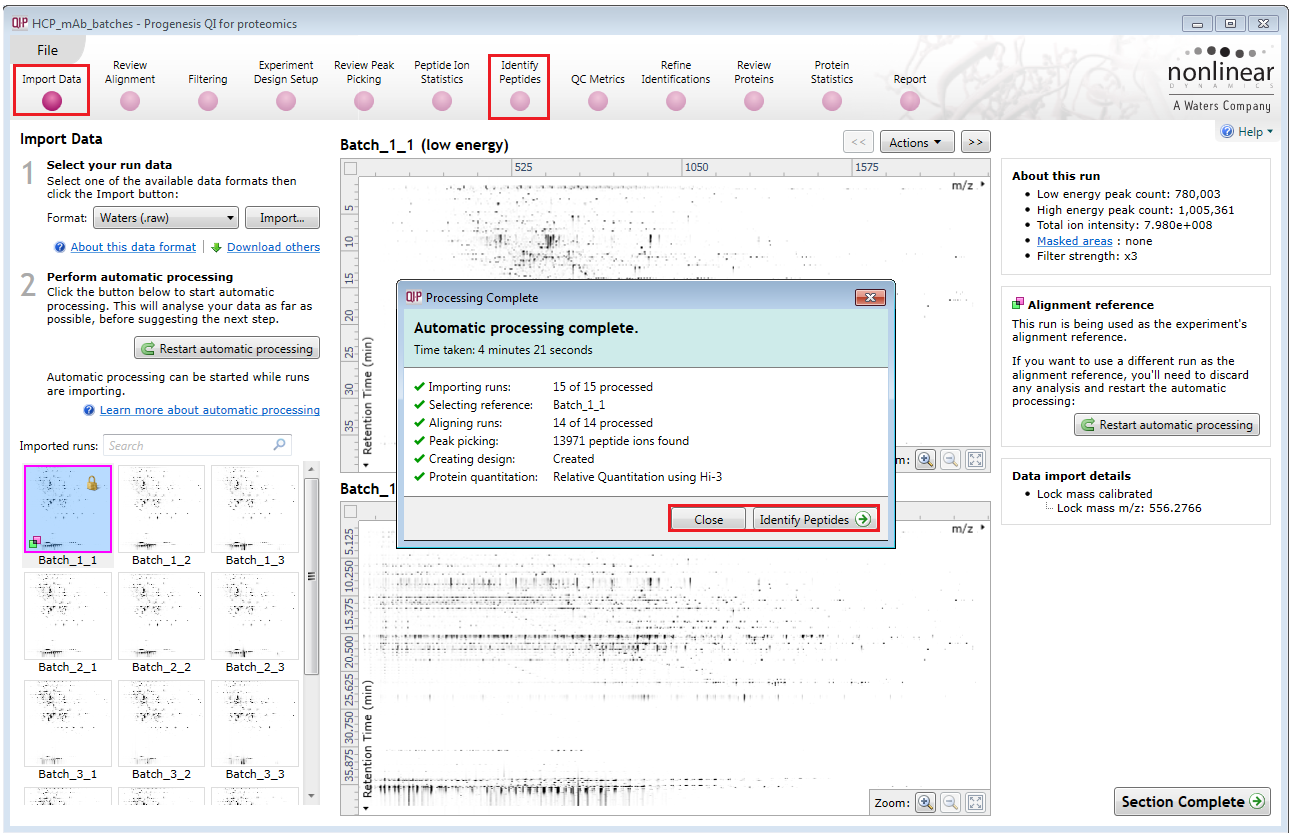


Do not perform identification by deselecting **Use MS^E data…….. untick this option**

Use default settings for Quantitation (Relative Quantitation using Hi-N) and Use protein grouping

Click **Finish** to start the analysis.

The automatic processing completes in approx. 5min (depending on your machine spec).



Having repeated the analysis you can either **Close** it, where it will remain at the Import Data stage and you can step through the Alignment and other stages or you can open it directly at the **Identify Peptides** stage.

Here we will close and then click on **Review Alignment** on the workflow at the top of the screen.

Note: the high quality of retention time alignment of the runs as indicated by the high % Score.

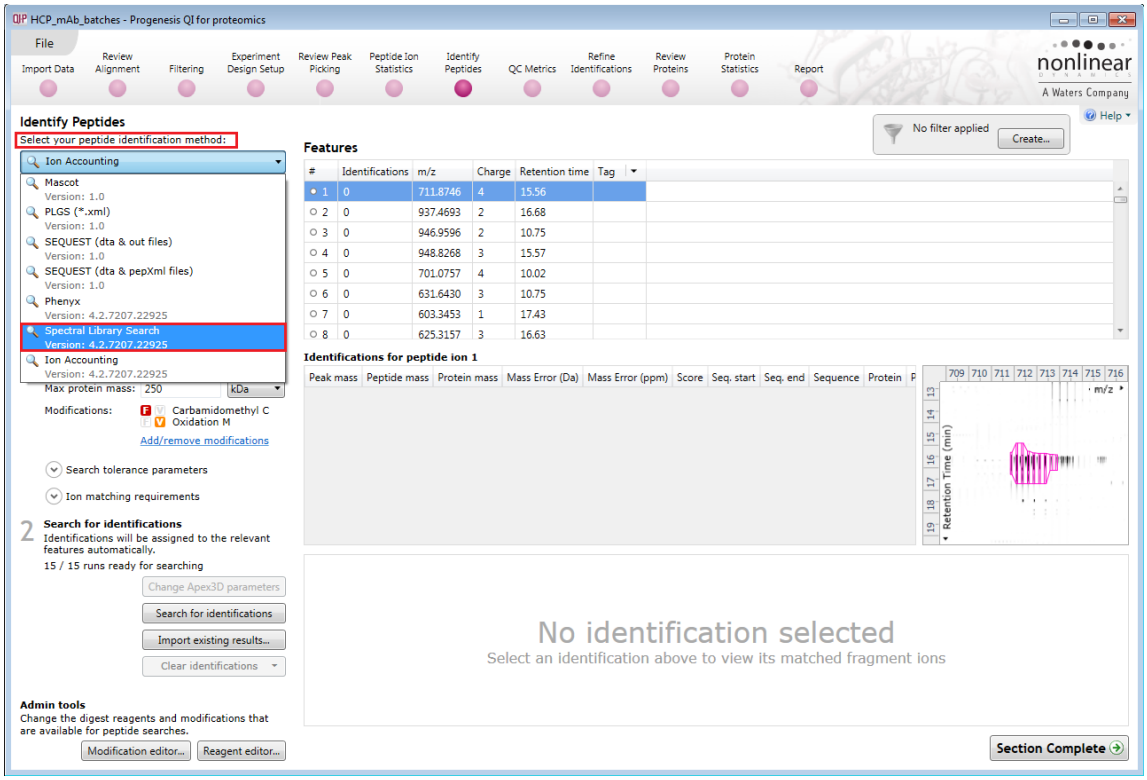
Now click on Identify Peptides and go to the next section.

Stage 11: Identifying and measuring HCP proteins using a spectral library

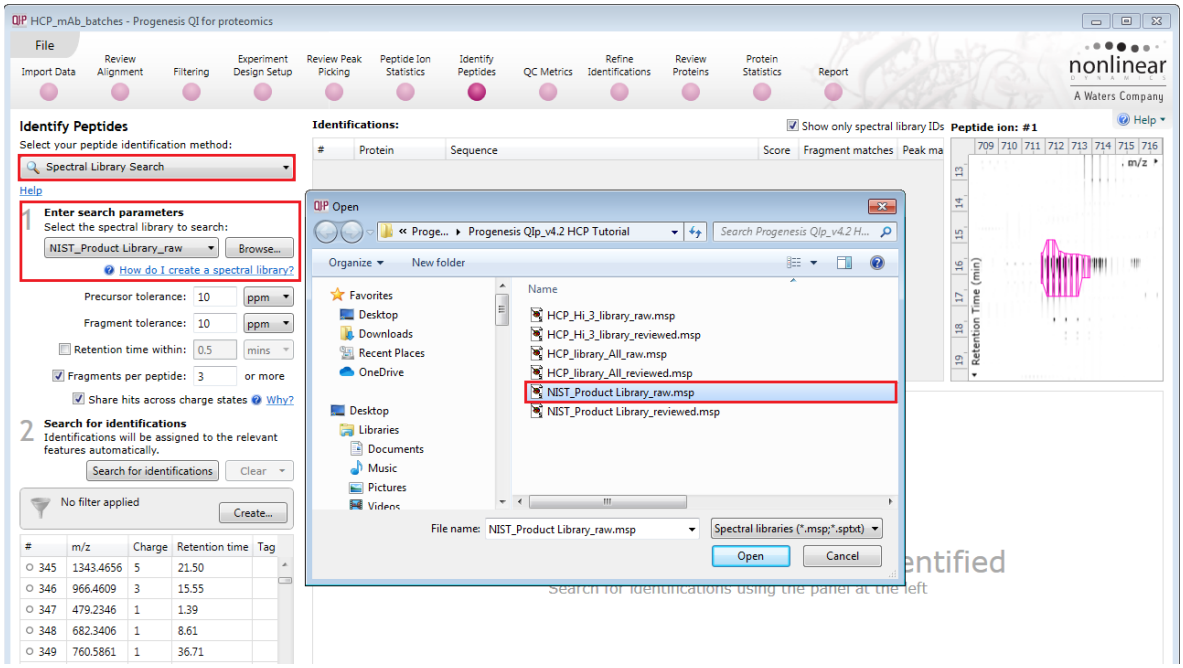
As an example of using the spectral libraries you have just created use it to identify and measure the Host Cell Proteins (HCP) present in the **HCP_mAb_Batches** of NIST Ab product. To do this use the second tutorial archive that you restored at the beginning of this tutorial.

If you have just reanalysed this you will be currently at the Identify Peptide stage in the workflow.

Select Spectral Library Search from the peptide identification methods

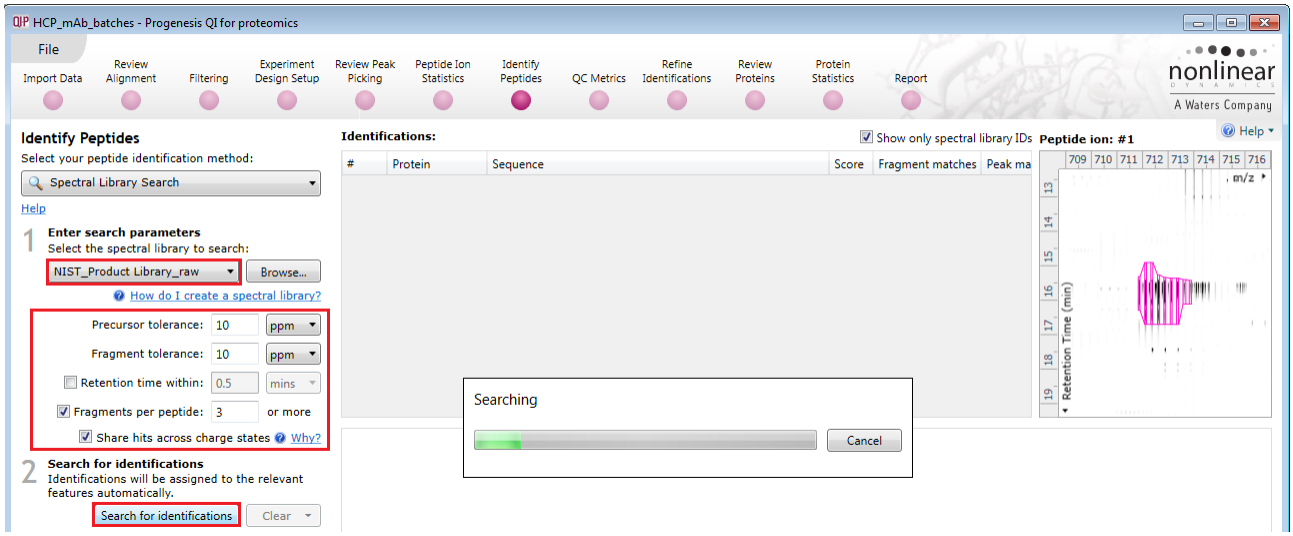


As no searches have been performed there will be no libraries to select. Click **Browse** and select the **NIST_Product Library_raw.msp** from the folder you restored the Tutorial data.



Now check the tolerances as shown below

The **NIST_Product Library_raw** will appear as the current spectral library



The Spectral library search parameters are described below

Precursor tolerance

Specifies the mass error below which a precursor is considered a match to a spectral library entry.

Fragment tolerance

Specifies the m/z error below which a fragment is considered a match to a spectral library entry's fragments.

Retention time within

Specifies the retention time error, below which a precursor is considered a match to a spectral library entry. If this is not selected, or a spectral library entry has no retention time information, it has no effect.

Fragments per peptide

The minimum number of fragment matches required for a match to be accepted. If not selected, all matches are accepted, even those with no fragment matches. If a spectral library entry has fewer fragments than the given value, all of its fragments must match. If a spectral library entry has no fragments, matches to that entry will be accepted regardless of this value.

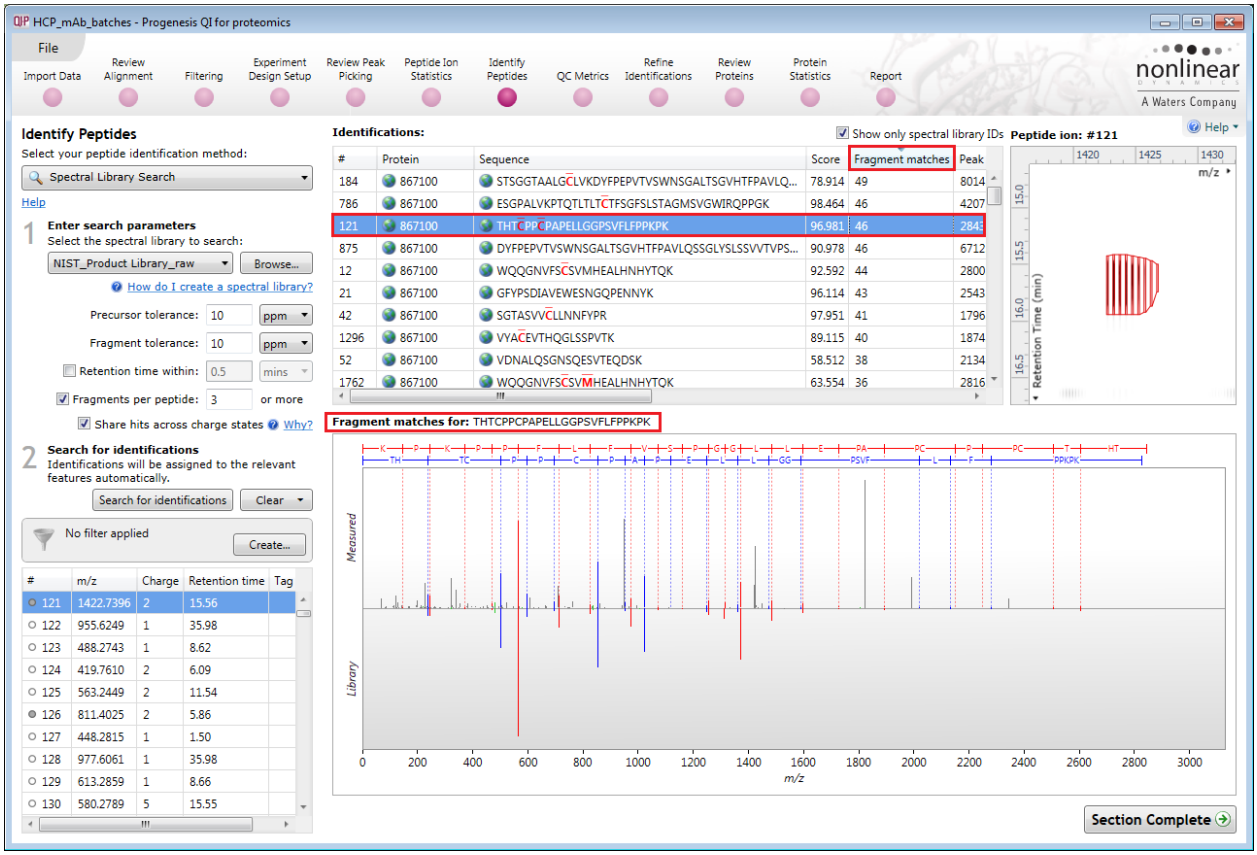
Share hits across charge states

If selected, charge state deconvolution of identifications will be performed. This means that if only some charge states of a given peptide were identified by the spectral library search, the other charge states will inherit the same identifications.

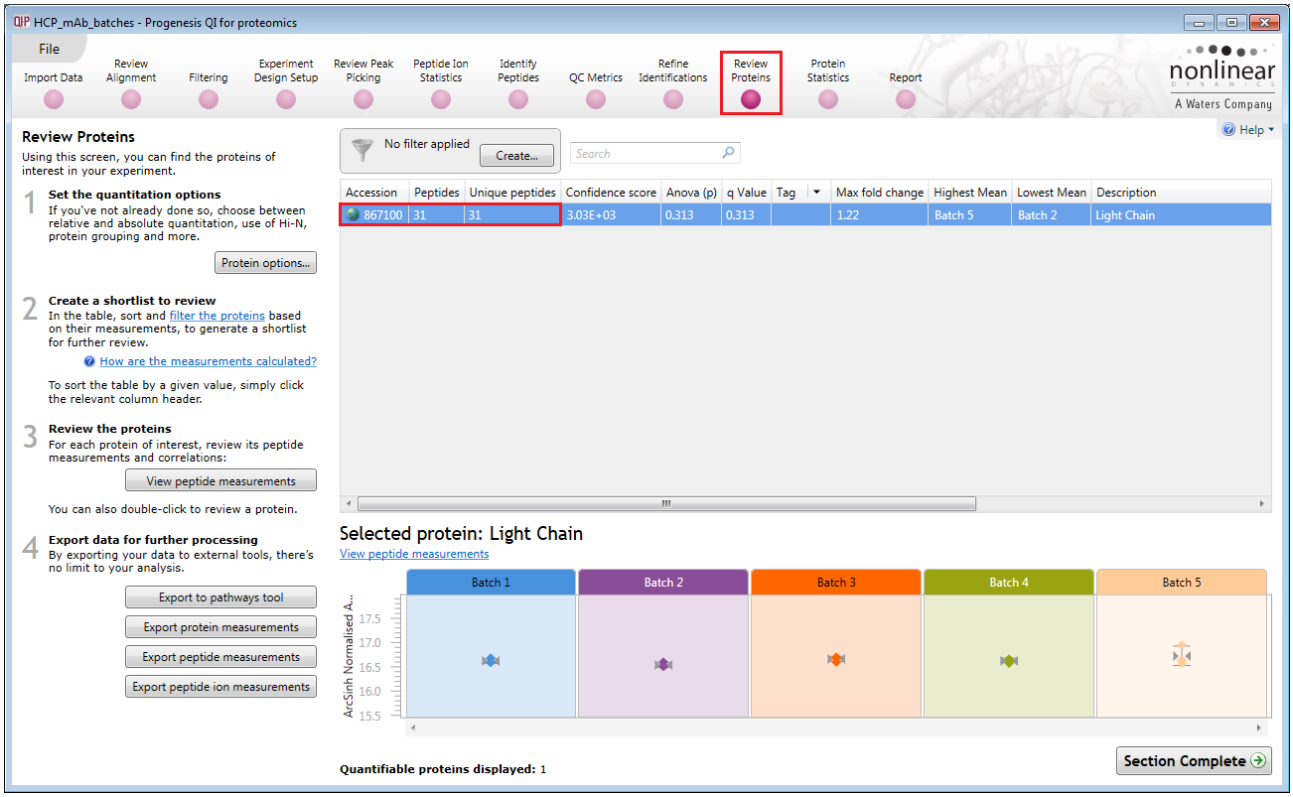
Tick the tolerances as shown above

Start the search by clicking **Search for identifications**

When the search completes order the table on Fragment matches, and select an example in the list. The 'mirror' plot will now display the fragment matches for the current peptide sequence showing the Measured and Library b and y ions.



Now click on **Review proteins** on the workflow to examine the quantified proteins. As the Product library only contained the spectra for one protein (867100) then only one protein is displayed with 31 Peptides.

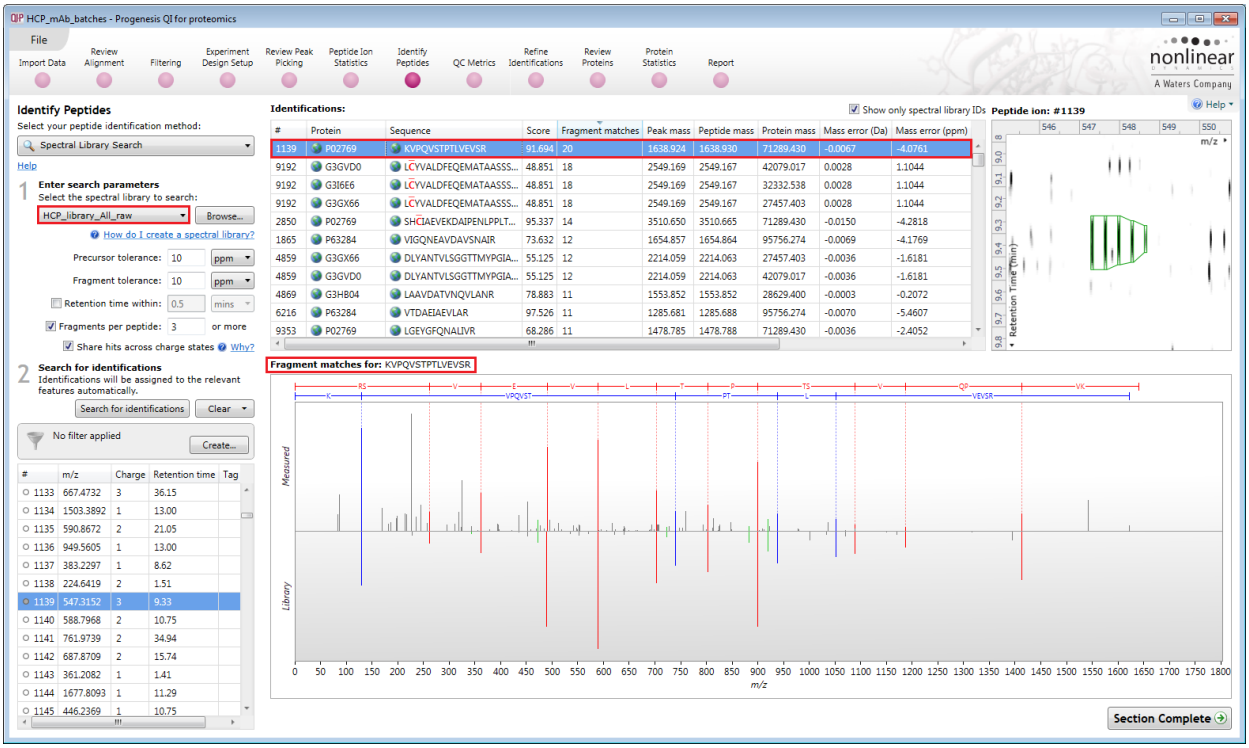


The protein quantitation (options) are currently set to Relative Quantitation based on the 3 most abundant peptides (Hi-N).

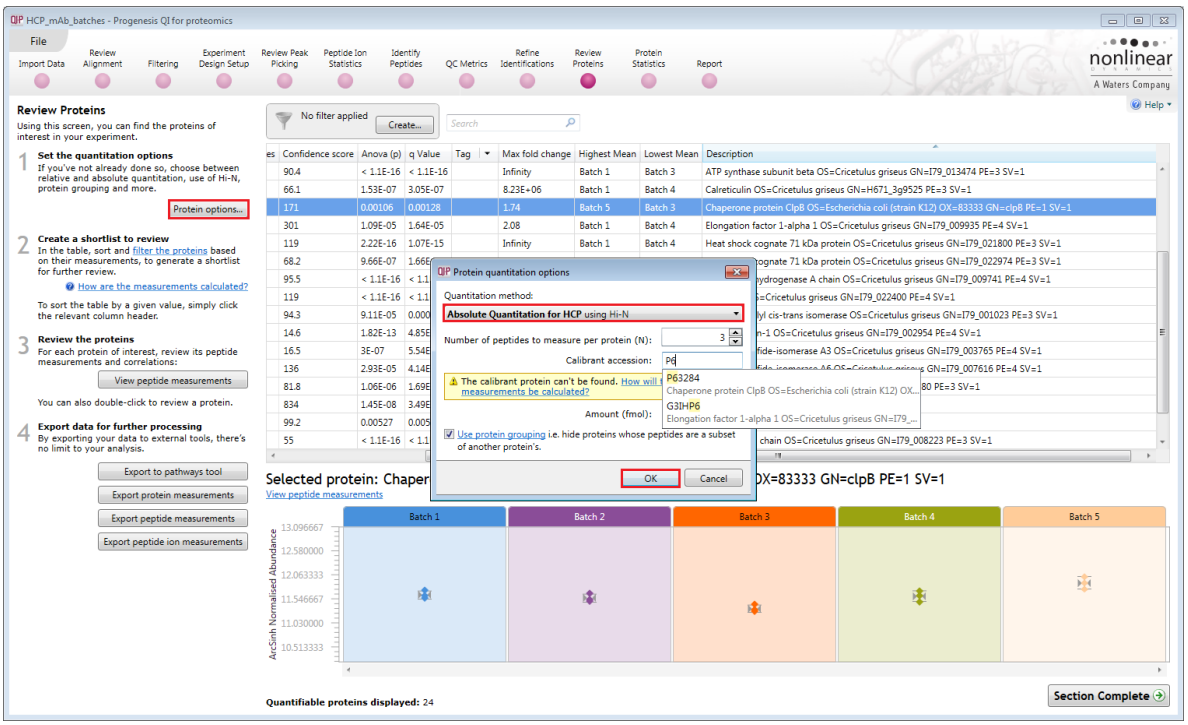
Note: having searched using the NIST_ product Library_raw you can set the normalisation to use only the peptide ions for this protein, as the same amount of this protein is present in all the batches, refer to page 15 for details on how to change the normalisation).

Now use the **HCP_library_All_raw.msp** to search the batches by first browsing for the new library then

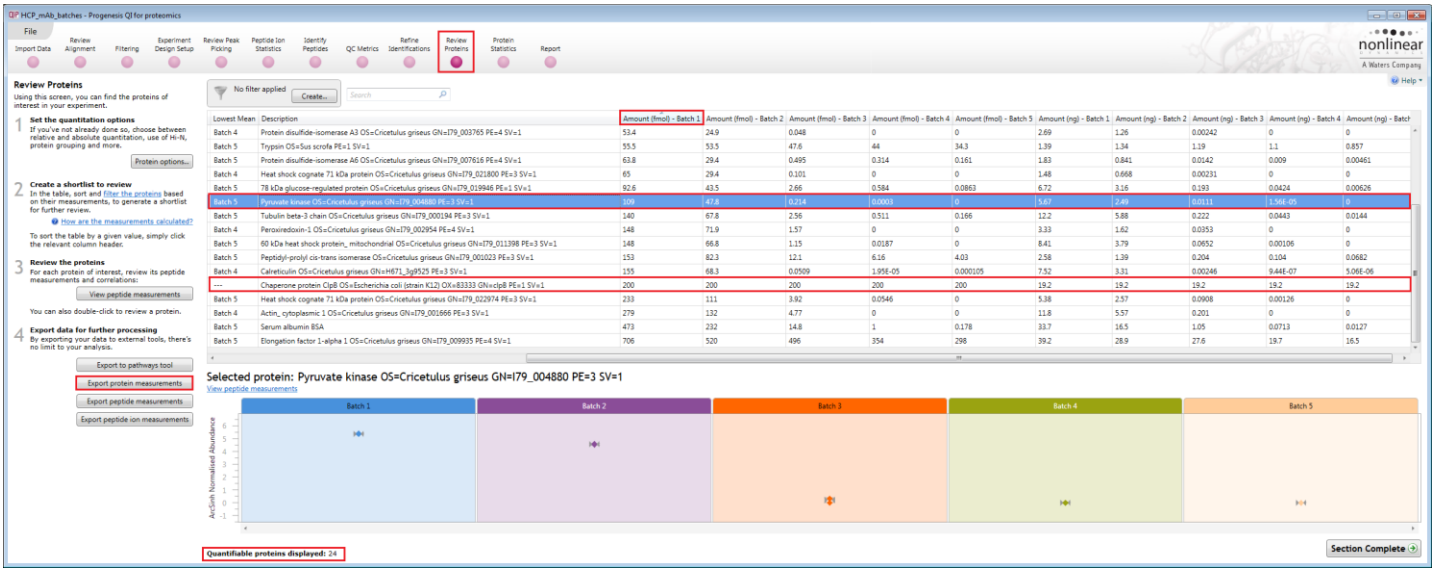
Click **Search for identifications**



Change the method for Protein Quantitation to **Absolute Quantitation for HCP** using Hi-N, by clicking on **Protein options** and use the peptides identified as belonging to the Chaperone Protein ClpB from E.coli, that was spiked into each sample at 200fmol, as the Calibrant (Accession P63284).

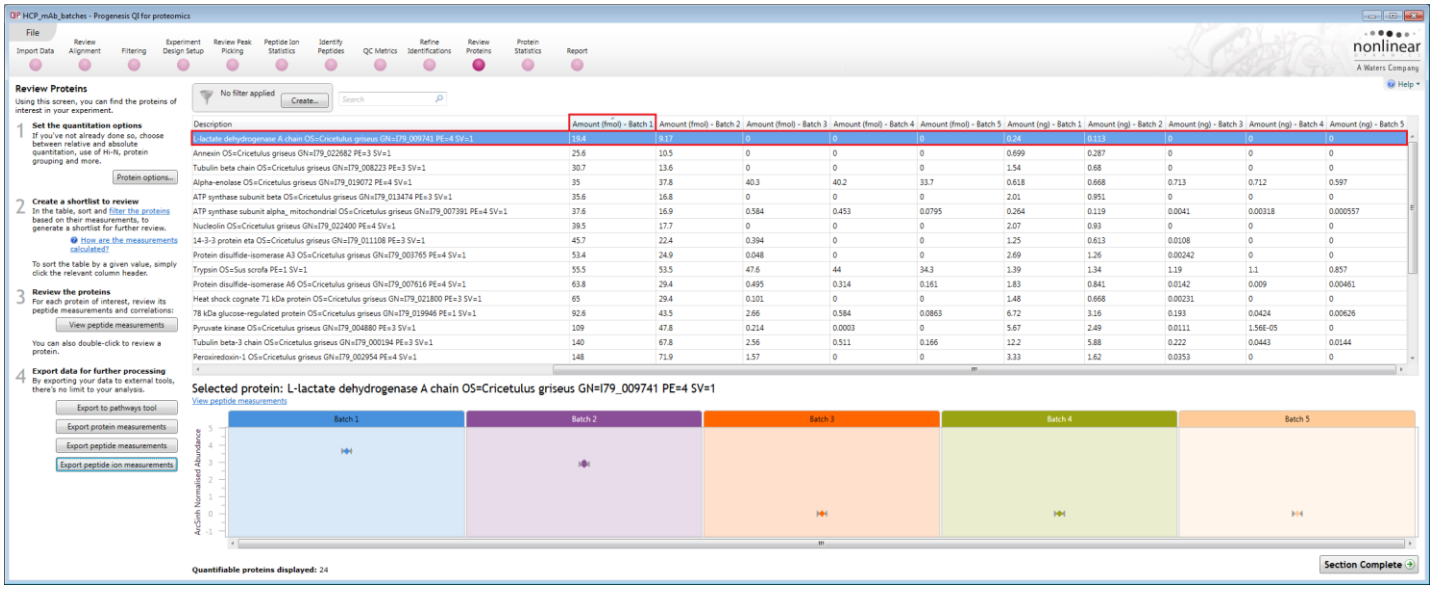


There are 24 quantifiable proteins identified, across the batches that you are testing



Note: having used Absolute Quantification for HCP using Hi-N, the spiked protein (Calibrant (Accession P63284) will show the same Amount (either fmol or ng) across all Batches under test.

Now order the data at the **Review Proteins** stage, starting with Batch 1. The first protein in the list shows decreasing presence as you go from Batch 1 to 2 to essentially undetected in Batch 3.

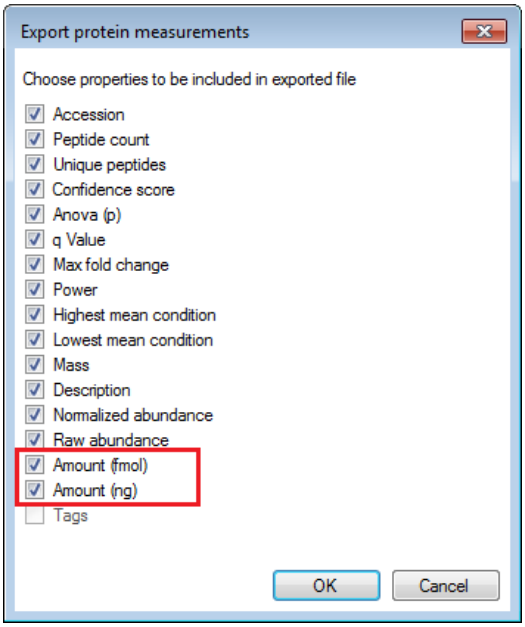


As you order on Batch 3, 5 of the proteins originally present in Batch 2 are no longer measurable in Batch 3, this rises to 13 in Batch 5.

Protein measurements are exported as .csv the content being dependant on the choices made on the dialog shown to the right.

You can export these measurements to Excel by clicking on **Export Protein Measurements**

Alternatively, right click on the table and select **Add to clip gallery…** (refer to Appendix 7 (page 59) for more details)



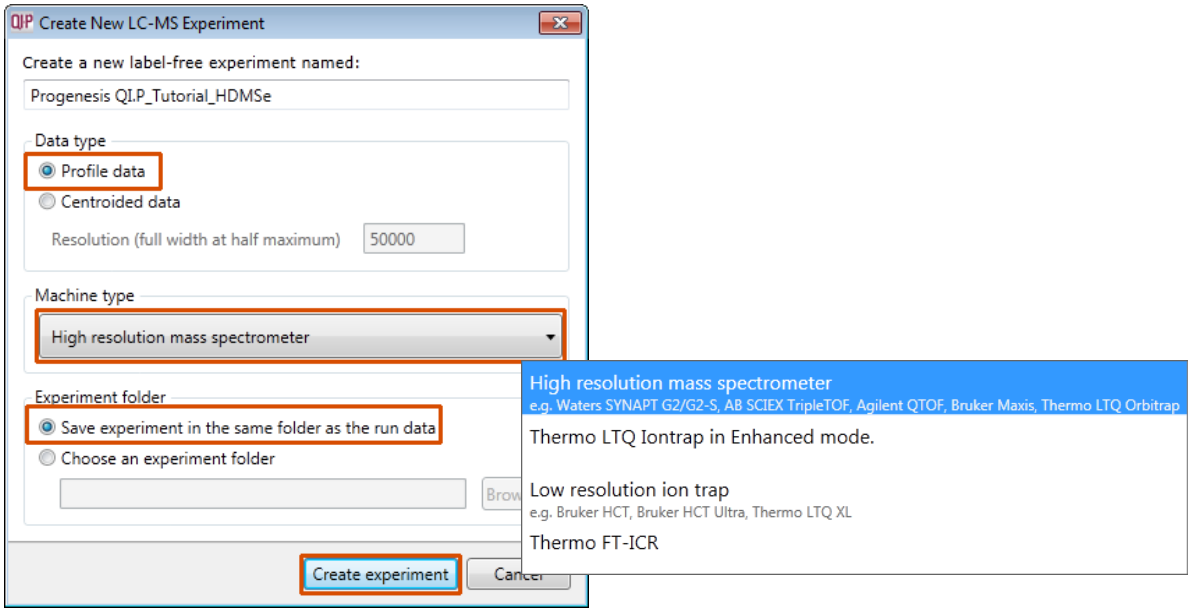
You can clear the current searches and repeat the searches with the other libraries provided or try the ones you have created.

Appendix 1: Stage 1 Data Import and QC review of LC-MS data set

You can use your own data files, either by directly loading the raw files (Waters, Thermo, Bruker, ABSciex and Agilent) or, for other Vendors, convert them to mzXML or mzML format first.

To create a new experiment with your files: open Progenesis QI for proteomics and click **New,** bottom left of the **Experiments** page and give your experiment a name. Then select data type, the default is 'Profile data'.

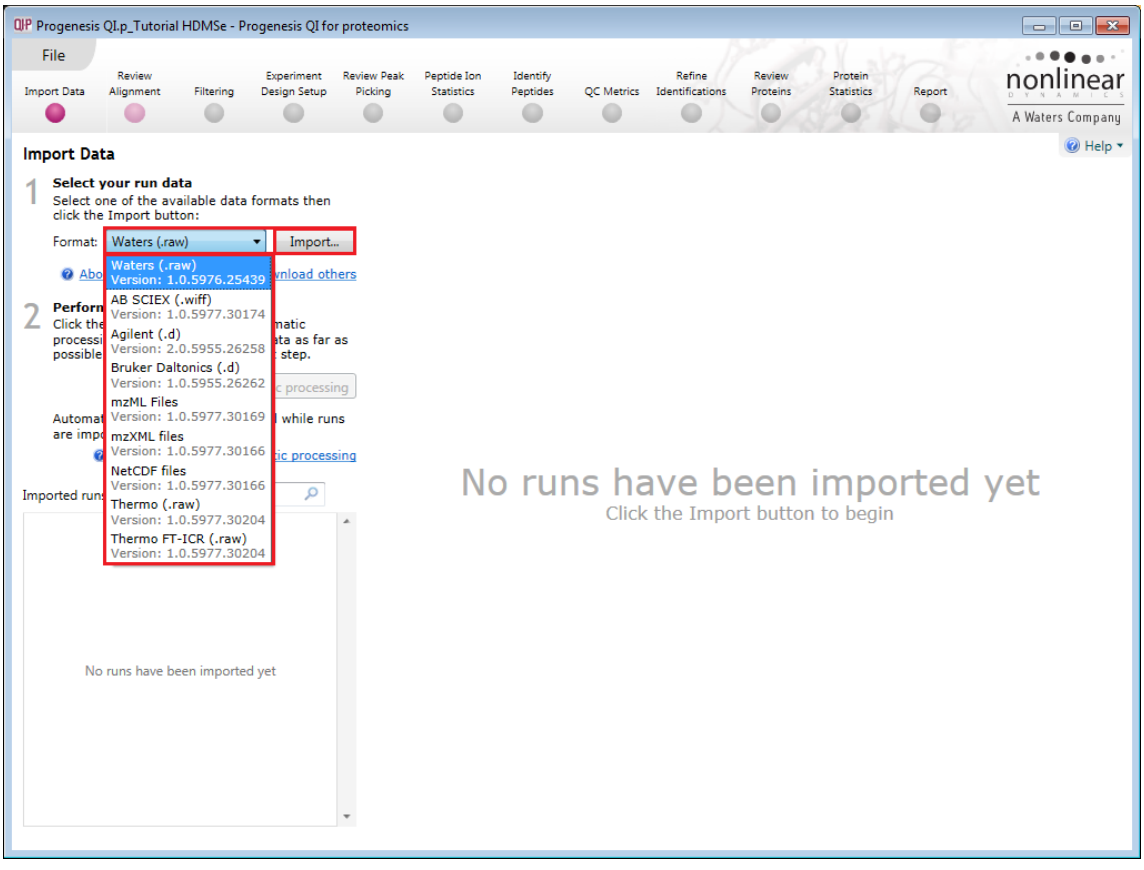
Note: if you have converted or captured the data as centroided then select Centroided data and enter the Resolution for the MS machine used.



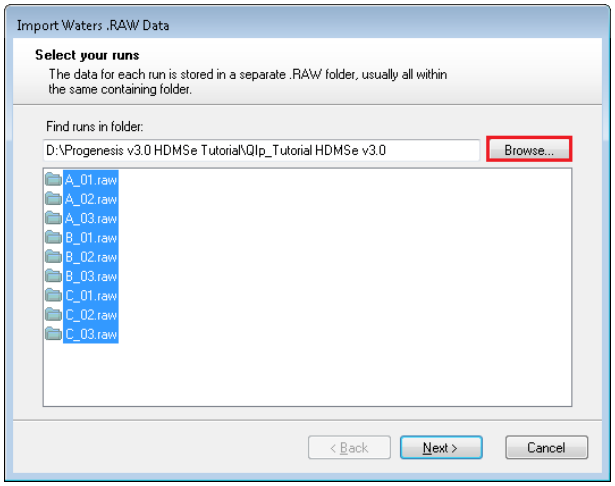
Click **Create experiment** to open the LC-MS Data Import stage of the workflow.

Select the 'Import Data file format', in this example they are Waters/SYNAPT data

Then locate your data files using **Import...**

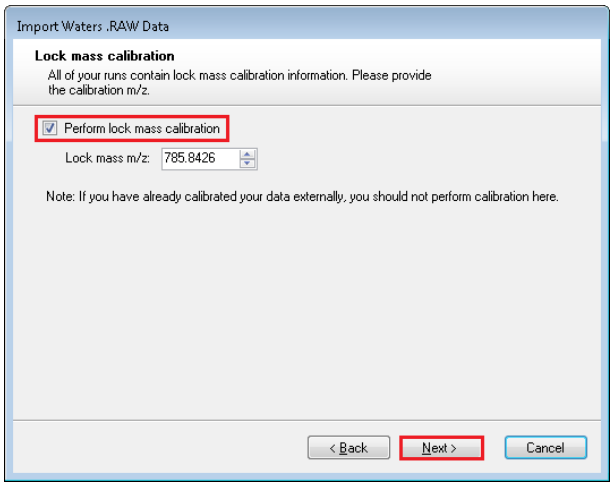


Locate and select all the .RAW folders (A_01 to C_03).



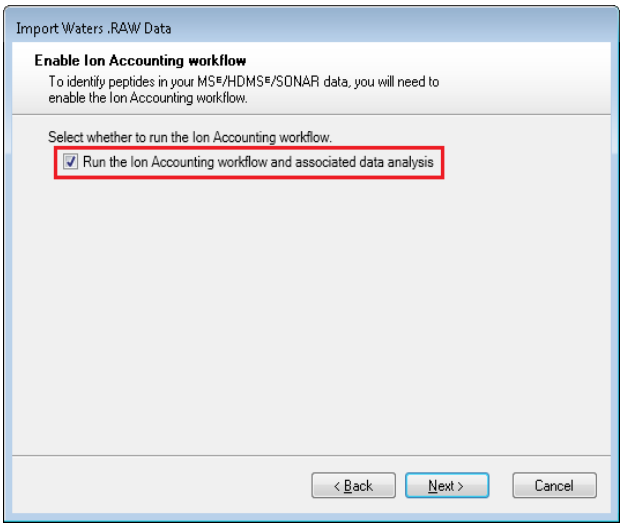
On importing, the lock mass calibration is read and presented on this dialog

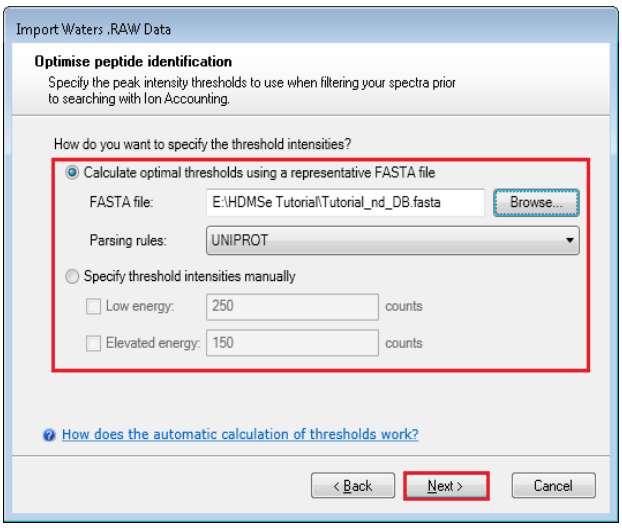
You can, if required, alter the lock mass calibration at this step.



For MSe, HDMSe and SONAR data formats the Ion accounting workflow is selected as default if your computer has a GPU.

Click **Next**. You can either choose to calculate optimal thresholds using an appropriate FASTA file for your data set or set the Thresholds manually.





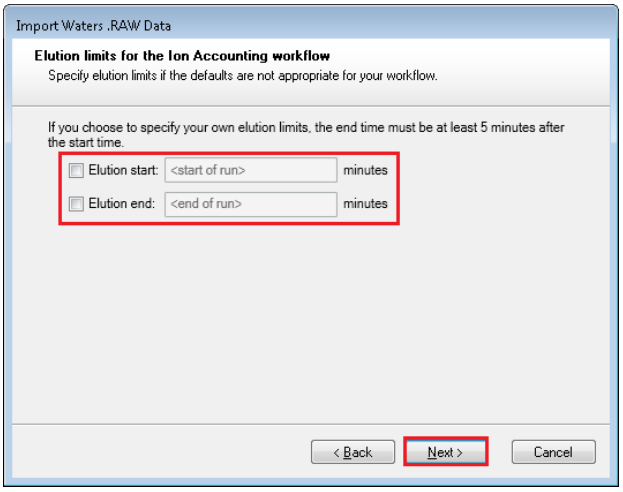
If you choose to determine the thresholds automatically then Progenesis determines appropriate thresholds by sampling each run and finding the thresholds that yield the most protein identifications in the sample area. For each run, Progenesis performs the following steps:

- It finds the 5-minute retention time window that contains the highest total intensity.
- It extracts the ions within this window and performs multiple Ion Accounting searches, each one using a different set of threshold values.

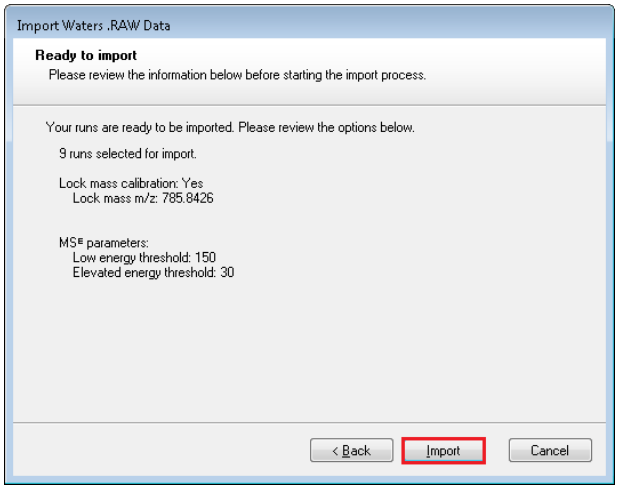
- Finally, it selects the thresholds that resulted in the largest number of protein identifications and applies those to the whole run. If more than one set of thresholds results in similar numbers of identifications (within 10% of the maximum), it will choose the highest thresholds as a way of optimising system performance.

Note: for HDMSe the settings are 150 and 30 and for MSe the settings are 250 and 150 for the Low and Elevated energies respectively.

Having selected how to handle the thresholds you will get the option to specify your own elution limits, the default is Start and End of the run, accept or make changes as necessary.



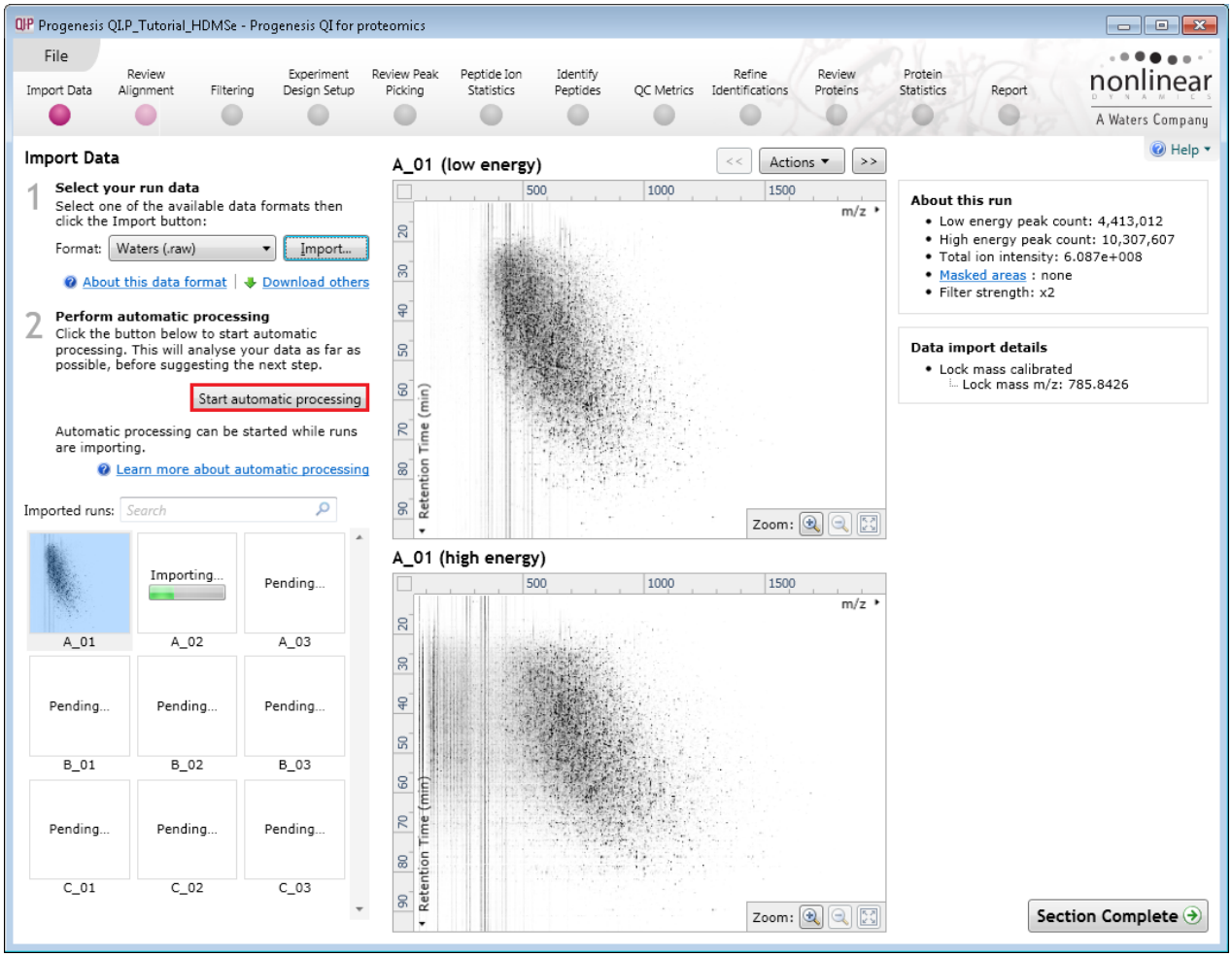
A summary of the loading parameters is provided before you click Import



On loading the selected runs your data set will be automatically examined and the size of each file will be reduced by a 'data modelling routine', which reduces the data by several orders of magnitude but still retains all the relevant quantitation and positional information.

Note: For a large number of files this may take some time.

Note: you can start the automatic processing before the loading has completed.

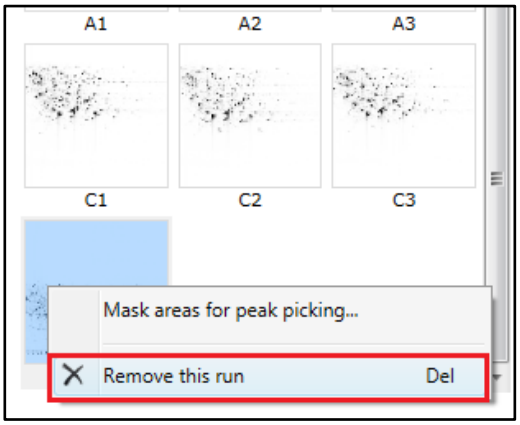


For details of setting up the steps in the automatic processing wizard return to Stage 2A page 9.

Review Chromatography

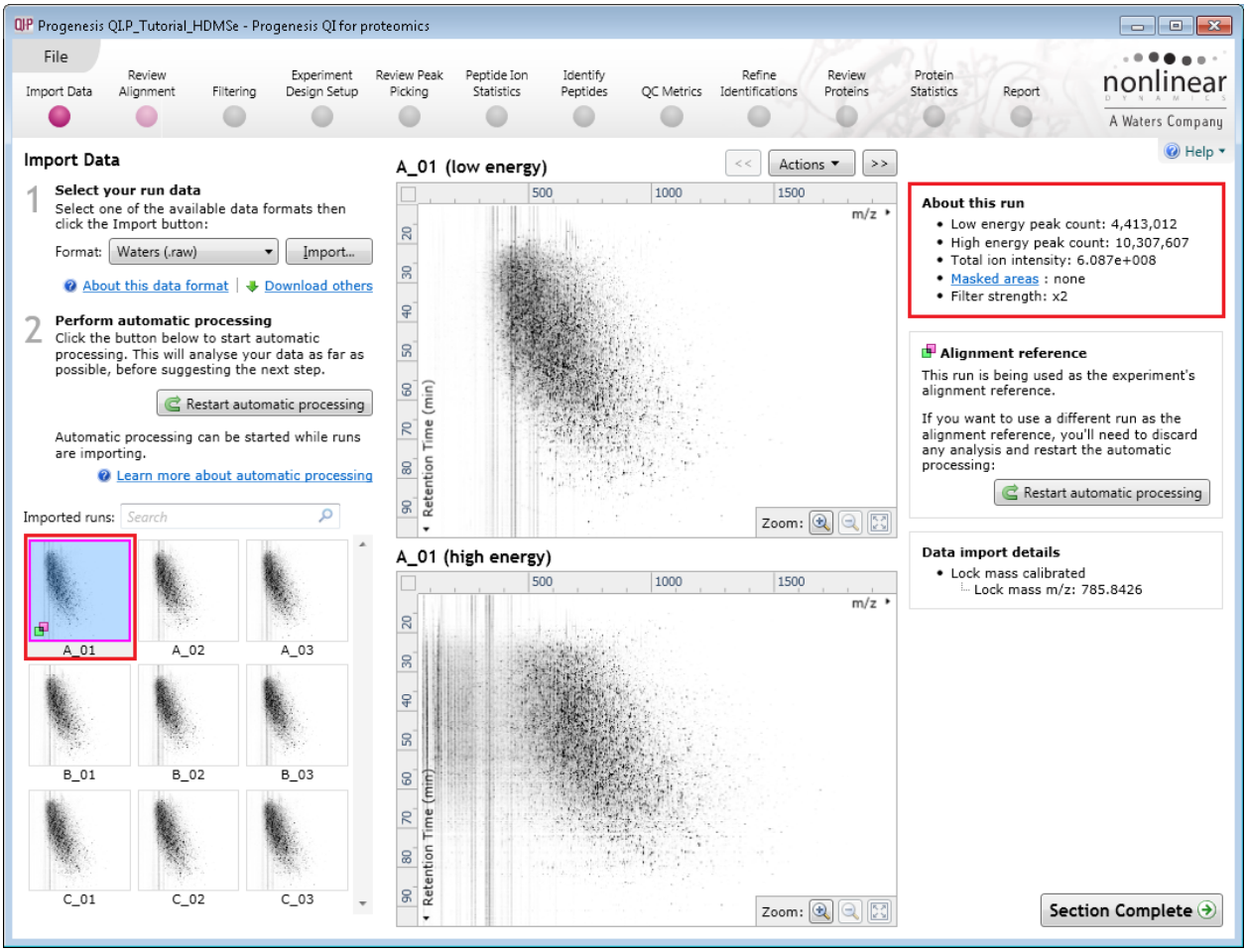
Each data file appears as a 2D representation of the run. If you created a **profile** experiment, at this stage you will be warned if any of the data files have been 'centroided' during the data acquisition and conversion process, as files must be of one format or the other.

You can delete run(s) by right clicking on the run in the list.



Note: you can also multi-select runs to remove by holding down the Ctrl key.

At the Import Data stage you can examine the quality of the imported runs using the 2D representation of the runs



Note: details of the current run appear on the top right of the view.

Once you have reviewed the imported runs click on **Review Alignment** on the workflow or **Section Complete** to move forward to the Review Alignment Stage.

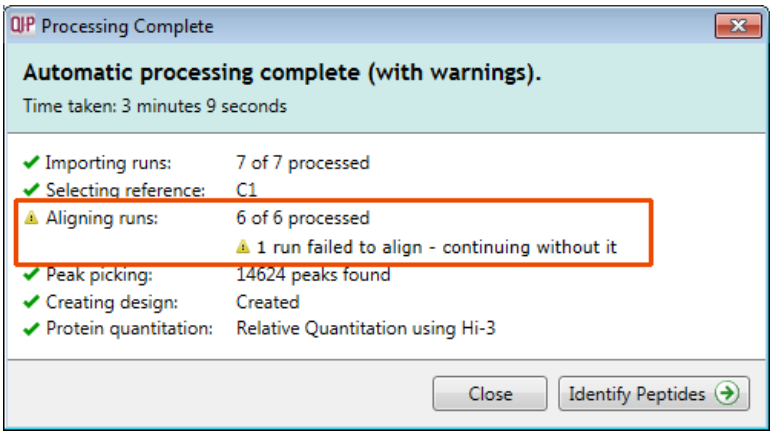
Note: you will be offered the automatic alignment if you have not performed it automatically already.

Now move to the next stage in the workflow (page 9 in this user guide) by clicking **Section Complete.**

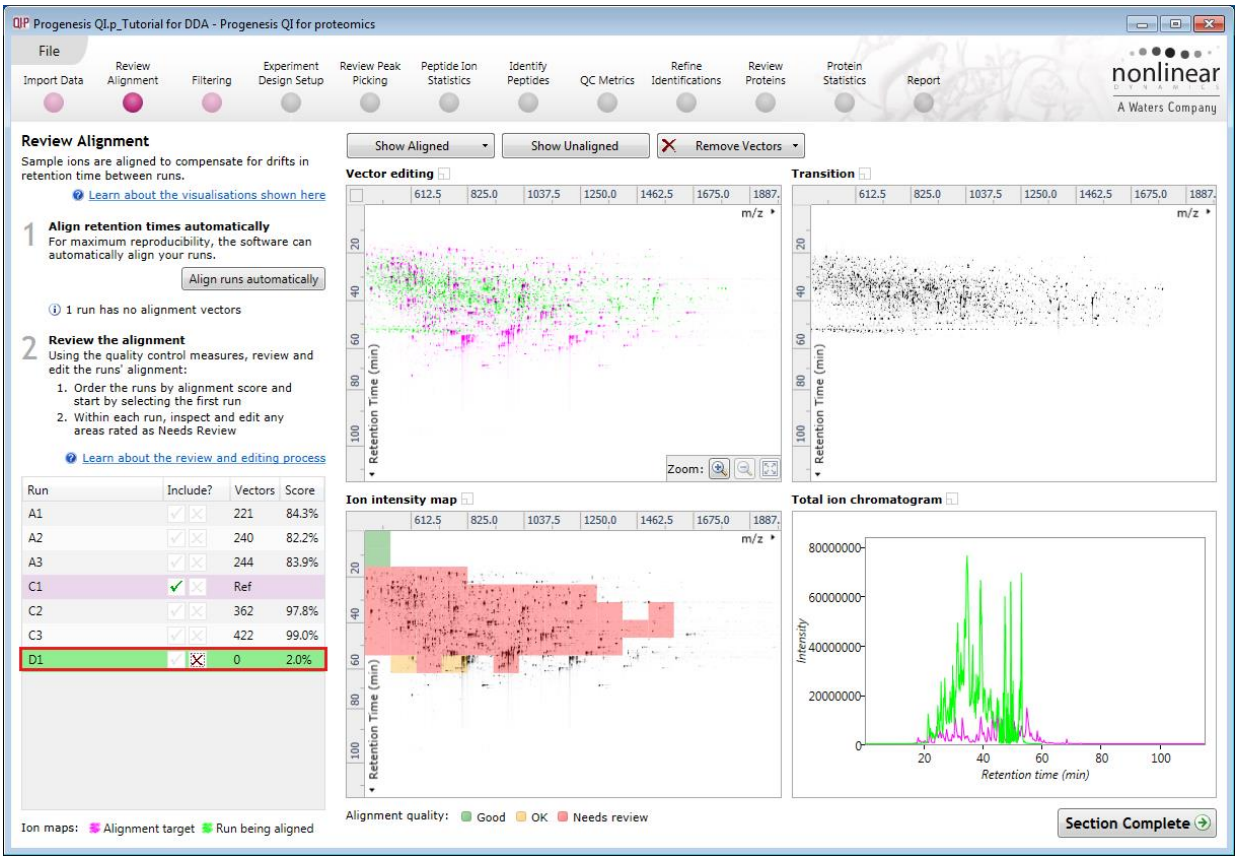
Appendix 2: Stage 2 Processing failures

If a stage fails to complete successfully or only partially completes, the automatic processing dialog will warn you of the problem. This may or may not allow the automatic processing to complete.

For example, a run that fails to automatically align will trigger a warning, although analysis will continue; however, the automatic processing dialog will prompt you to 'drop-off' at the **Review Alignment** stage on completion to investigate the problem.



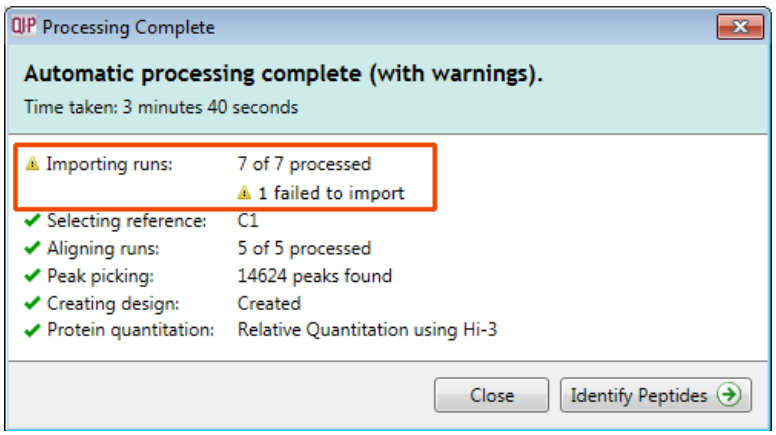
Note: in this example the run that failed to align will not contribute to the peak picking and will be excluded at the alignment stage (a cross appears in the include column).



You can either remove the run from the experiment at the **Import Data** or add it back in at the **Review Alignment** stage once the alignment of the run has been corrected.

As another example, runs that import successfully but with warnings at the **Import Data** stage will cause a flag in the readout to notify you of the potential quality issue.

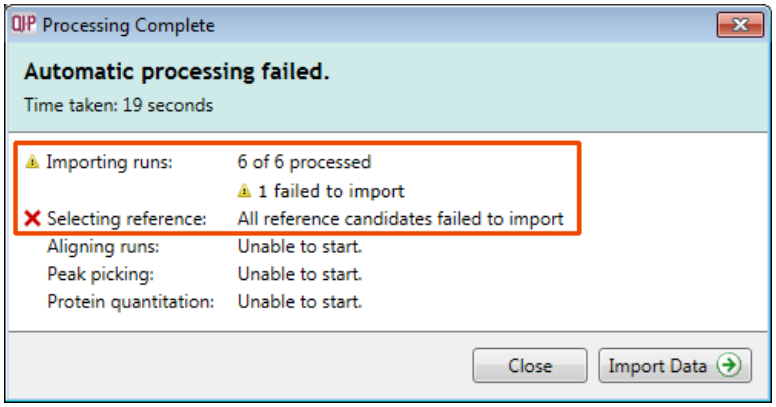
If some runs in a data set fail to import (but not all), the automatic processing will continue informing you that one or more runs have failed to import.



In this case you can remove the runs at Import Data and if appropriate replace them with additional runs.

Note: adding additional runs will then be aligned and peak picking should be re-done to include data from the added runs in the generation of the aggregate.

An example of a problem that would halt the automatic processing would be the failure to successfully import all the potential reference candidates, (for example: while importing, you specified the selection of the alignment reference to be made from several runs before they were fully imported and set the processing underway, and they later failed to import owing to problems with the runs).



In this case, the processing dialog would halt and prompt you to select another reference.

Appendix 3: Licensing runs

When setting up a **New experiment** if you are evaluating Progenesis QI for proteomics with unlicensed runs then the licensing page will open after **Import Data section**.

If you already have a programmed dongle attached to your machine then the License Runs page will not appear.

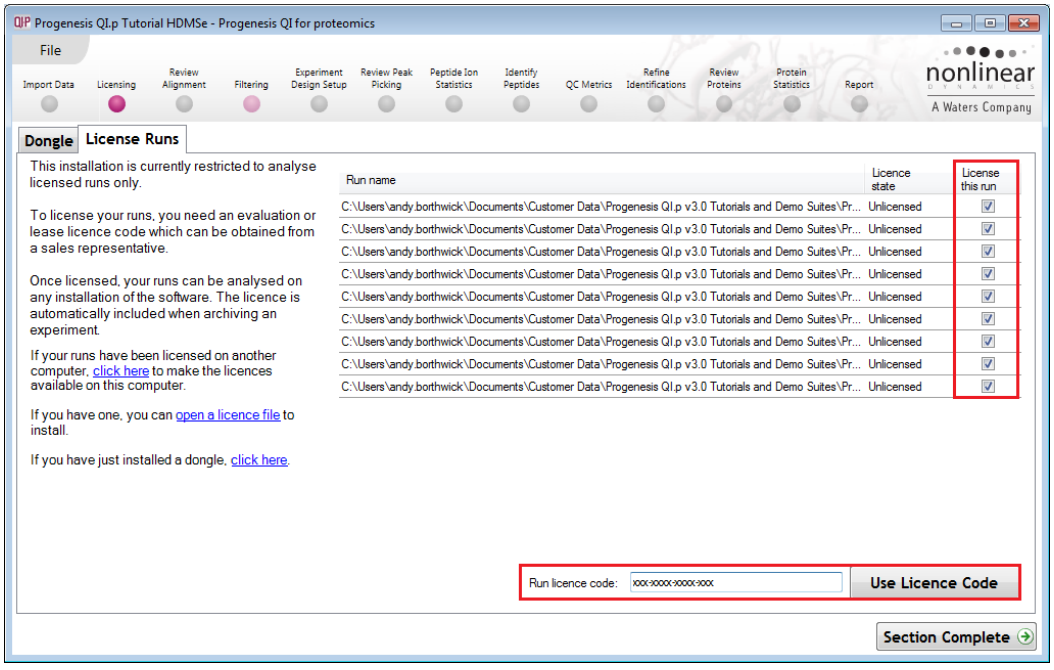
To use this page to License your Runs you must first either obtain an **'Evaluation' Licence Code from a Sales Person or purchase a licence code directly**.

Each code will allow you to license a set number of runs.

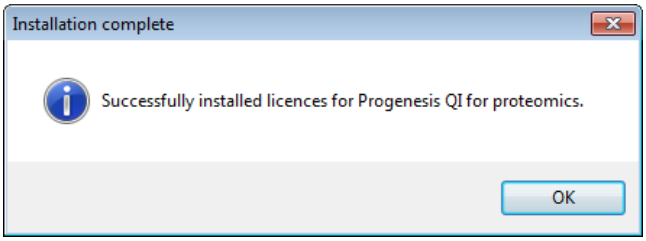
The runs in your experiment will be listed as shown below.

To activate license(s) for the selected runs enter the code in the space provided and click **Use Licence code**.

Note: you will need an internet connection to use this method.



A message confirming successful installation of your licences will appear.

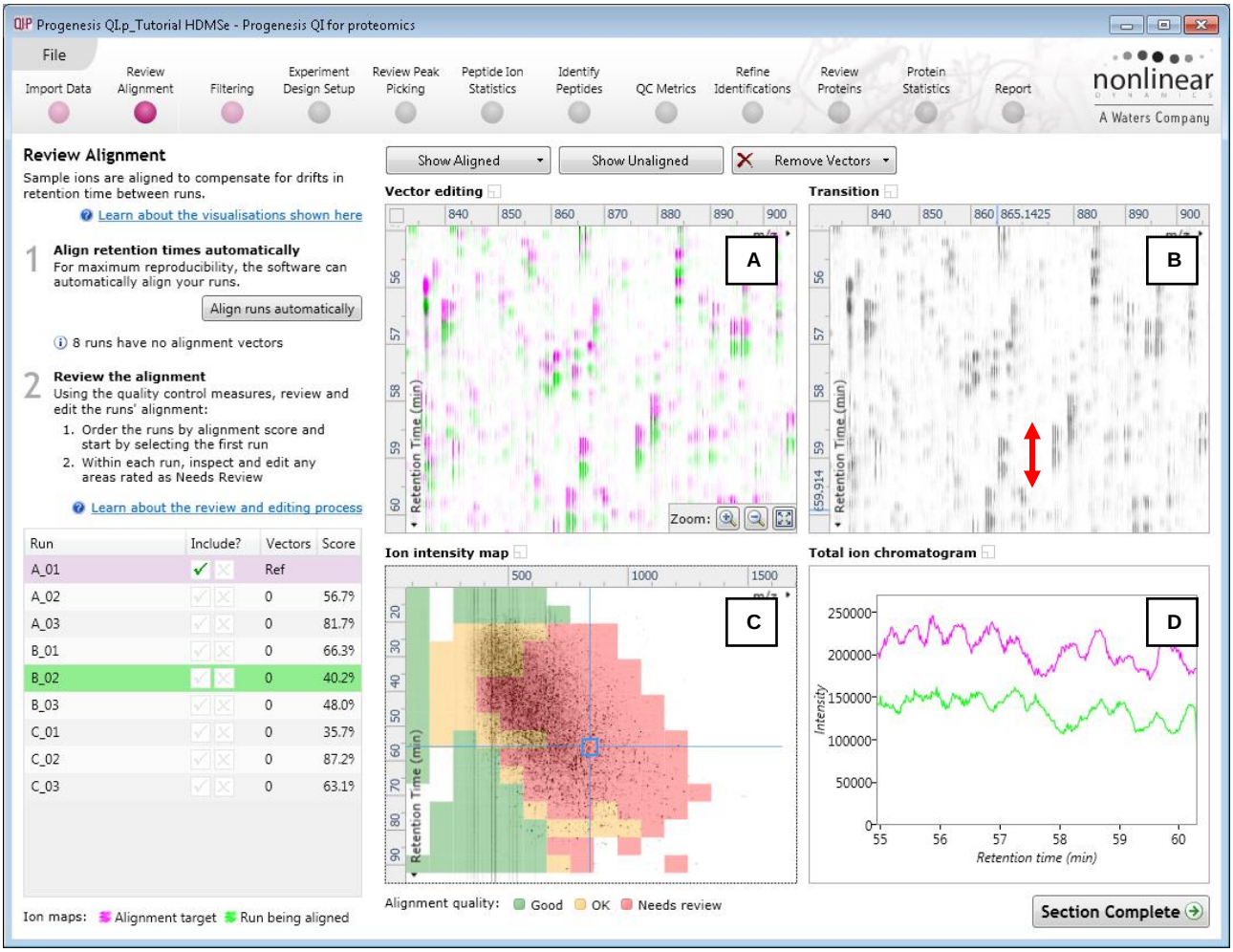


Click **OK**, the view will update and Alignment, the next stage in the workflow, will open with the licensed files.

Appendix 4: Manual assistance of Alignment

Approach to alignment

To place manual alignment vectors on a run (B_02 in this example):



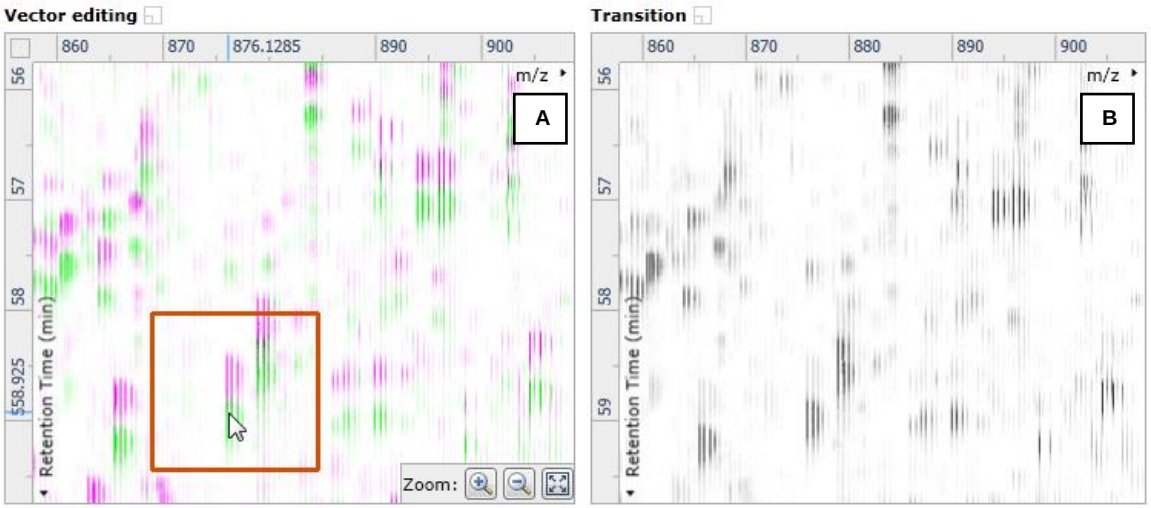
1. Click on Run B_02 in the **Runs** panel, this will be highlighted in green and the reference run (A_01) will be highlighted in magenta.
2. You will need to place approximately 5 - 10 **alignment vectors** evenly distributed from top to bottom of the whole run (RT range).
3. First drag out an area on the **Ion Intensity Map** (C), this will reset the other 3 windows to display the same 'zoomed' area

Note: the peptide ions moving back and forwards between the 2 runs in the **Transition** window (B) indicates the misalignment of the two runs.

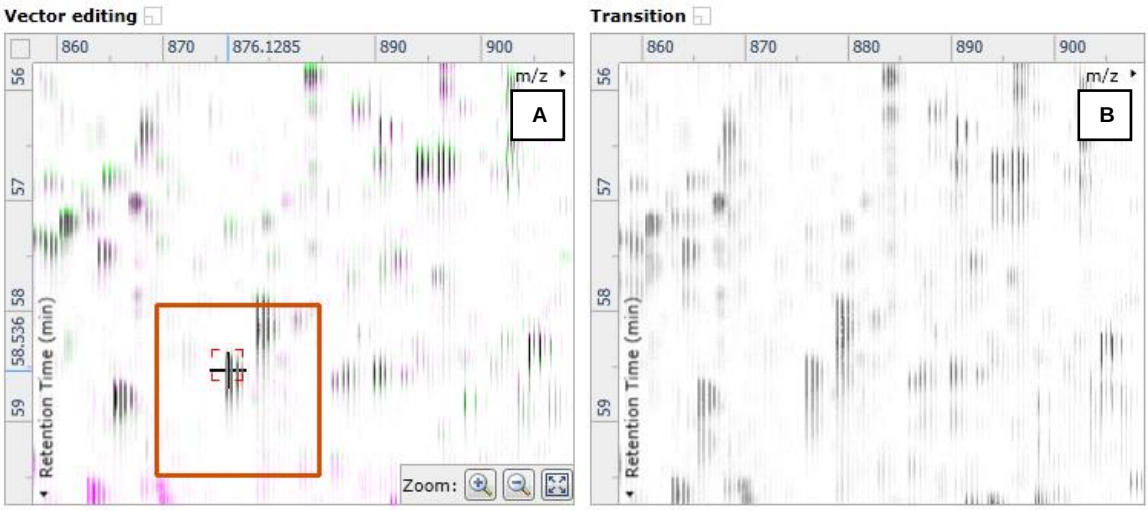
Note: the Ion Intensity Map gives you a colour metric, visually scoring the current alignment and an overall score is placed next to the Vectors column in the table. With each additional vector added this score will update to reflect the 'changing' overall quality of the alignment. The colour coding on the Ion intensity Map will also update with each additional vector.

Note: The **Total Ion Chromatograms** window (D) also reflects the misalignment of the 2 runs for the current Retention Time range (vertical dimension of the current Focus grid in the **Ion Intensity Map** window).

4. Click and hold on a green peptide ion in Window A as shown below.

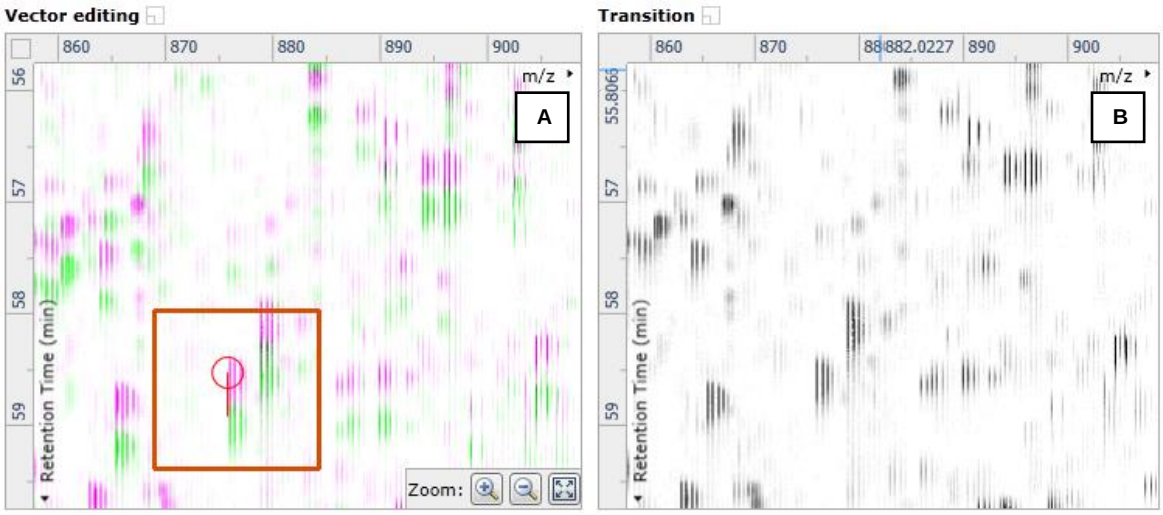


5. As you are holding down the left mouse button (depending on the severity of the misalignment), the alignment vector will automatically find the correct lock. If not, drag the green peptide ion over the corresponding magenta peptide ion of the reference run. The red box will appear as shown below indicating that a positional lock has been found for the overlapping peptide ions.



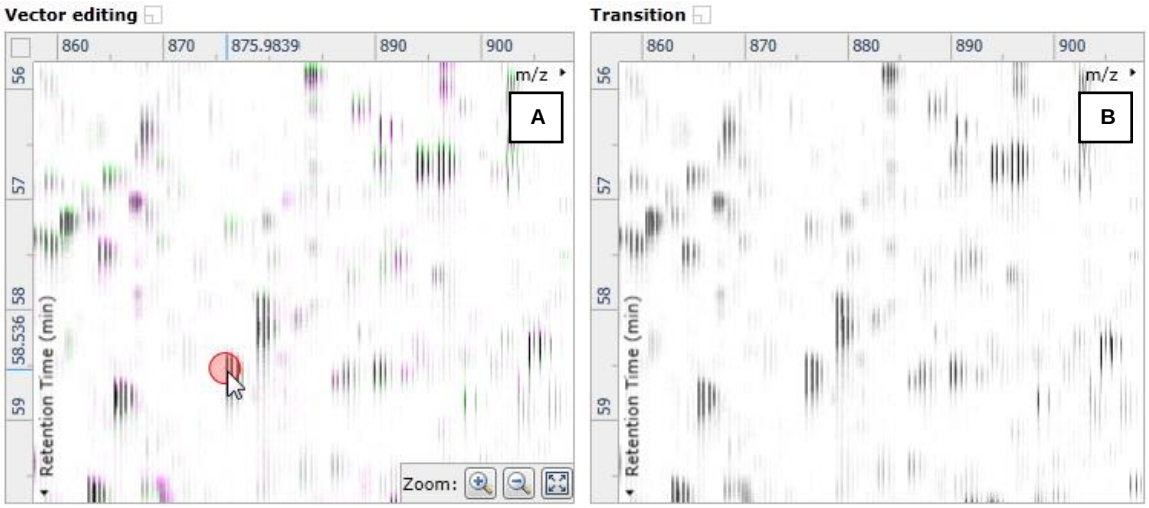
***Tip*:** while holding down the mouse button hold down the **Alt** key. This will allow smooth movement of the cursor as the **Alt** key allows you to override the 'automatic alignment' performed as you depress the mouse button.

6. On releasing the left mouse button the view will 'bounce' back and a red vector, starting in the green peptide ion and finishing in the magenta peptide ion will appear.

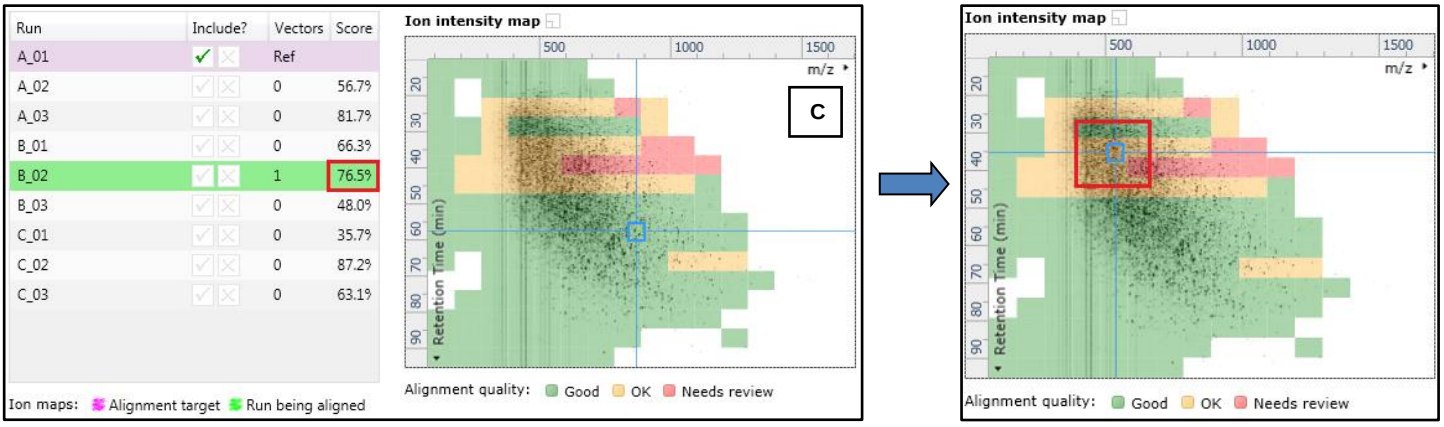


Note: an incorrectly placed vector is removed by right clicking on it in the **Vector Editing** window and selecting delete vector.

7. Now click **Show Aligned** on the top tool bar to see the effect of adding a single vector.



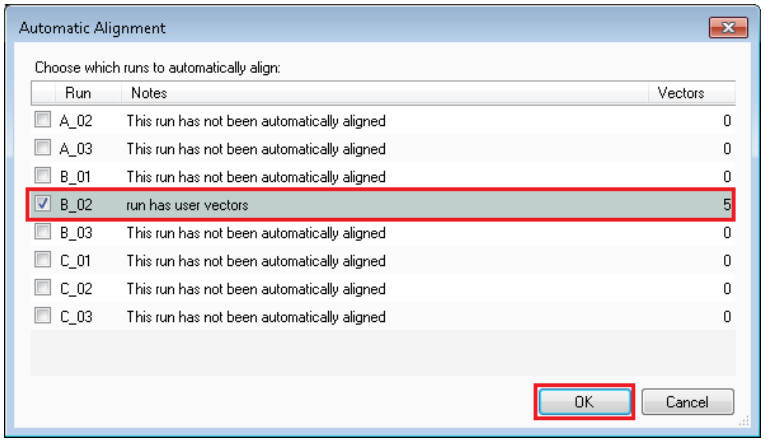
8. With the placement of a single manual vector the increase in the proportion of the **Ion Intensity Map** (C) showing green is reflected in the improved alignment score in the table. Now click in the Ion Intensity Map to relocate the focus in order to place the next manual vector.



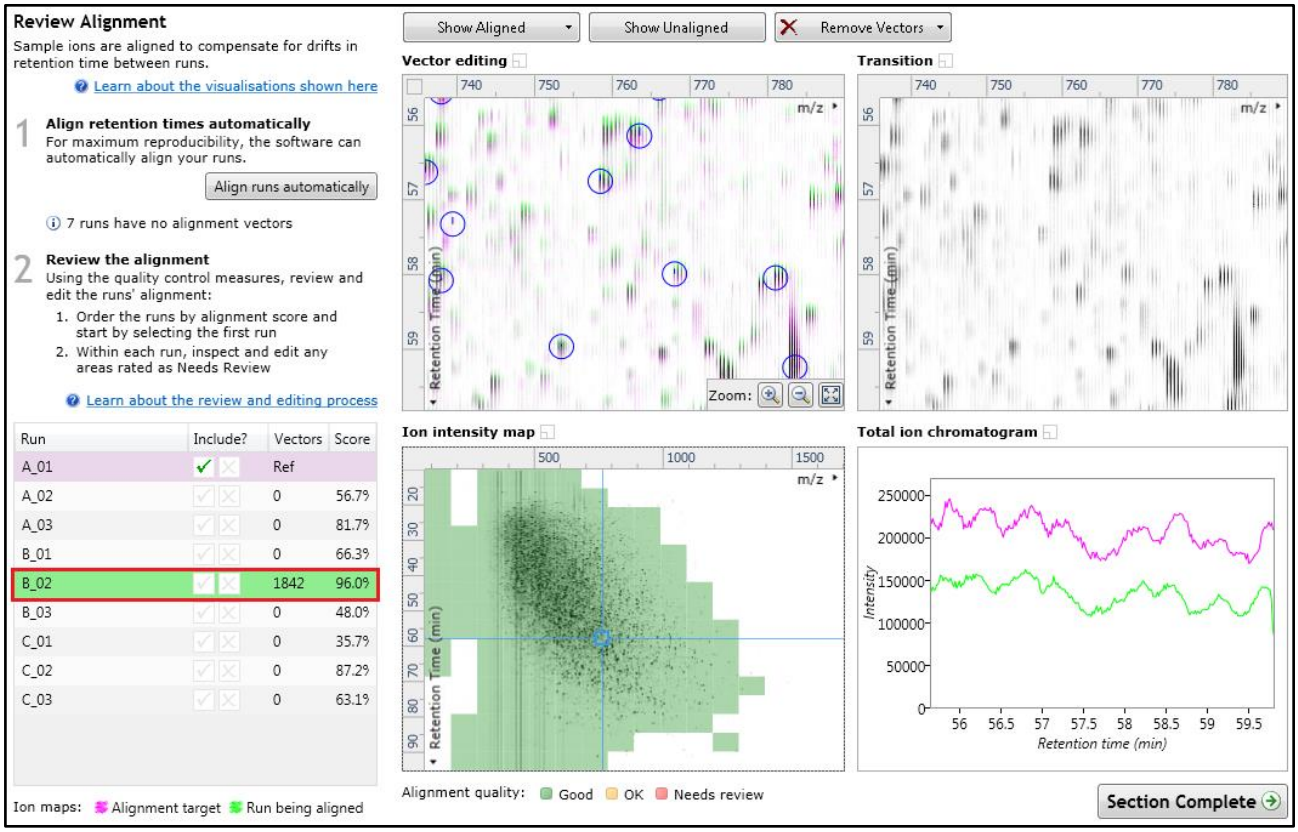
9. Adding an additional vector will improve the alignment further as shown below.

10. The shift in the Retention Time (RT) is as a result of incorrect running of the chromatography. In many of these cases if the Automatic Alignment fails to generate a good alignment then removing all the alignment vectors for this run and placing a single manual vector to act as a 'seed' for the Automatic Alignment algorithm maybe all that is required to generate a good alignment.
11. In the case of the example shown above placing a small number of vectors from the top to the bottom of the run is sufficient to markedly improve the alignment.

12. At this point you would redo the automatic alignment of this image by selecting automatic alignment. **Note**: if you are focusing only on the alignment of one run, then un-tick the other runs in the alignment dialog.



13. On pressing OK the Automatic Alignment will run for the selected run. On completion the table and views will update to display the automatically generated vectors (shown in blue).



14. Repeat this process for all the runs to be aligned.

The number of manual vectors that you add at this stage is dependent on the misalignment between the current run and the Reference run.

Note: In many cases only using the Automatic vector wizard will achieve the alignment.

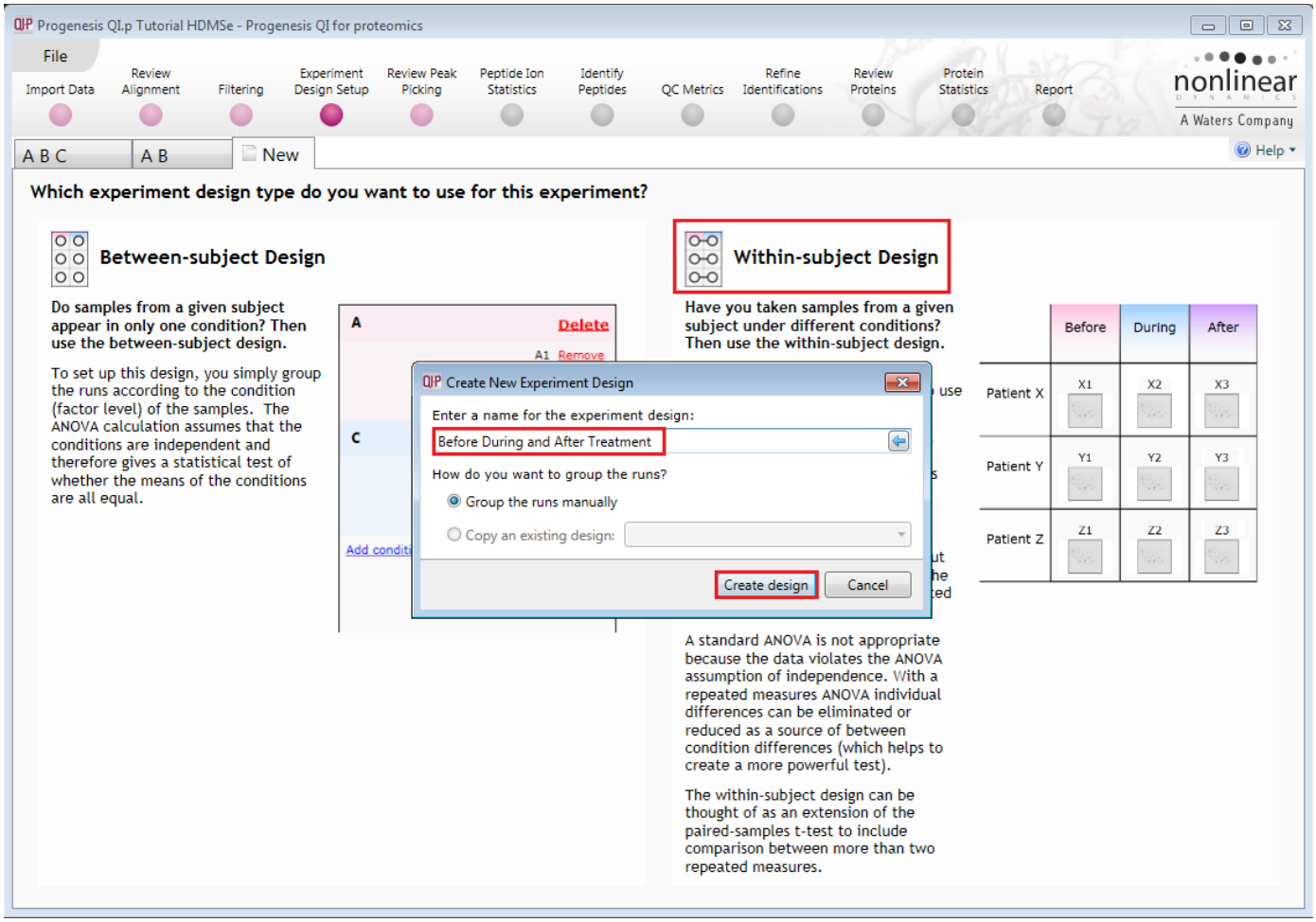
Tip: a normal alignment strategy would be: to run the automatic alignment first for all runs, then order the alignments based on score. For low scoring alignments remove all the vectors and place 1 to 5 manual vectors to increase the score then perform automatic alignment. Then review the improved alignment score.

Also the 'ease' of addition of vectors is dependent on the actual differences between the LC-MS runs being aligned.

Appendix 5: Within-subject Design

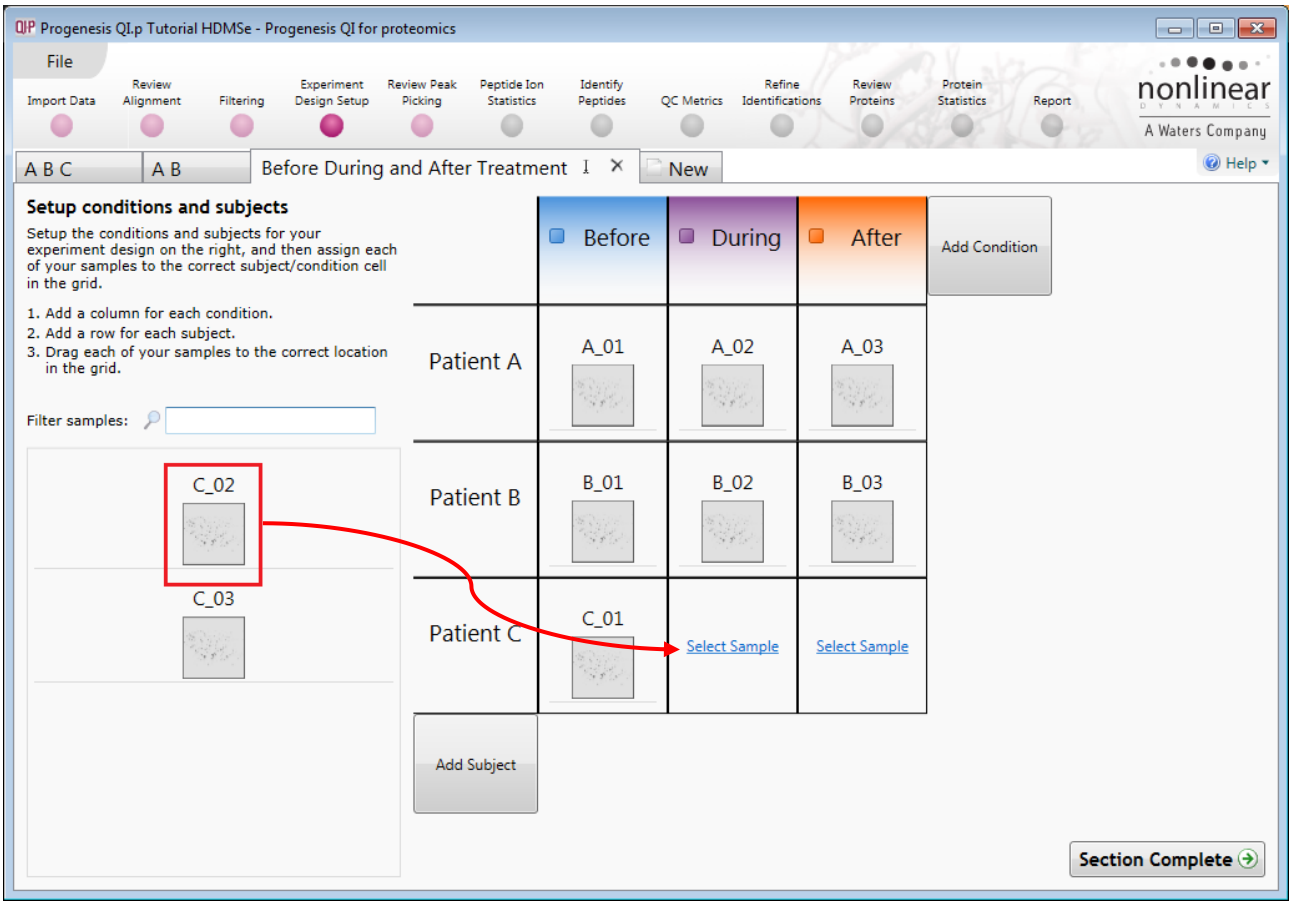
To create a **Within-subject Design** for your data set select this option on the **Experiment Design Setup** page and enter the name of the design.

In this example there are 3 Subjects (i.e. patients A, B and C) who have been individually sampled: Before(1), During (2) and After (3) treatment



When the design page opens use the **Add Subject** and **Add Condition** buttons to create the matrix that fits your experimental design, over typing the names as required.

Then Drag and drop the Samples on to the correct 'cell' of the matrix.



You can create additional Experimental Designs using the New tab

All of these Experimental Designs are available at the later stages of the workflow with the exception of **Identify Peptides**, and **Refine Identifications** (including Resolve Conflicts).

Appendix 6: Resolve Conflicts

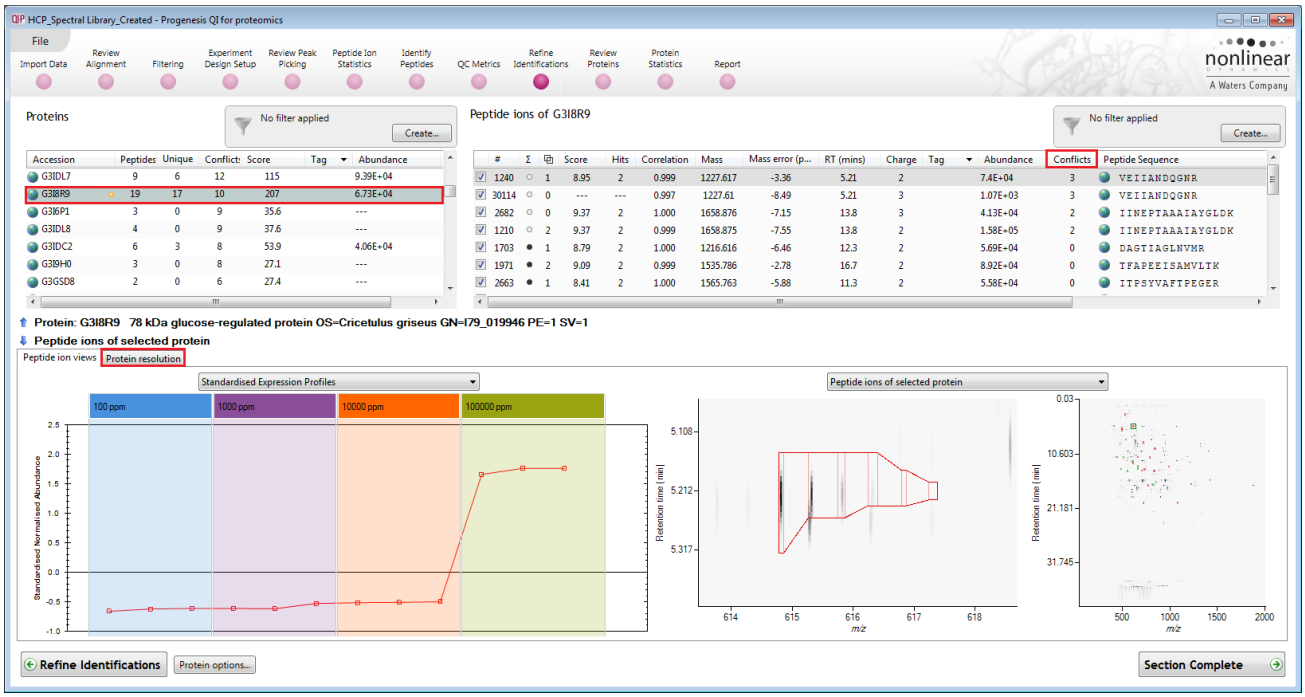
This stage allows you to examine the behaviour of the identified peptides and choose to resolve any conflicts for the various peptide assignments at the protein level.

The **Resolve Conflicts** stage is now accessed at the bottom left of the Refine Identifications stage.

The number of conflicts you have to resolve will depend on the scope and stringency of the filters you apply at the **Refine Identifications** stage.

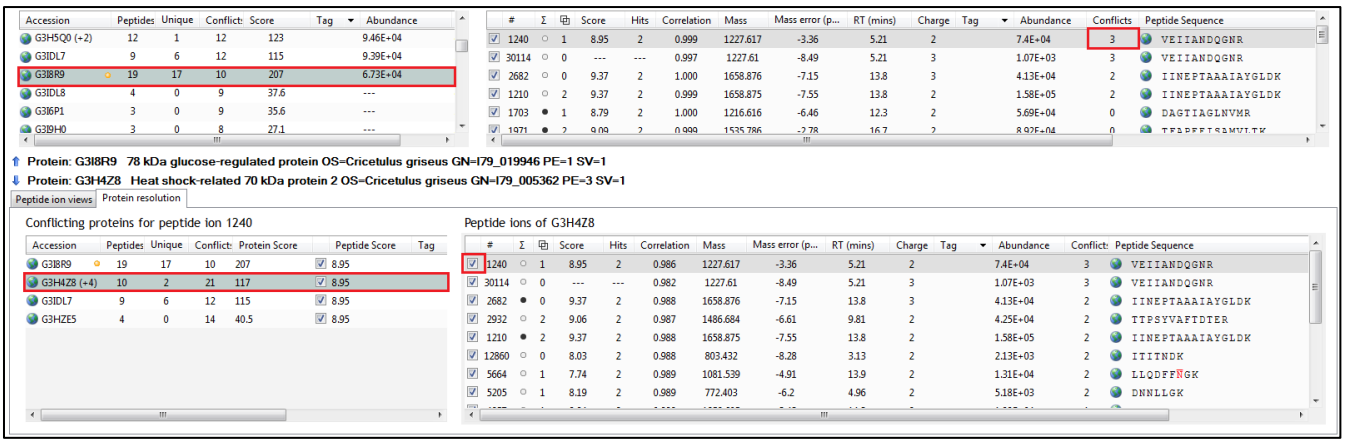
Currently no Conflict resolution was performed on the HCP_Spectral Library_Created data set.

Example of performing conflict resolution on G3I8R9 (left hand table), which has a total number of 19 peptides, 17 of which are uniquely assigned to this protein, the remaining 2 have a total of 10 conflicting protein assignments.



On the peptides table order on conflicts and select the first peptide (which has 3 conflicts) and click on the Protein resolution tab to show the conflicting proteins

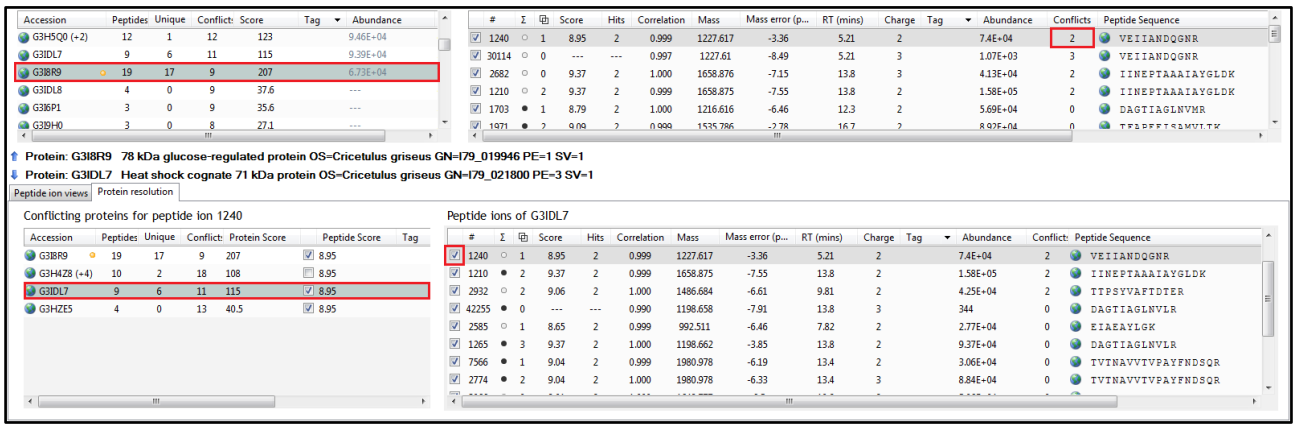
The Conflicting proteins are ordered on the basis of Protein Score in the bottom left table



Untick the peptide on the bottom right table to favour the current protein based on the highest protein score

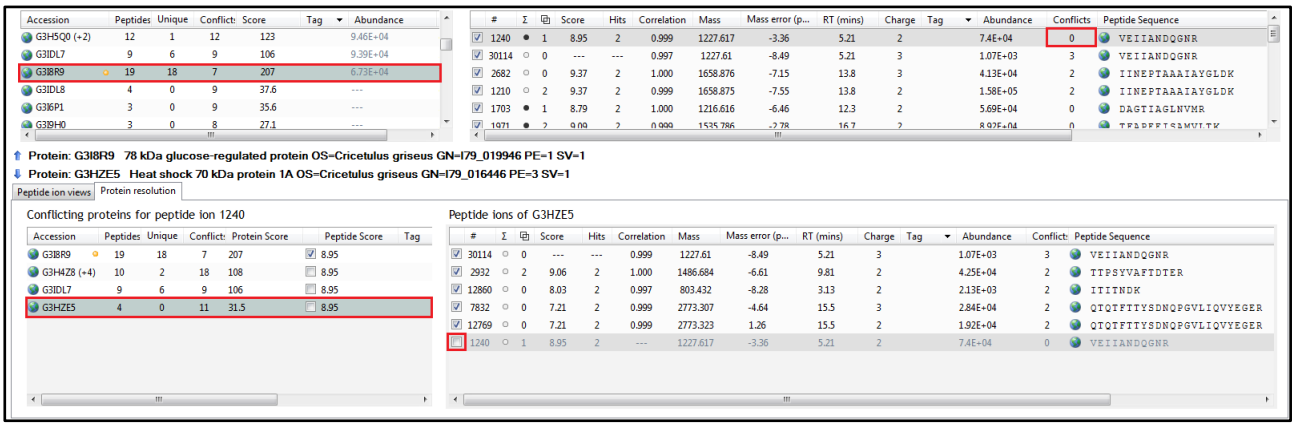
Then move down the Conflicting proteins table to the next protein.

Here also untick the peptide in the bottom right table.



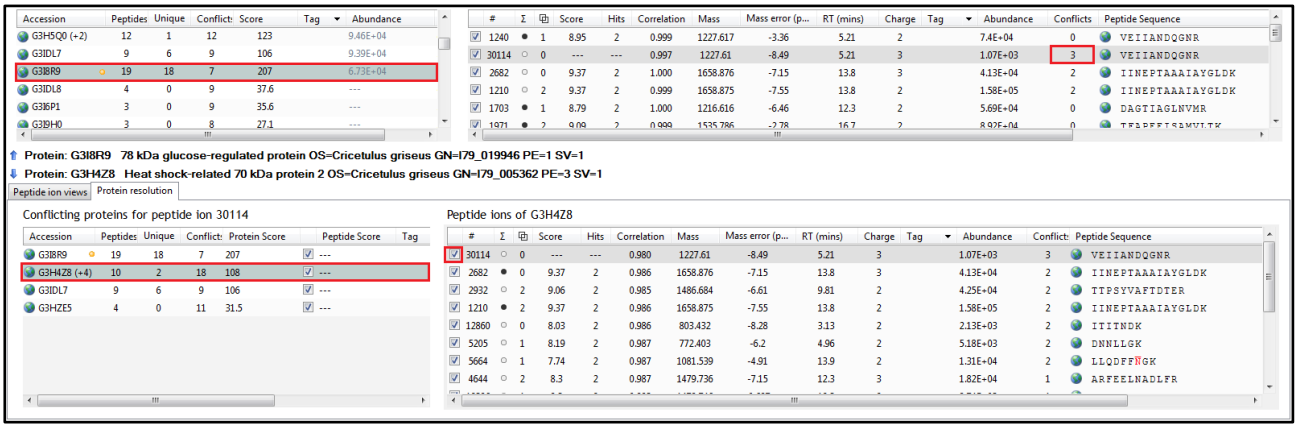
Note: as you untick each Peptide ion for the conflicting protein the number of conflicts is reduced in the top 2 tables

Repeat this process for all the conflicting proteins for this peptide ion

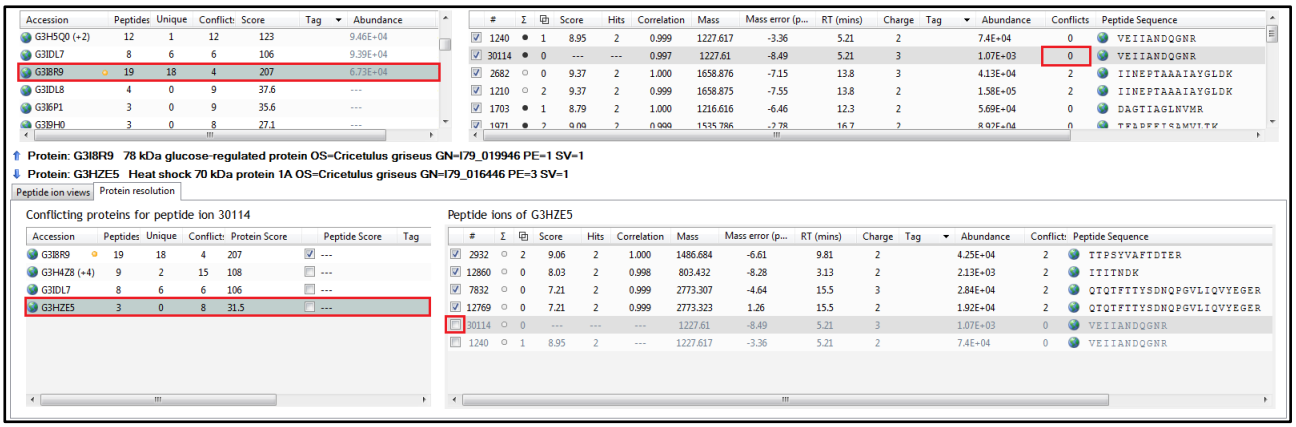


Now move to the second Peptide ion (with 3 conflicts) on the top right table

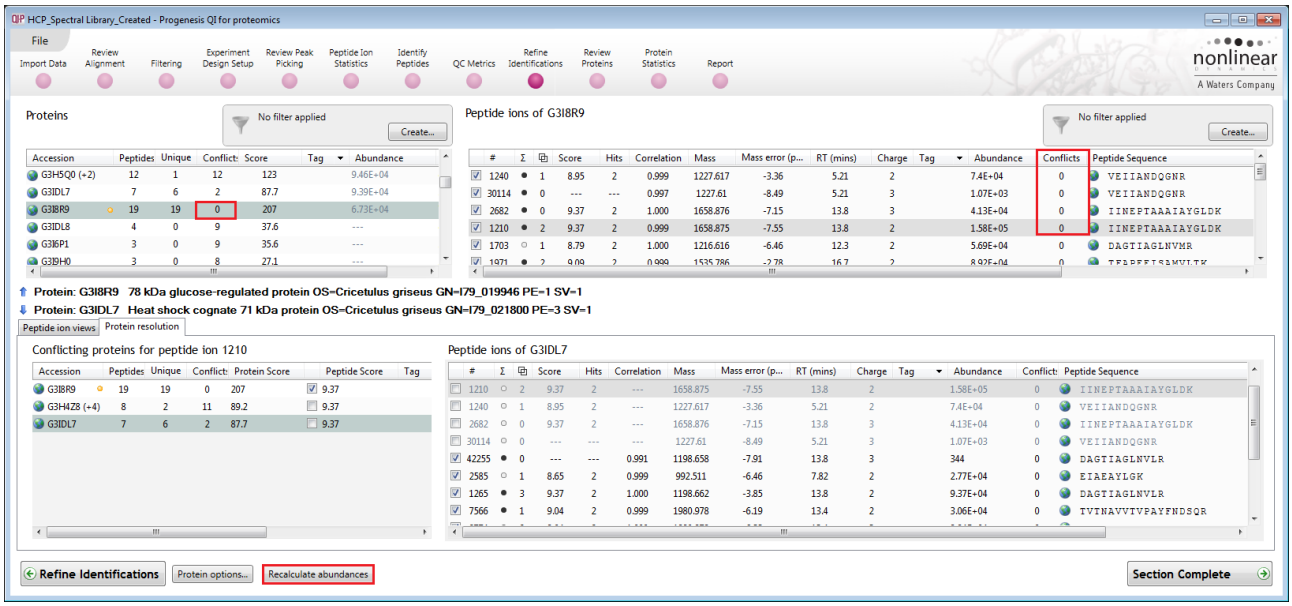
Then move through the Conflicting proteins on the bottom left table unticking the peptide ions on the right hand tables as appropriate



The tables will update to reflect the resolved conflicts.

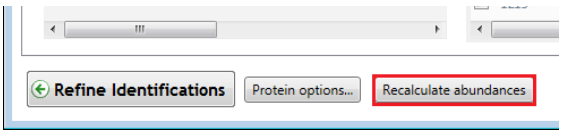


Repeat this process until there are no remaining conflicts on the top 2 tables for the current protein G3I8R9.



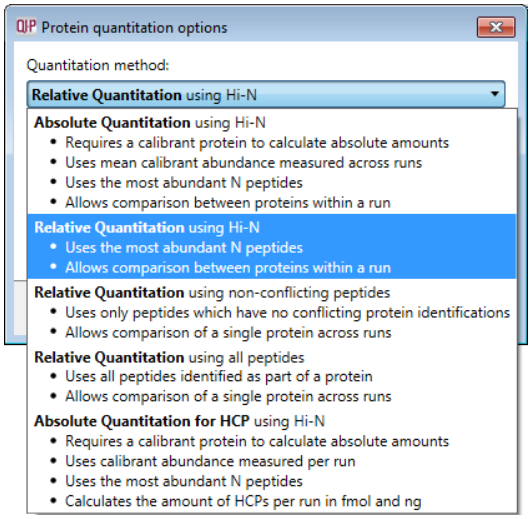
To Resolve the conflicts for the whole data set work through this process with all of the proteins in the top left hand table, that display conflicts, until there are no remaining conflicts.

Note: the abundances will need to be recalculated as a result of performing Conflict resolution. This is achieved by clicking on the Recalculate abundances**,** which appears during Conflict resolution



Protein Quantitation options

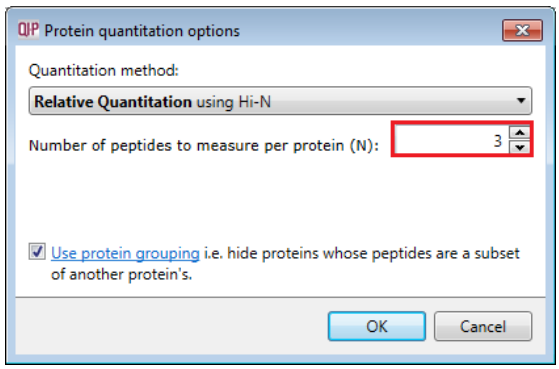
There are 5 options with which to control how the Protein Quantification is performed by Progenesis QI for proteomics.



The default option that will be applied is **Relative Quantitation using Hi-N**.

If you have selected one of the other options during the setup of the Auto Processing of your data (Stage 2) then this option will be applied.

Hi-N in Progenesis QI for proteomics is an implementation of Hi-3 as described by Silva *et al.* [References]. After peptide and protein identification, the abundance of each peptide is calculated from all its constituent peptide ions.

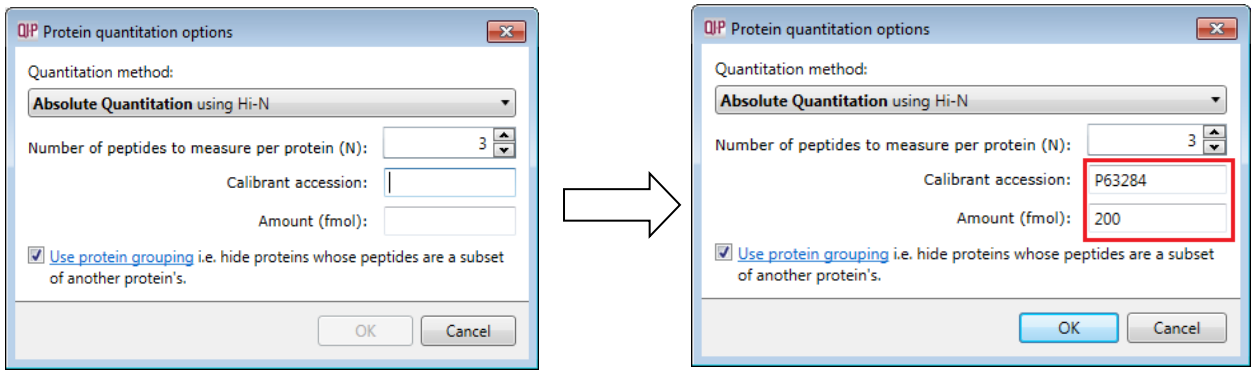


For each protein, the N most abundant peptides (N being set according to the user selection) have their **abundances averaged** to provide a reading for the protein signal.

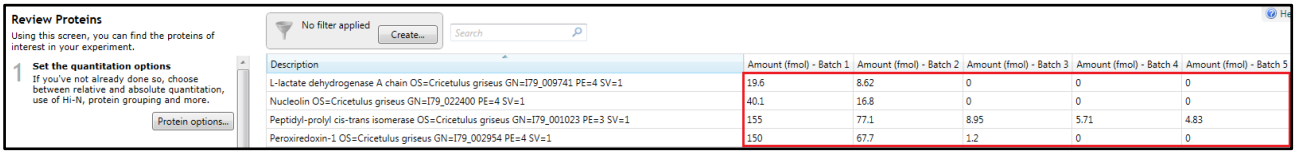
The ranking of peptide abundance is based on the integrated value across all the runs, allowed by the accurate alignment and lack of missing values. This gives added confidence in the peptide selection, taking all runs into account to make the ranking robust.

The averaged abundance readings not only make possible the **relative quantitation** of the same protein across all runs to be determined but also allow, with the inclusion of a known amount of a calibrant protein in each run, this to be converted to an **absolute** reading for protein amount.

To generate values for **absolute quantitation** enter the accession number and amount for the calibrant.



The absolute amounts, based on the calibrant used, are reported at the Review Proteins stage as additional columns (one for each condition) following the protein description.



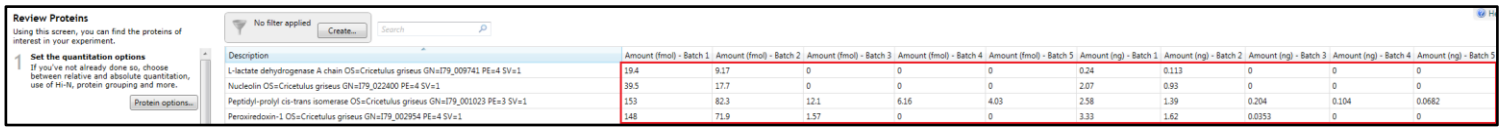
The ranking of peptide abundance is based on the integrated value across all the runs, allowed by the accurate alignment and lack of missing values. This gives added confidence in the peptide selection, taking all runs into

account to make the ranking robust. These Hi-N methods allow the relative and absolute comparison of proteins within the same run.

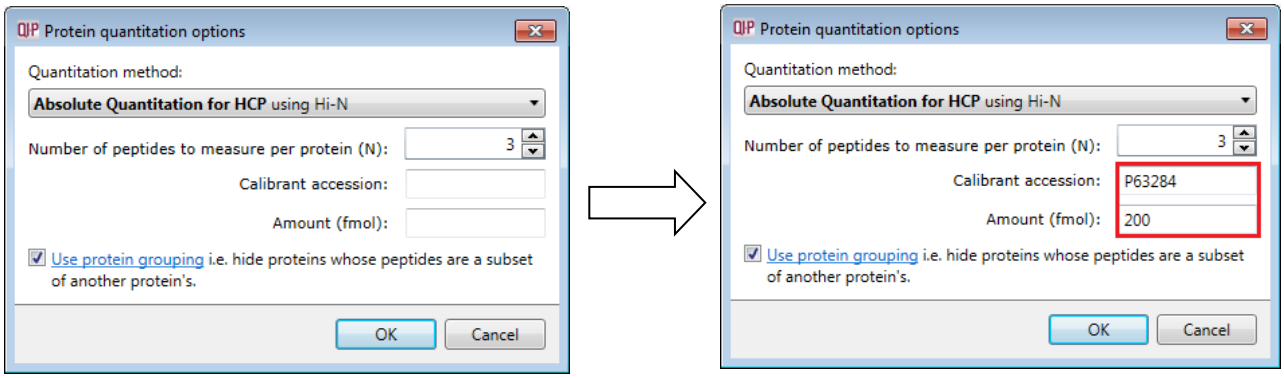
To generate values for **Absolute Quantitation for HCP** (Host Cell Proteins) select the 5th option on the drop down at the beginning of this section.

Note: there are 2 differences between this HCP-specific option and the Absolute Quantitation option:

- The mass of protein present is also reported in ng in addition to the amount in fmol
- The calculation of protein amounts and masses present is based on measurements of the calibrant protein present in the same run, and not a pooled measurement over all the runs. It does still assume a specified amount of a calibrant in every run. However, it does not pool information across the runs to derive its relationship between the calibrant and observed abundance. The amount of a contaminant is instead calculated directly by relation to the observed amount of the calibrant in the same run.



As for **Absolute Quantitation** enter the accession number and amount for the calibrant.



The absolute amounts, based on the calibrant used, are reported at the Review Proteins stage as additional columns (one for each condition) following the protein description.

How the values for the calibrant behave across runs comparing Absolute Quant and Absolute Quant for HCP using the same calibrant

Description	Amount (fmol) - Batch 1	Amount (fmol) - Batch 2	Amount (fmol) - Batch 3	Amount (fmol) - Batch 4	Amount (fmol) - Batch 5
Calreticulin OS=Cricetulus griseus GN=H671_3g9525 PE=3 SV=1	158	65.1	0.0418	1.92E-05	0.000136
Chaperone protein ClpB OS=Escherichia coli (strain K12) OX=83333 GN=clpB PE=1 SV=1	203	188	151	196	262
Elongation factor 1-alpha 1 OS=Cricetulus griseus GN=I79_009935 PE=4 SV=1	715	489	371	344	390

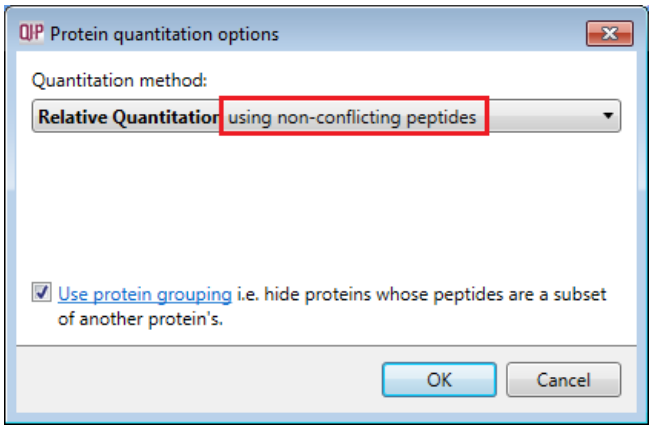
Description	Amount (fmol) - Batch 1	Amount (fmol) - Batch 2	Amount (fmol) - Batch 3	Amount (fmol) - Batch 4	Amount (fmol) - Batch 5	Amount (ng) - Batch 1	Amount (ng) - Batch 2	Amount (ng) - Batch 3	Amount (ng) - Batch 4	Amount (ng) - Batch 5
Calreticulin OS=Cricetulus griseus GN=H671_3g9525 PE=3 SV=1	155	68.3	0.0509	1.95E-05	0.000105	7.52	3.31	0.00246	9.44E-07	5.06E-06
Chaperone protein ClpB OS=Escherichia coli (strain K12) OX=83333 GN=clpB PE=1 SV=1	200	200	200	200	200	19.2	19.2	19.2	19.2	19.2
Elongation factor 1-alpha 1 OS=Cricetulus griseus GN=I79_009935 PE=4 SV=1	706	520	496	354	298	39.2	28.9	27.6	19.7	16.5

Note: When there are **peptide conflicts** (a peptide is shared between two proteins, for example) it is important to assign the signal correctly for absolute quantitation. To do this, Progenesis QI for proteomics carries out a two-step process. Firstly, Hi-N is carried out only on the N most abundant **unique** (non-conflicting) peptides of the proteins concerned. This provides a ratio estimate for the two proteins based only on unshared peptides. The abundance of any shared peptides is then divided and allotted in this ratio between the two proteins, and the full Hi-N calculation is then applied using the divided values for conflicted peptides.

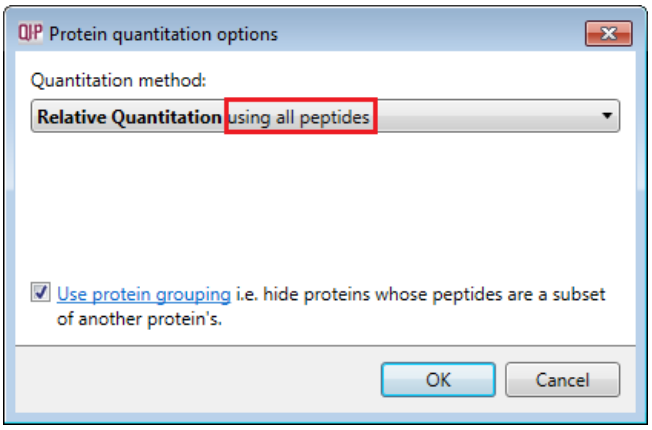
Naturally, if the conflicted peptides are not among the N most abundant in either protein initially, then this will not cause any difference in the result.

Relative Quantitation can also be performed comparing a single protein across all the runs using only the unique or non-conflicting peptides. Select the third method from the drop down

Using non conflicting peptides



The relative Quantitation can also be performed using all peptides.



Note: if you have performed conflict resolution then there will be no difference between these methods.

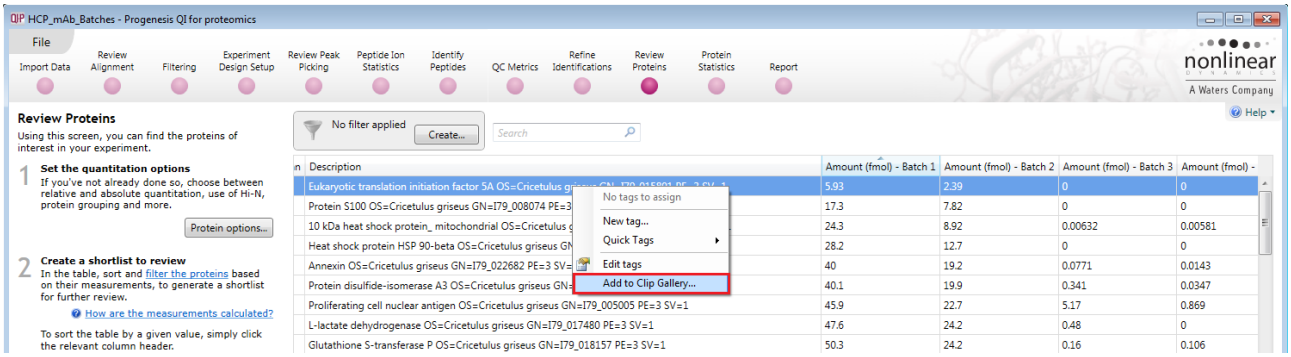
Now move to the Review Proteins section by clicking on **Review Proteins** icon on the workflow at the top of the screen.

Appendix 7: Using Clip Gallery to Save and Export Pictures and Data

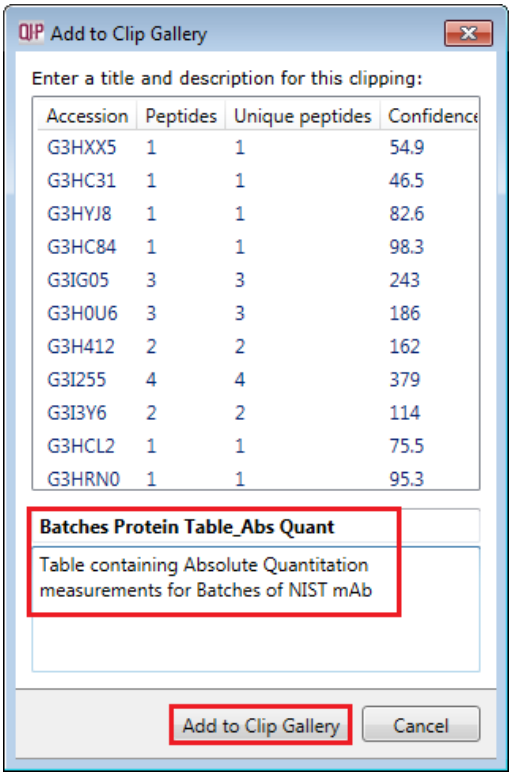
At nearly every stage of the Progenesis QI for proteomics workflow the views and data tables can be added to the Clip Gallery.

The saved images of the Views and the tables are retained as part of the experiment and are stored accordingly. This facility allows you to capture (high resolution) images that can be used in the development of specific reports and/or used as part of the process of publishing your experimental findings.

As an example of using the Clip Gallery, at the **Review Proteins** stage right click on the Protein Table and select **Add to clip gallery…**

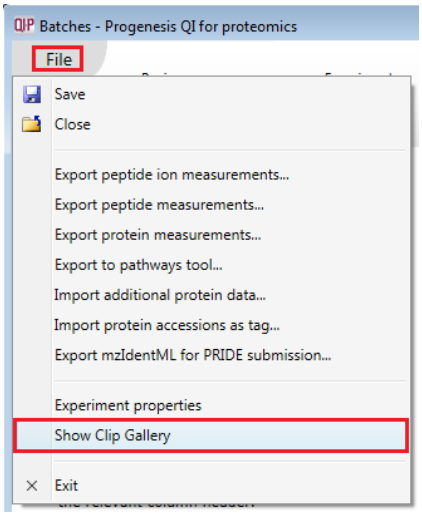


This will open a dialog displaying what is to be saved and allows you to alter the title and provide a description of the item for later reference.



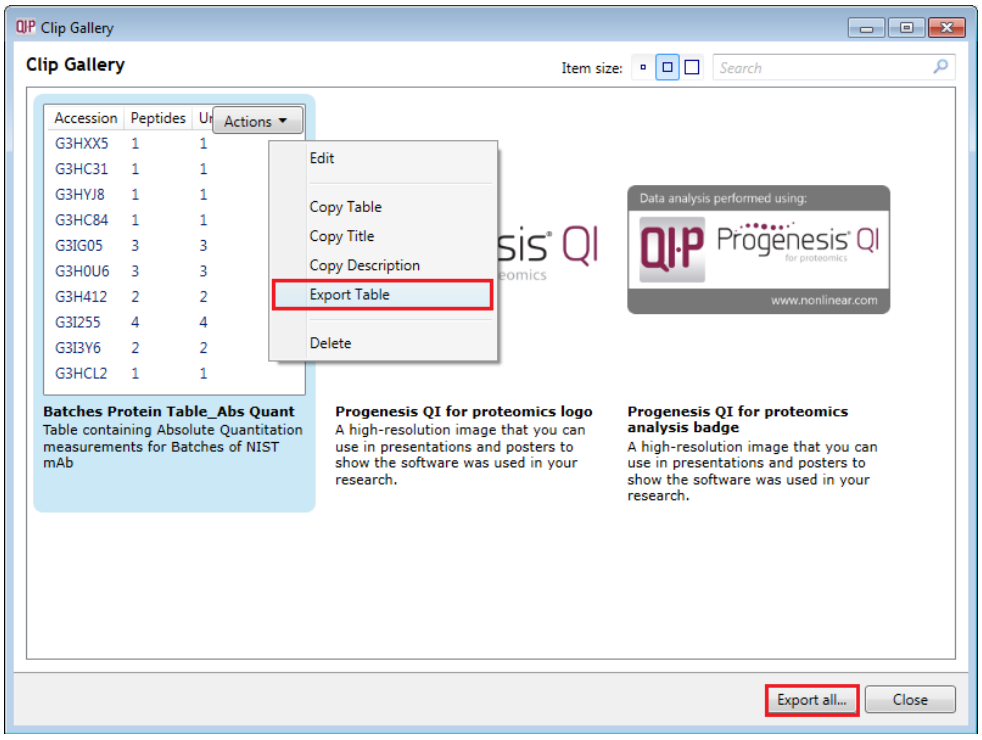
Enter details as required and click **Add to clip gallery**

To view, edit and/or export from the clip galley the gallery can be accessed from the **File** menu.



Click **Show Clip Gallery**

Selecting an item in the gallery makes available an **Actions** menu that allows you to manage the output of the item.



Note: there is also the capacity to **Export all…** the items in the experiments clip gallery which creates a list of files in a folder of your choice where the file name is based on the item title.

Note: right clicking on a table and adding it to the Clip Gallery allows you to export the current content to Excel as a .csv file.

References

Silva, JC, Gorenstein, MV, Li, G-Z, Vissers, JPC and Geromanos. Molecular and Cellular Proteomics (2006); 5 : 144-156 Absolute Quantification of Proteins by LCMS^{E}