

Progenesis QI for proteomics User Guide

Analysis workflow guidelines for DDA data

THE SCIENCE OF WHAT'S POSSIBLE.

Contents

Introduction	3
How to use this document	3
How can I analyse my own runs using LC-MS?	3
LC-MS Data used in this user guide	3
Workflow approach to LC-MS run analysis	4
Restoring the Tutorial	5
Stage 1: Import Data and QC review of LC-MS data set	6
Stage 2A: Automatic Processing of your data	7
Stage 2B: After Automatic Processing	12
Stage 3: Licensing	13
Stage 4: Review Alignment	14
Reviewing Quality of Alignment	15
Stage 5A: Filtering	18
Stage 5B: Reviewing Normalisation	22
Stage 6: Experiment Design Setup for Analysed Runs	25
Stage 7: Review Peak Picking and editing of results	28
Stage 8: Peptide Statistics on selected peptide ions	37
Stage 9: Identify peptides	41
Stage 10: QC Metrics	45
Stage 11: Refine Identifications	47
Stage 12: Resolve Conflicts	49
Protein Grouping	53
Protein Quantitation options	54
Stage 13: Review Proteins	56
Stage 14: Exporting Protein Data	60
Exporting Protein Data to Pathways Tool(s)	61
Stage 15: Protein Statistics	64
Stage 16: Reporting	65
Creating an Inclusion list	67
Congratulations!	68
Appendix 1: Stage 1 Data Import and QC review of LC-MS data set	69
Appendix 2: Stage 1 Processing failures	73
Appendix 3: Stage 1 Data QC review and addition of exclusion areas	75
Appendix 4: Licensing runs (Stage 3)	76
Appendix 5: Manual assistance of Alignment	77
Appendix 6: Within-subject Design	81
Appendix 7: Power Analysis (Peptide Stats)	83
Appendix 8: Using Clip Gallery to Save and Export Pictures and Data	84
Appendix 9 Search engine parameters (Stage 9) Mascot	86

Introduction

This user guide takes you through a complete analysis of 6 LC-MS runs with 2 groups (3 replicate runs per group) using the unique Progenesis QI for proteomics workflow. It starts with LC-MS data file loading then Alignment, followed by Analysis that creates a list of interesting peptide ions (peptides) which are explored within Peptide Ion Stats using multivariate statistical methods then onto Protein identity and Reporting.

To allow ease of use the tutorial is designed to start with the restoration of an Archived experiment 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.

If you are using your own data files please refer to Appendix 1 (page 69) then start at page 7.

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 (dependant on PC spec) and is divided into two sections. 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. Alternatively if you would like to arrange a demonstration in your own laboratory contact <u>support@nonlinear.com</u> and we will help you.

LC-MS Data used in this user guide

NLD would like to thank Dr Robert Parker and Prof Haroun Shah at the Health Protection Agency, London, UK for providing the example data used in this user guide as well as invaluable discussion on the handling of the data.

Workflow approach to LC-MS run analysis

Progenesis QI for proteomics adopts an intuitive **Workflow** approach to performing comparative LC-MS data analysis. The following user guide describes the various stages of this workflow (see below) focusing mainly on the stages from Alignment to Report.

Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report
•	0		•	•	0	•	•		0			0

Stage	Description	Page
	LC-MS Import Data: Selection and review of data files for analysis	6
Import Data	Automatic Processing: setting up steps for automatic processing	7
	After Automatic Processing: how to work with auto analysed data	12
Licensing	Licensing : allows licensing of individual data files when there is no dongle attached (Appendix 3)	13
Review Alignment	Review Alignment: automatic and manual LC-MS run alignment	14
Filtering	Filtering : defining filters for peaks based on Retention Time, m/z, Charge State and Number of Isotopes.	18
	Review Normalisation: exploring LC-MS normalisation	22
Experiment Design Setup	Experiment Design Setup: defining one or more group set ups for analysed aligned runs	25
Review Peak Picking	Review Peak Picking : review and validate results, edit peak detection, tag groups of peaks and select peaks for further analysis	28
Peptide Ion Statistics	Peptide Ion Statistics : performing multivariate statistical analysis on tagged and selected groups of peptide ions	37
Identify Peptides	Identify Peptides : managing export of MS/MS spectra to, and import of peptide ids from Peptide Search engines.	41
QC Metrics	QC metrics: quality control charts for experimental/analysed data	45
Refine Identifications	Refine Identifications: manage filtering of peptide ids	47
Resolve Conflicts	Resolve Conflicts : validation and resolution of peptide id conflicts for data entered from Database Search engines	49
Review Proteins	Review proteins: review protein and peptide identity and data export	56
Protein Statistics	Protein Statistics: multivariate statistical analysis on proteins	64
Report	Report: generate a report for proteins and/or peptides	65

Waters

Restoring the Tutorial

Open Progenesis QI for proteomics and download the Compressed (.zip) Tutorial Archive file from the 'User guide and tutorial' link shown below, placing it in a **new folder** on your desktop. Before restoring the tutorial in the software **you must** first right click on the (.zip) file and extract it to the same folder.

Now you can restore the uncompressed Tutorial archive file. To do this, first locate the **Progenesis QIP_Tutorial DDA.Progenesis QIP Archive** file using the **Open** button and press Open.

QP Progenesis QI for proteomics			
File			nonlinear
Experiments			A Waters Company
Perform analysis Combine analysed fr QIP Open Experiment QP QP A Progenesis QLp v2 > Proge		Ql.p Tutoria 👂	New to Progenesis QI for proteomics? Here are some resources to help you get started with Progenesis QI for proteomics: • The Progenesis QI for proteomics workflow
Organize 🔻 New folder	911 ·	• 🔳 🔞	User guide and tutorial data Frequently-asked guestions
ShareFile	Name Date modified Progenesis QLP_Tutorial DDA.Progenesis 05/10/2014 19:2	Type 7 Progenesis	Progenesis at HUPO 2014
E Desktop E			Are you heading to Spain for this year's global HUPO gathering? Join us for a look at Progenesis QI for proteomics v2.0.
Documents Music Pictures			HUPO 2014, Madrid, Spain 5-8 October 2014
Videos Andy Borthwick Computer			Latest blog posts • <u>Out now - Progenesis QI for</u> proteomics v2.0
🗣 Network 📴 Control Panel 👻 🗸	m	•	 How to save your samples before it's too late!
File name: Progenesis QI.	P_Tutorial DDA.ProgenesisQIPArchive Experiments and Arr	chives (*.Prc ▼ Cancel	 Back to basics - No missing values "Making decisions is easy. Getting them right is the hard part." Three years and a million thanks!
Other experiments		2.0.5387.52102	

This opens the 'Import from archive' dialog.

Select the **Create a new experiment** option and select the folder in which you placed the archive, using the icon (to the right).



nport from	n archive	
Import	Progenesis QI.P_Tutorial DE)A from archive
🔘 Rep	lace an existing experiment	
Experin	nent to replace: Progenesis QI.P	_Tutorial DDA 🔹
Creating	te a new experiment	
Name:	Progenesis QI.P_Tutorial DDA	
Folder:	mer Data\Progenesis QI.p v2_Tu	torials and Demo Suites\Progenesis QI.p Tutorial for DDA
		Import Cancel
		Import Cancel

Then press Import.

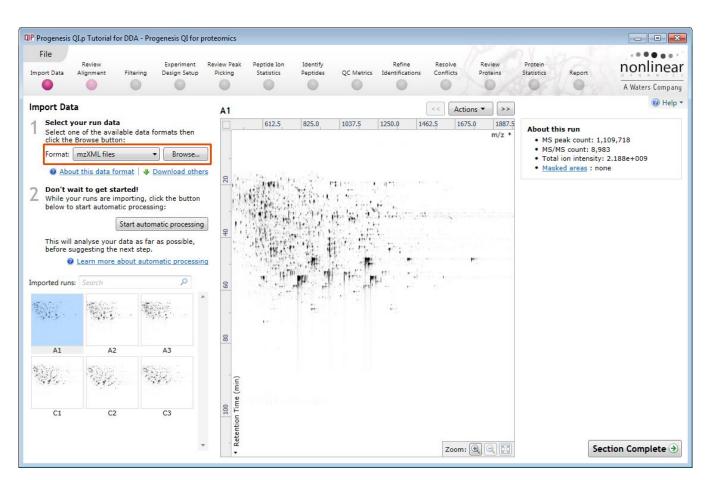
Loading: Progenesis QI.P_Tutorial DDA

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

The tutorial will now open at the Import Data stage (see below).



Each data file appears as a 2D representation of the run. At this stage you will be warned if any of the data files have been 'centroided' during the data acquisition and conversion process.

Note: the **Experiment Properties** are available from the File menu. These were selected when the experiment was created (see Appendix 1, page 69).

Tip: the **'Exclude areas from selected run'** facility allows you to examine and exclude areas (usually early and/or late in the LC dimension (Retention Time)) that appear excessively noisy due to capture of data during column regeneration (see Appendix 3, page 75). This is not required for this data set.

<<	Acti	ons 🔹 >			
1500	Mask areas for peak picking				
	×	Remove run	Delete		
	_		 rotal ion intensity: 2.1 <u>Masked areas</u>: none 	88e+009	

OP Experiment Properties

Runs in this experiment: 6

Peak processing: Profile data

Machine resolution: High resolution

Note: use the Remove Run to remove run(s) from the current experiment.

Now start the Automatic Processing.

×

Close

Stage 2A: 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.

-	Perform automatic processing Click the button below to start automatic processing. This will analyse your data as far as possible, before suggesting the next step.			
	Start automatic processing			
	Automatic processing can be started while runs are importing.			
	Learn more about automatic processing			

Setup of processing steps in the Analysis Workflow, up to and including Identify Peptides, can be performed in the Automatic Processing wizard, these include:

- Automatic alignment of all runs to a reference run
- Automatic peak picking
- Creating an Experiment design
- Choosing a Quantitation method
- Identification of peptides (only available for MSe and HDMSe data formats)

In this tutorial example you have 6 Data Dependently Acquired (DDA) LC-MS runs, so the automatic identification of peptides is unavailable in the automatic processing.

As the runs have already been imported, click **Start automatic processing** to setup the Automatic processing wizard.

QPP Start automatic processing	
Select an alignment reference To compensate for drifts in retention time, all runs in the ex to a single reference run.	periment must be aligned
How do you want to choose your alignment reference?	
Assess all runs in the experiment for suitability	
\bigcirc Use the most suitable run from candidates that I select	
🔘 Use this run:	
🗌 A1 👻	
For information on choosing the alignment reference, and w select your own candidates, please see the <u>online guidance</u> .	
< Back	Next > Cancel

Progenesis QI for proteomics provides three methods for choosing the alignment reference run:

1. Assess all runs in the experiment for suitability

This method compares every run in your experiment to every other run for similarity, then select the run with the greatest similarity to all other runs as the alignment reference.

If you have no prior knowledge about which of your runs would make a good reference, then this choice will normally produce a good alignment reference for you. This method, however, can take a long time for a large number of runs.

2. Use the most suitable run from candidates that I select

This method asks you to make a selection of reference candidates; the automatic algorithm then chooses the best reference from this subset of runs.

This method is appropriate when you have some prior knowledge of your runs suitability as references: i.e when all the candidate runs are pooled samples or

i.e when all the candidates are from a condition that displays the largest set of common peptide ions.

3. Use this run

This method allows you to manually choose the reference run.

Manual selection gives you full control, but there are a couple of risks to note:

- If you choose a pending run which subsequently fails to load, alignment will not be performed.
- If you choose a run before it fully loads, and it turns out to have chromatography issues, alignment will be negatively affected (for this reason we recommend that you let your reference run fully load and assess its chromatography before loading further runs).

For this tutorial we will select the first option (See Appendix 1, page 69 for more details on using the other options).

You will now be asked if you want to align your runs automatically.

QIP Start automatic processing	- • ×
Automatic alignment After selecting the experiment's alignment reference, the software of automatically align all runs.	can also
After the alignment reference is chosen, do you want to start automatic Ves, automatically align my runs	: alignment?
< Back Next :	Cancel

The default is for automatic alignment, click Next.

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

QP Start automatic processing
Peak picking Peak picking is the process by which we locate the peptide ions and their isotopic peaks in your samples.
After the automatic alignment is finished, do you want to start automatic peak picking? Perform peak picking Set parameters
< Back Next > Cancel

For the purposes of this User guide we will use the default settings for peak picking except in the case of Retention Time limits where we will limit the peak picking to between 10 and 75 min.

Note: for more details on setting Peak Picking parameters refer to the section on Filtering (page 18) Click on **Set parameters** and select the fourth tab to set **Retention time limits** for the detection. The default limits are displayed in the unticked boxes and detection will use these values whenever the box(s) are unticked. Enter values of 10 and 75 min and tick the boxes as shown below.

Peak Picking Paramete	ers				IP Peak Picking Paramete	ers		
Runs for peak picking	Peak picking limit	s Maximum charge	Retention time limits		Runs for peak picking	Peak picking limits	Maximum charge	Retention time li
Choose runs for p	eak picking -				Retention time li	mits		
ou can tick or un-tic ontrol which will be		📝 Run			You can set the minin maximum retention ti			
eak picking algorithr	n. Although any	✓ A1			picking. Ions that elut	te before or	Ignore ions befor	e 10 mir
un which is left un-ti affect the feature out	lines, it will still	✓ A2			after these values will		Ignore ions after	75 mir
have outlines added t available in the exper	o it and will be	A3				L		
setup.		✓ C1						
Learn more about wh	y you might not	 ✓ C2 ✓ C3 						
want to select all runs	5.	✓ C3						
			OK Cance	:				ОКС

Click **OK** to return to the Automatic Processing Wizard and click **Next** to Define an Experiment design.

OP Start automatic processing						
Experiment design Experiment designs allow you to group and compare your samples according to their experimental conditions.						
By defining an experiment design in advance, statistical measures such as ANOVA can be calculated automatically.						
Set up an experiment design						
Enter a name for the experiment design:						
AC						
Load the <u>criteria for grouping runs</u> rom this file:						
Browse						
Group runs by: <pre><no groups="" valid=""></no></pre>						
< Back Next > Cancel						

To handle the grouping of your run data you can make use of **sample tracking information** that has been stored in a spread sheet at the time of sample collection and/or preparation.

Note: if a spreadsheet file of your data is not available you can create your experiment designs after the automatic analysis of the runs

For this example there is a **Tutorial Groups.csv** file available with the Tutorial Archive you restored at the beginning of this tutorial exercise.

Sample Name	Conditions	Date of Collection	Location
A1	А	02/03/2011	Fridge A
A2	Α	02/03/2011	Fridge A
A3	А	02/03/2011	Fridge B
C1	С	06/03/2011	Fridge B
C2	С	06/03/2011	Fridge B
C3	С	06/03/2011	Fridge A

Give the experiment design a name (i.e. AC) and then use the **Browse** function to locate the Tutorial Groups.csv file.

The 'Group runs by' drop down will update to reflect the possible fields in the csv file that you can use to 'group' your runs by: Conditions, Date of collection or Location.

QIP Start automatic pr	rocessing	•					
Experiment design Experiment designs allow you to group and compare your samples according to their experimental conditions.							
	By defining an experiment design in advance, statistical measures such as ANOVA can be calculated automatically.						
🔽 Set up an expe	eriment design						
Enter a name fo	or the experiment design:						
AC		~					
	ia for grouping runs from this file: .borthwick\Documents\Customer Data\Progenesis QI.p_Tutc	Browse					
		browsen					
Group runs by:	Conditions	-					
	Date of Collection						
	Location	- H					
	< Back Next >	Cancel					

Select Conditions and then click Next.

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

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.

QP Start automatic processing	OP Start automatic processing
Protein Quantitation If you've included a known amount of a calibrant protein in each of your samples, you can calculate the absolute amounts of each identified protein.	Protein Quantitation If you've included a known amount of a calibrant protein in each of your samples, you can calculate the absolute amounts of each identified protein.
Quantitation method:	Quantitation method:
Relative Quantitation using Hi-N	Relative Quantitation using Hi-N
Number of peptides to measure per protein (N):	Absolute Quantitation using Hi-N • Requires a calibrant protein to calculate absolute amounts • Uses the most abundant N peptides • Allows comparison between proteins within a run
	Relative Quantitation using Hi-N Uses the most abundant N peptides Allows comparison between proteins within a run
	Relative Quantitation using non-conflicting peptides • Uses only peptides which have no conflicting protein identifications • Allows comparison of a single protein across runs
Group proteins using the parsimony principle, i.e. describe the minimum set of protein sequences that account for all observed peptides.	Relative Quantitation using all peptides • Uses all peptides identified as part of a protein • Allows comparison of a single protein across runs sequences that account for all observed peptides.
< Back Finish Cancel	< Back Finish Cancel

Use the drop down to reveal the alternative methods for protein quantitation

Select the Default option Relative Quantitation using Hi-N and click Finish.

The Alignment process starts with the automatic selection of C1 as the alignment reference

✓ Importing runs: 6 of 6 processed Selecting reference: Choosing an alignment reference Aligning runs: Pending Peak picking: Pending Creating design: Pending Protein quantitation: Pending Cancel	QIP Automatic Processing Automatic processi Current step: Choosing a	ing	×			
Creating design: Pending Protein quantitation: Pending ✓ Selecting reference: C1 ♥ Aligning runs: 4 of 5 processed Peak picking: Pending Creating design: Pending Protein quantitation: Protein quantitation: Protein quantitation: Pending	Selecting reference: Aligning runs: Peak picking:	Choosing an alignment reference Pending Pending		Automatic process Current step: Aligning 'C	ing 2'	<u>x</u>
		-	Cancel	 Selecting reference: Aligning runs: Peak picking: Creating design: 	C1 4 of 5 processed Pending Pending	

Once Alignment completes Peak Picking commences

QIP Automatic Processing Automatic process Current step: Analysing	ing			7			
 Importing runs: Selecting reference: Aligning runs: Peak picking: 	6 of 6 processed C1 5 of 5 processed Picking			QIP Processing Complete Automatic processi Time taken: 3 minutes 3	• •		X
Creating design: Protein quantitation:	Pending Pending	Cance	21	 Importing runs: Selecting reference: Aligning runs: Peak picking: Creating design: Protein quantitation: 	6 of 6 processed C1 5 of 5 processed 14624 peaks found Created Relative Quantitation o	using Hi-3	
						Close	Identify Peptides 🌖

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

When Processing completes, depending on what stages you selected to perform, the Wizard displays (on the right) what stage the workflow will open at.

In this example, as the data is DDA it is going to open at **Identify Peptides** if all possible processing steps are selected.

You can either:

- Continue with the analysis, as the Processing dialog is not displaying any warnings, and perform **Identify Peptides**. In which case you can go to page 41
- Open the analysis at Identify Peptides and immediately return to the Review Alignment stage by clicking on it in the Workflow to review alignment quality (page 14)
- Alternatively you can Close the dialog. This will not move you to a later stage in the workflow but
 instead will allow you to review the 'Run Chromatography' then navigate systematically through all
 the stages yourself or jump from stage to stage.

File									12	1	W al	L.F.	
Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	nonlinear
•	F	•	•	0	0	•	•	•	0		•	190	A Waters Company
Identify					MS/MS Spe	ctra							

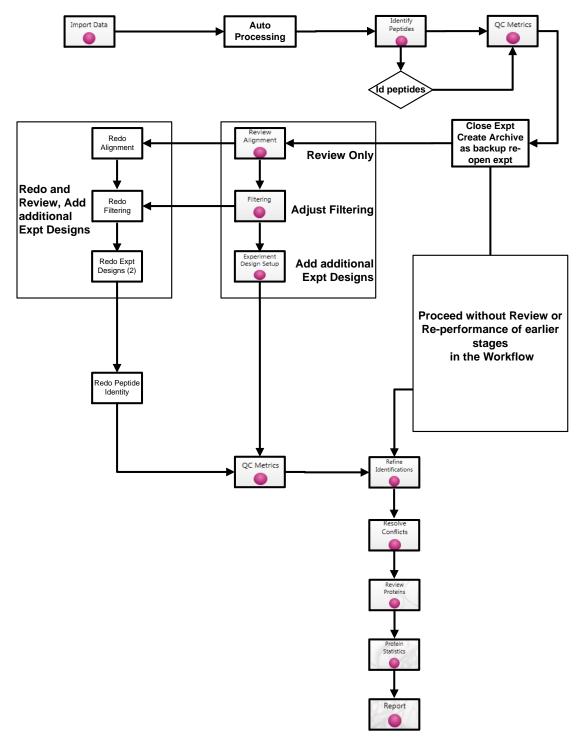
Note: if processing fails to complete successfully there are a number of suggested strategies you can use to proceed with your analysis. These are outlined in Appendix 2 (page 73)

The next stage in this document gives you a graphical view of how to proceed with your analysis following automatic processing.

Stage 2B: After Automatic Processing

When Processing completes, depending on what stages you selected to perform, the Automatic Processing Dialog displays what stage the workflow will open at.

The flow chart gives you an overview of the various steps you can take to proceed with your automatically processed data.



In this example, as the data is DDA it is going to open at Identify Peptides so you can either:

- Continue with the analysis, as the Processing dialog is not displaying any warnings, and perform **Identify Peptides**. In which case you can go to page 41
- Open the analysis at Identify Peptides and return to the Review Alignment stage by clicking on it in the Workflow (page 14).

Stage 3: Licensing

This stage in the analysis workflow will **only** appear if you are using 'Unlicensed' data files to evaluate the software and have no dongle attached.



If you have performed an analysis using Automatic Processing without a valid dongle or do not have the appropriate code to licence your runs, and you close Progenesis QI for proteomics you will be warned that the analysis will be lost.

File	Licensing	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	A Waters Compar
ongle	License	Runs												
	tallation is driven the state of the state o	currently re	estricted to	analyse	Run name	•							Licence state	License this run
Tolicon		ns, vou nee	d an ava	luction	C:\Users\a	andy.borthwid	k\Documer	nts\Customer	Data\Progene	sis QI.p_Tu	torials and De	mo Suites\QI,	p Unlicensed	J 🗸
				om a sales	C:\Users\a	andy.borthwid	k\Documer	nts\Customer	Data\Progene	sis QI.p_Tu	torials and De	emo Suites\QI,	p Unlicensed	
represer	ntative.				C:\Users\a	andy.borthwid	k\Documer	nts\Customer	Data\Progene	sis QI.p_Tu	torials and De	erno Suites\QI,	p Unlicensed	
Once lice	ensed vo	ur runs can	he analy	sed on	C:\Users\a	andy.borthwid	k\Documer	nts\Customer	Data\Progene	sis QI.p_Tu	torials and De	emo Suites\QI;	p Unlicensed	
any insta	allation of t	he software	e. The lice	ence is	C:\Users\a	andy.borthwid	k\Documer	nts\Customer	Data\Progene	sis QI.p_Tu	torials and De	emo Suites\QI,	p Unlicensed	
automatically included when archiving an experiment.				C:\Users\a	andy.borthwid	k\Documer	nts\Customer	Data\Progene	sis QI.p_Tu	torials and De	mo Suites\QI,	p Unlicensed		
compute	er, <u>click her</u>	een license re to make f			QIP Una	ble to save (experiment					×		
compute available If you ha install.	er, <u>click her</u> e on this co ave one, yo	re to make t	the licence	es <u>file</u> to	QP Una		t save the		hout a valid li	icense. If ye	ou close nov			
compute available If you ha install.	er, <u>click her</u> e on this co ave one, yo	re to make f imputer. iu can <u>open</u>	the licence	es <u>file</u> to	OP Una	You canno	t save the		hout a valid li	icense. If yo OK	ou close nov	v your		

Note: although the analysis will be lost (Alignment Peak Picking etc) the experiment file and all the 'loaded pre-processed runs are retained'. This allows you to reopen the experiment, when you have a licenced code and/or dongle available, and redo the analysis steps without having to reload the data.

For more details on managing the licencing of your data refer to Appendix 4 (page 76)

If you are using the tutorial archive, this page will not appear as the data files are licensed.

Stage 4: Review Alignment

At this stage Progenesis QI for proteomics Alignment opens displaying the alignment of the runs to the Reference run (C1).

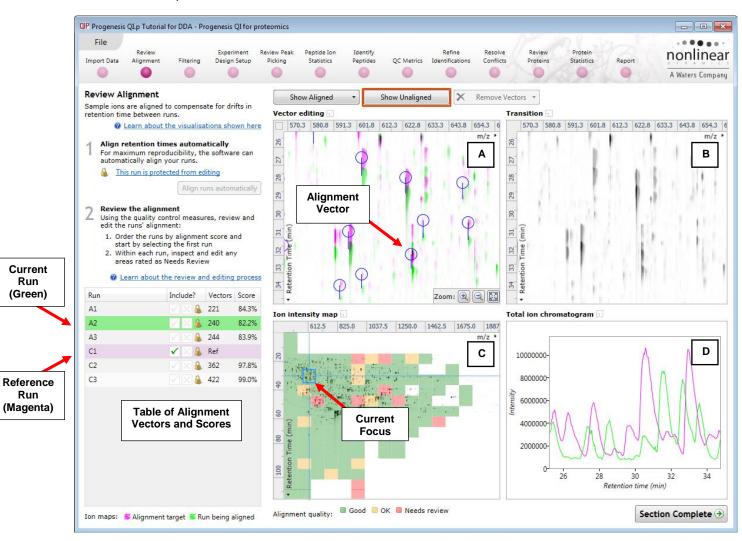
Layout of Alignment

To familiarize you with Progenesis QI for proteomics Alignment, this section describes the various graphical views used in the alignment of the LC-MS runs

To setup the display so that it looks similar to the one above:

• In the Run table click on Run A2 to make it current. You will now be looking at the alignment of A2 to C1 in the unaligned view. Now drag out an area to review on the **Ion intensity map**. The other 3 views will update to reflect the new focus.

Run	Include?	Vectors	Score
A1		221	84.3%
A2 A3	🗸 📈	240	82.2%
		244	83.9%
Cl	\checkmark \times	Ref	
C2		362	97.8%
C3	X	422	99.0%



Vector Editing (Window A): is the main alignment area and displays the area defined by the current **focus** rectangle shown in Window C. The current run is displayed in green and the reference run is displayed in magenta. Here is where you can review in detail the vectors and also place the manual alignment vectors when required.

Transition (Window B): uses an **alpha blend** to animate between the current and reference runs. Before the runs are aligned, the peptide ions appear to move up and down. Once correctly aligned, they will appear to pulse. During the process of adding vectors, this view can be used to zoom in and also navigate thus helping with accurate placement of manual vectors.

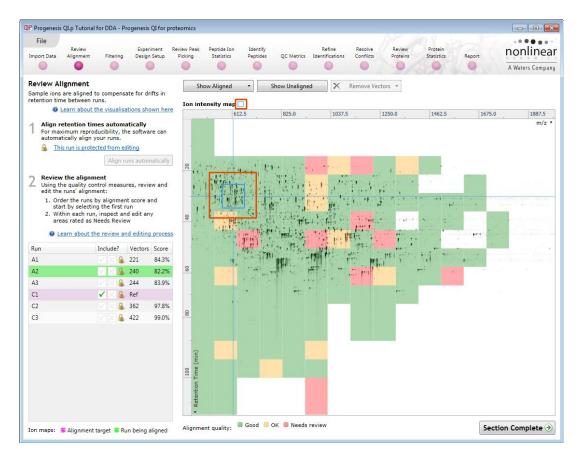
Ion Intensity Map (Window C): shows the **focus** for the other windows. When you click on the view the blue rectangle will move to the selected area. The focus can be moved systematically across the view using the cursor keys. The focus area size can be altered by clicking and dragging out a new area with the mouse. This view also provides a visual quality metric for the Alignment of the runs, from green through yellow to red.

Total Ion Chromatogram (Window D): shows the current **total ion** chromatogram (green) overlaid on the Reference chromatogram (magenta). As the peptide ions are aligned in the **Vector Editing** view the chromatograms become aligned. The retention time range displayed is the vertical dimension of the Focus Grid currently displayed in the **Ion Intensity map** (Window C).

Reviewing quality of alignment vectors

After **Automatic alignment** the number of vectors and Quality Scores will be updated on the **Runs** panel and the vectors will appear (in blue) on the **Vector editing** view.

If the alignment has worked well then the Ion Intensity Map will appear predominantly green and a score of greater than 80% will be reported in the table. Also when **Show Aligned** is selected, in Window A (vector editing) vector length should appear minimal and in Window B (Transition) will show peptide ions pulsing slightly but not moving up and down.



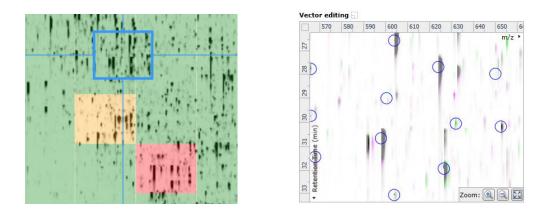
Note: you can use the icon to the right of the panel name to expand or contract each view

Reviewing Quality of Alignment

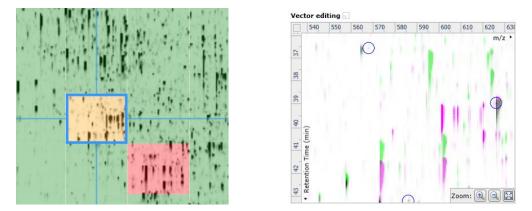
At this point the quality metric, overlaid on the Ion Intensity Map as coloured squares, acts as a guide drawing your attention to areas of the alignment. These range from Good (Green) through OK (Yellow) to Needs review (Red). When reviewing individual squares double click on a coloured square to set the focus.

Three example squares are examined here.

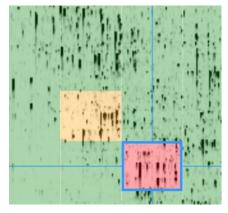
For a 'green' square, the majority of the data appears overlapped (black) indicating good alignment. When viewed in the Transition view the data appears to pulse.

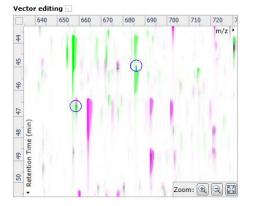


For a 'yellow' square, some of the data appears overlapped (black) indicating OK alignment. When viewed in the Transition view some of the data appears to pulse.



For a 'red' square little of the data appears overlapped (black) indicating questionable alignment. When viewed in the Transition view little data appears to pulse.

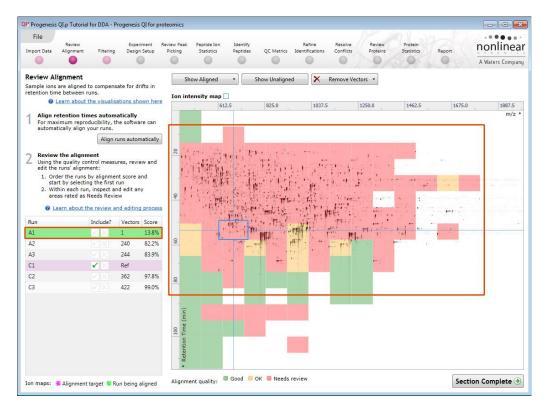




Note: the coloured metric **should be used as a guide**. In cases where there are a few 'isolated' red squares this this can also be indicative of 'real' differences between the two runs being aligned and should be considered when examining the overall score and surrounding squares in the current alignment.

The weighted average of the individual squares gives the overall percentage score for each run alignment.

Note: a marked area of red combined with a low score clearly indicates a 'miss alignment' and may require some manual intervention (see Appendix 5, page 77).



Having performed the analysis automatically, in the course of reviewing the quality of alignment you decide that the alignment requires editing then you can unlock the analysis.

To do this click on the **This run is protected from editing** link above the 'greyed out' **Align runs automatically** button.

Review Alignment Sample ions are aligned to compensate for drifts in	
retention time between runs.	QIP Protected from editing
Learn about the visualisations shown here	
Align retention times automatically For maximum reproducibility, the software can automatically align your runs. This run is protected from editing Align runs automatically	Discard analysis for all runs? Changing the alignment reference will reset ALL analysis, requiring alignment, peak picking and all subsequent analysis to be repeated. If you want to backup the current analysis state, you should archive this experiment before resetting the reference choice.
 Review the alignment Using the quality control measures, review and edit the runs' alignment: Order the runs by alignment score and start by selecting the first run Within each run, inspect and edit any areas rated as Needs Review Learn about the review and editing process 	Discard all analysis and continue Cancel

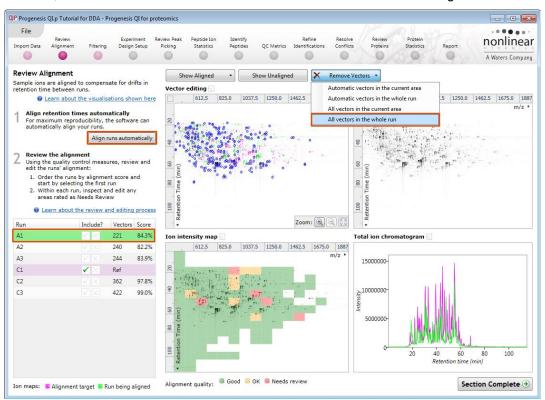
Note: as you click on the link, you will be warned that you are discarding the current analysis (all steps beyond alignment).

Details on editing alignment are described in Appendix 5 (page 77)

The alignment quality of this tutorial data set does not require any manual intervention.

If you have chosen to discard the current automatic analysis and have been exploring the alignment of one or more of the runs using manual vectors (for the purposes of this tutorial) make sure you first remove all manual vectors and then re-perform the Automatic alignment.

To do this for A1, first select Remove 'All vectors in the whole run' and then click Align runs automatically.



Having re-performed the Automatic alignment the Vectors and scores will appear as shown above.

Since you have unlocked your analysis you must now perform the Peak Picking that was originally performed as part of the Automatic processing.

To move to Peak Picking click on Filtering on the Workflow or Section Complete (bottom right).

Stage 5A: Filtering

The Peak picking Parameters dialog opens if Picking has not been performed if it has been performed move to section 5B.

Peak Picking Parameters

The Peak Picking Parameters dialog opens, showing all the runs in the current experiment and a tick against each run. This is the default setting, where the peak picking algorithm uses information from all of the runs to contribute to the pattern of peptide ion outlines.

QIP Peak Picking Parameters	×	P Peak Picking Parameters				×
Runs for peak picking Peak picking limits	s Maximum charge Retention time limits	Runs for peak picking Peak	ak picking limits	Maximum charge	Retention time limits	L
control which will be used by the peak picking algorithm. Although any run which is left un-ticked will not affect the peptide ion outlines, it will still have outlines added to it and will be available in the experiment design setup.	 ✓ Run ✓ A1 ✓ A2 ✓ A3 ✓ C1 ✓ C2 ✓ C3 	Sensitivity You can adjust the sensitiv the peak picking algorithm these different methods. Ei sensitivity method examine intensities of groups of MS to judge whether they are form part of an ion or whet they represent noise and s should be ignored. Peaks ti rejected as noise will not b to build ion outlines.	n using Each S peaks e likely to ether so that are be used		ivity method uses a orithm to determine e data. The higher the more peptide	
	OK Cancel	Chromatographic pea The chromatographic peak gives the length of time ov which an ion has eluted. If a minimum peak width, an that has eluted over a shor period will be rejected.	ak width k width ver if you set ny ion	fewer def	ault more 3 cak width OK Cance	
Wators						18



Tip: It may be appropriate **only** to pick peaks that are present in a limited number of your runs. In which case un-tick the runs that you do **NOT** want to contribute to the peptide ion detection pattern. This may be important when one or more of the 'replicate' runs appear noisy due to non-optimal chromatography or sample handling.

Note: peptide ions outlines will be added to 'un-ticked' runs; although these runs will **not** contribute to the peak picking pattern.

Tip: depending on run quality, a suggested minimum number of ticked runs should include at least one replicate of each experimental condition.

The sensitivity of the peak picking can be controlled by adjusting settings under the **Peak picking limits** tab.

Note: for all 3 **Sensitivity** modes a Chromatographic peak width (Retention time window) for the peaks **can** be set by applying a minimum retention window or peak width in minutes.



For the runs in this user guide we will use the default settings for the Automatic method and NOT apply a minimum peak width (as shown above).

The third tab allows you to set the **maximum charge** of the peptide ions, which will be detected. The default setting is a charge state of 20. For this example, leave this set as default.

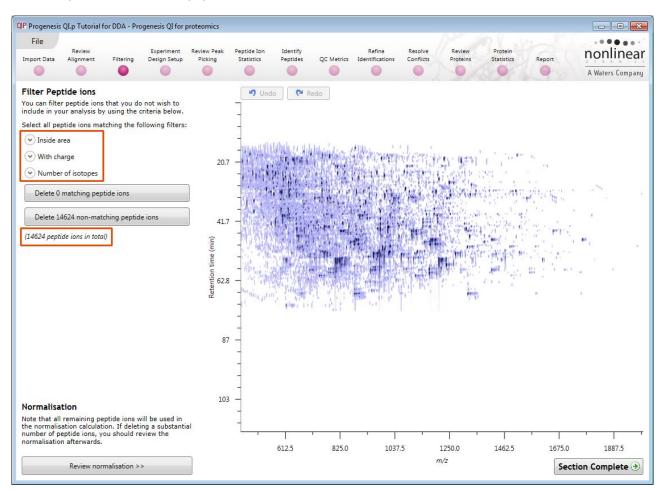
Note: you can either, leave this set as default and remove the high charge state peptide ions at the Filtering stage or you can choose not to detect them in the first place by reducing the charge state threshold here.

Finally, you can set **Retention time limits** for the detection. The default limits are displayed in the unticked boxes and detection will use these values whenever the box(s) are unticked. Enter values of 10 and 75 min and tick the boxes as shown below.

QIP Peak Picking Parameters	QIP Peak Picking Parameters
Runs for peak picking Peak picking limits Maximum charge Retention time limits	Runs for peak picking Peak picking limits Maximum charge Retention time limits
Maximum allowable charge	Retention time limits
You can set the maximum charge of ions to be detected. Ions with a charge greater than this value will be rejected.	You can set the minimum and maximum retention time for peak picking. Ions that elute before or after these values will be ignored.
	✓ Ignore ions after 75 minutes
OK Cancel	OK Cancel

Click **OK** to start the detection process.

On completion of detection, the Filtering stage will open displaying the number of peptide ions detected, in this example there are 14624 peptide ions.



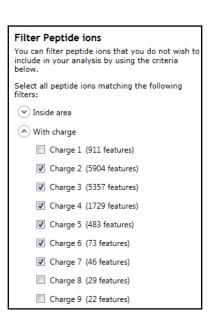
If required you can remove peptide ions based on position, charge state, number of isotopes or combinations of these peptide ion properties.

As an example, we will filter the peptide ions based on charge 'charge state'.

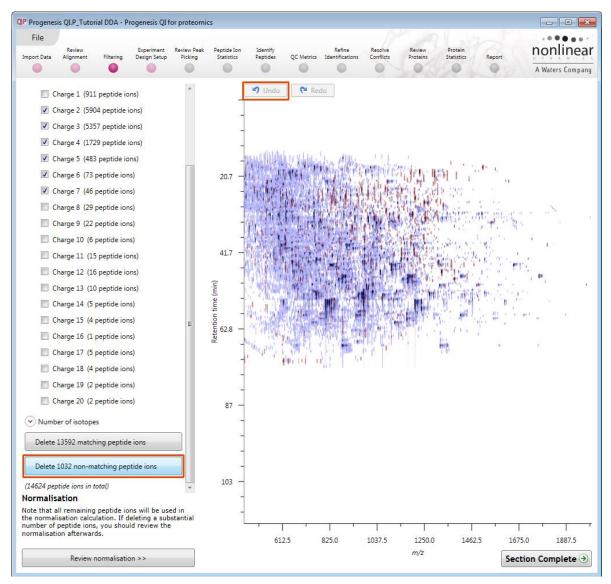
When **With charge** is selected the number of peptide ions present at each charge state is displayed, these can be selected accordingly. In this case we will retain peptide ions with a charge state of 2 to 7.

Area limits, charge state and number of isotopes can be combined to refine the peptide ion selection.

Tip: when filtering on only one property of the peptide ion i.e. charge state, make sure you have 'collapsed' the other filters (see right)



Hence all peptide ions with a charge state of 1 or 8 and above will appear red on the main view as you hold the cursor over the appropriate delete button.



To remove these peptide ions press Delete 1032 Non Matching Peptide ions.

You can use the **Undo** button to bring back deleted peptide ions, however, when you move to the next section you will lose the capacity to undo the filter. Before moving on from filtering you can review the normalisation of the data.

Tip: When you have reached the filtering stage, it is good practice to close the experiment and save an archive. This can be used to restore the unfiltered state if the filtering you have performed is too 'stringent'.

Stage 5B: Reviewing Normalisation

Review normalisation is accessed from the button at the bottom left corner of the filtering page.

Normalisation Note that all remaining peptide ions will be used in the normalisation calculation. If deleting a substantial number of peptide ions, you should review the normalisation afterwards.	103 -	612.5
Review normalisation >>		

If you have filtered out a number of peptide ions from the original detection pattern then the normalisation will update.

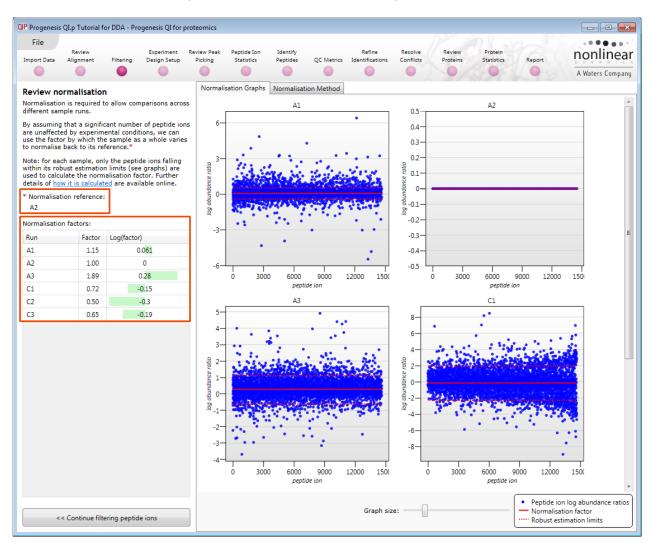
Recalculating normali	sation	

The **Review Normalisation** page will open displaying plots for the normalisation of all the peptide ions on each run.

This page in the workflow **does not** allow you to alter the Normalisation of your data but provides you with individual views for each run showing the data points used in the calculation of the normalisation factor for the run.

Alternatively, if you do not believe normalisation is necessary, you can opt to 'Don't use any normalisation' for the rest of the analysis (Normalisation Method tab).

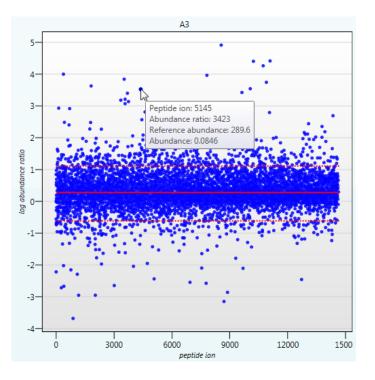
Normalisation factors are reported in the table to the left of the plots.



Calculation of Normalisation Factor:

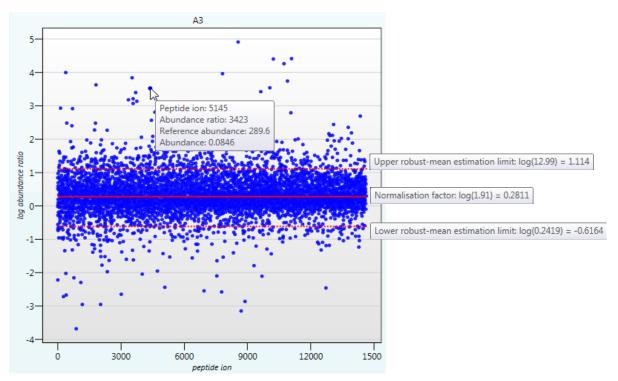
Progenesis QI for proteomics will automatically select one of the runs that is 'least different' from all the other runs in the data set to be the 'Normalising reference'. The run used, is shown above the table of Normalisation factors.

For each sample run, each blue dot shows the log of the abundance ratio for a different peptide ion (normalisation target abundance/run abundance).



The details for individual peptide ions can be viewed as you hold the cursor over the dots on the plot.

On the graph the peptide ions are shown ordered by ascending mean abundance. The normalisation factor is then calculated by finding the mean of the log abundance ratios of the peptide ions that fall within the 'robust estimated limits' (dotted red lines). Peptide ions outside these limits are considered to be outliers and therefore will not affect the normalisation.



Note: if you do not wish to work with normalised data then Select 'Don't use any normalisation'.

QIP Progenesi	s QI.p Tutorial	for DDA - Pr	ogenesis QI for	proteomics									
File Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	A Waters Company
Review n	ormalisatio	n		Norma	lisation Graphs	; Normalis	ation Method						
Normalisation different same		to allow cor	mparisons acro	Norma	lise to all prote								
are unaffect use the fact	ted by experir	nental cond he sample a	er of peptide io itions, we can is a whole varie	Ins Norma	lise to all prote lise to a set of use any normal	housekeepir	na proteins						

Note: once you have identified a set of proteins, you can then apply the **Normalise to a set of housekeeping proteins** by using this option to locate and select the peptide ions.

For this experiment, you should leave the Normalise to all peptide ions option selected.

	8-
< Continue filtering features	

For this example, we **DO NOT** do any additional Filtering so click on **Section complete**.

Now return to filtering by clicking on the button

on the bottom left of the screen

Note: if you do any extra filtering then Normalisation recalculates as you move to the next stage in the Workflow.

Stage 6: Experiment Design Setup for Analysed Runs

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

There are two basic types of experimental designs:

Between-subject design: here samples from any given subject appear in only one condition. (i.e. control versus various drug treatments). The ANOVA calculation assumes that the conditions are independent and applies the statistical test that assumes the means of the conditions are equal.

QIP	Progenesis	QI.p Tutorial f	or DDA - Pro	genesis QI for p	proteomics										
	File	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	<u>D</u>	Onlinear Waters Company
	New														Ø Help ▼
\ \	Vhich ex	xperiment	design ty	/pe do you	want to u	se for this	experime	nt?							
		Between-	subject I	Design			Create	C	Within	-subject	Design				
	appear	ples from a in only one between-su	condition?	Then	A		Delete	SL	ave you taken Ibject under d hen use the w	lifferent co	onditions?		Before	During	After
	the run: (factor	up this desigr s according t level) of the calculation a	o the condi samples. T	tion The t the			A2 <u>Remove</u> A3 <u>Remove</u>	ev a	ote: you must very subject fo within-subject	r every cor design.	ndition to use	Patient X	X1	X2	X3
	condition therefo	ons are indep re gives a sta r the means	endent and itistical tes	l t of	c		Delete C1 Remove C2 Remove	of w	or example, yo f design for a t here every sub t each time poi	ime series ject has be	experiment	Patient Y	Y1	Y2	Y3
				1	Add condition		C3 <u>Remove</u>	so (f	o set up this de oftware not onl actor level) ea	ly which co ch run belo	ondition ongs to but	Patient Z	Z1	Z2	Z3
								so	so which subje oftware will the easures ANOVA	en perform					
								be as re di re co	standard ANO ecause the dat sumption of in peated measu fferences can educed as a sou ondition differe eate a more p	a violates i idependend res ANOVA be elimina urce of bet ences (whio	the ANOVA ce. With a individual ted or ween ch helps to				
								th pa co	ne within-subje lought of as an aired-samples to omparison betv peated measu	extension t-test to in veen more	of the clude				

Within-subject design: here samples have been taken from a given subject under different conditions (i.e. the same subject has been sampled over a period of time or after one or more treatments). Here a standard ANOVA is not appropriate as the data violates the ANOVA assumption of independance. Therefore by using a *repeated measures* ANOVA, individual differences can be eliminated or reduced as a source of between condition differences. This within-subject design can be thought of as a extension of the paired samples t-test, including comparison between more than two repeated measures.

Additional information on how to apply the Within-subject Design is in Appendix 6 page 81

This experiment contains 2 conditions: A and C and uses the **Between-subject design** to group the analysed runs to reflect the Biological conditions in the original study.

To create a new **Between-subject Design** move the cursor over this option and click to open the dialog.

QIP Create New Experiment Design
Enter a name for the experiment design:
AC How do you want to group the runs?
I Group the runs manually
Copy an existing design:
Import criteria from a file: Browse
Group runs by: <a>(< no valid groups>
What file formats are supported? Create design Cancel

Give the new experimental design a name and then click Create design.

File Review nport Data Alignment	Filtering Design Setup	riew Peak Picking	Peptide Ion Identify Statistics Peptides QC Metrics	Refine Resolve Identifications Conflicts		Protein Statistics Report	nonlinea
		•		• •		10 10 20	A Waters Compan
CI × 🗋 New							🔞 Help
etup conditions		Runs	Add Selected Runs to Condition	Search	Q		
etup the conditions that e.g., control, drug A, etc our samples to the corre	t you want to compare below c), and then assign each of ect condition.		Add to new condition	C2		C3	
A	Delete		A	1000 C			
_	A1 <u>Remove</u>			8.96).		and the second	
	A2 <u>Remove</u>						
	A3 Remove						
Add condition							

To create a new condition

- 1. Select the runs for the condition by clicking on the required icon in the **Runs** panel, as shown.
- 2. Press the 'black triangle' next to the Add Selected Runs to Condition button on the main toolbar.
- 3. Select Add to new condition... from the drop down menu.
- 4. A new condition will appear in the Conditions panel on the left.
- 5. Rename the condition (e.g. C) by over typing the default name.
- 6. Repeat steps 1 to 5 until all the required runs are grouped into conditions.

An alternative way to handling the grouping of this set and other larger (and more complex) experimental designs is to make use of **sample tracking information** that has been stored in a spread sheet at the time of sample collection and/or preparation.

AC	New	
Which e	xperiment	design type do y
	Between-	subject Design

For this example there is a **Tutorial Groups.csv** file available in the Experiment Archive you restored at the beginning of this tutorial exercise.

To use this approach select the **Import design from file** option from the New Experiment Design dialog. Then locate the Tutorial Groups file and select what to **Group by**, for example: **Conditions**.

QIP Create New Experiment Design	n 💌
Enter a name for the experime	nt design:
AC-2	(+)
How do you want to group the	runs?
Group the runs manually	
Copy an existing design:	AC v
Import criteria from a file:	ers Tutorial Tutorial Groups.csv Browse
Group runs by:	Conditions 🔹
	Conditions
What file formats are support	Date of Collection
	Location

When Create design is pressed the new tab refreshes to allow you to adjust the conditions.

Note: currently this second method is the only method of creating an Experiment Design that can be applied when using the Automatic Analysis process. Additional Designs can be applied post Automatic processing.

Use **Delete** on the Conditions panel to remove conditions that are not required in this particular design.

QIP Progenesis QI.p Tutorial for DDA - Progenesis QI for pro	eomics		
File			
	v Peak Peptide Ion Identify Refine cing Statistics Peptides QC Metrics Identifications	Resolve Review Protein Conflicts Proteins Statistics Report	nonlinear
		•	A Waters Company
AC AC-2 I X New			🔞 Help 🔻
Setup conditions	Runs Add Selected Runs to Condition	٩	
Setup the conditions that you want to compare below (e.g., control, drug A, etc), and then assign each of your samples to the correct condition.	C3		
A Delete			
A1 Remove	- Share		
A2 <u>Remove</u>			
A3 <u>Remove</u>			
C Delete			
C1 <u>Remove</u>			
C2 <u>Remove</u>			
Add condition			
		Sec	tion Complete 🏵

Note: On deleting a condition the runs will reappear in the Runs window.

Note: both designs are available as separate tabs.

To move to the next stage in the workflow, Review Peak Picking, click Section Complete.

Stage 7: Review Peak Picking and editing of results

The purpose of this stage in the Workflow is to review the list of peptide ions using the visual tools provided and edit peptide ions if required.

The review stage has 4 display modes: 1D, 2D, 3D and Peptide ion Details controlled by the tabs on the bottom left of the display and the expander bar to the right of the table. Each display has multiple views to allow comparative exploration of the detected peptide ions on the aligned LC-MS runs.

Exploring analysed data using the Data displays

Window A: shows the list of peptide ions ranked by the p value for the one way **Anova** using the current grouping.

Note: a value of 'Infinity' in the Fold column indicates 'Presence/Absence'



Note: by default all the peptide ions are included in the selection for the next section of the analysis.

To highlight a group of peptide ions drag out a selection on the table.

Run Expression profile

ArcSinh Normalised Abundance

The 1D Display

Window B: displays the Mass spectrum for the current peptide ion on the selected Run (in window D). Hold the cursor over the peak to indicate m/z and intensity

Window C: displays the Chromatogram for the current peptide ion on the selected Run (in window D). Hold the cursor over the peak to indicate Retention time and intensity

Window D: displays the details of the currently selected run. By default the selected run is an Aggregate of all the aligned runs.

Details of individual runs can be viewed by using the 'Run' link and selecting the run you wish to view.

The peptide ion editing tools are located in this window (see page 32 for functional explanation).

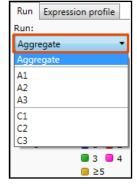
А

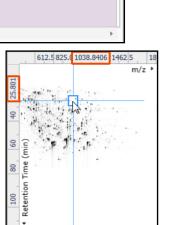
Clicking on the Expression Profile tab in Window D shows the comparative behaviour of the peptide ion across the various biological groups based on group average normalised volume. The error bars show +/- 3 standard errors.

Window E: shows where the current peptide ion is located on the LC-MS run by means of the cross hairs.

•••

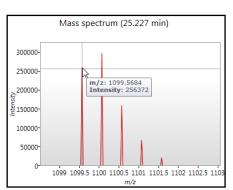
To change the current location, click on the image of the run (**note**: the retention time and m/z values update as you move the cursor around this view).

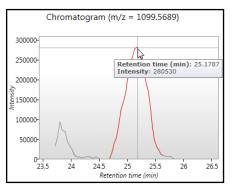




29

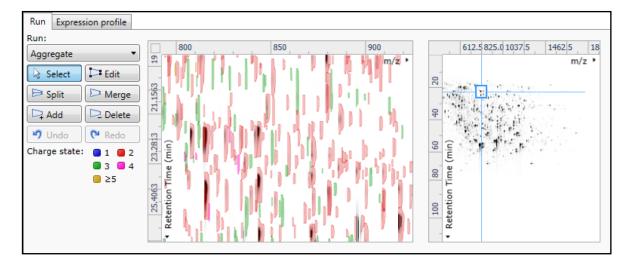
) (h)





Note: doing this updates the focus of all the other windows.

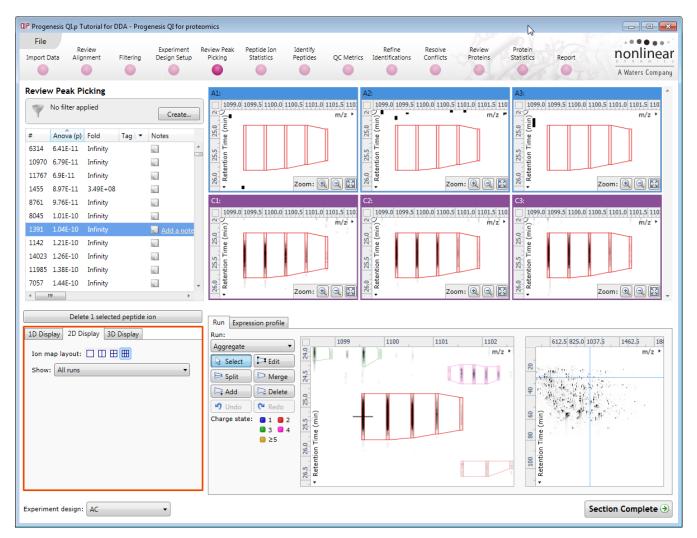
You can also drag out a larger area on this view that will refocus the other windows.



The 2D Display

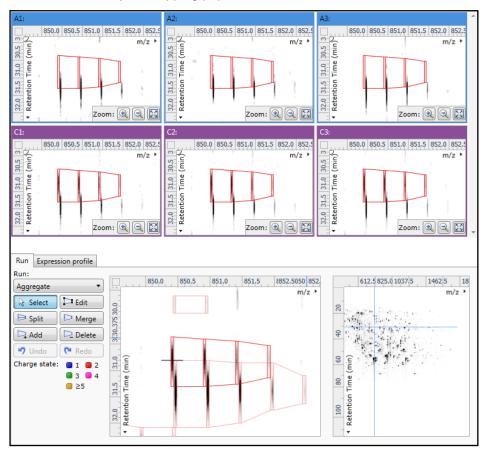
Windows A, D and E: perform the same functions across all 4 display modes.

In the 2D Display mode, Window B displays a montage of the current peptide ion across all the aligned LC-MS runs.



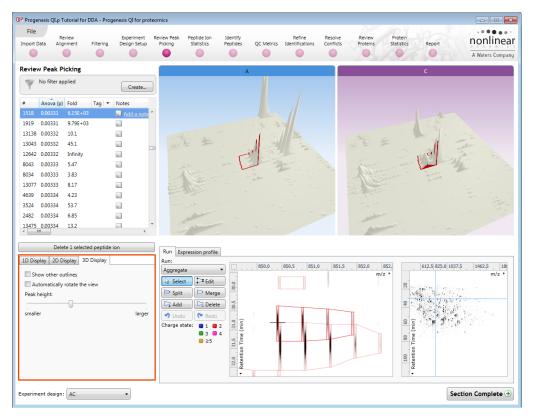
The appearance of the Montage (window B) is controlled by the panel on the bottom left of the display.

Using the the various views in the 2D display one can examine the peptide ion detection in detail to validate the correct detection of even fully overlapping peptide ions as shown above.



The 3D Display

Window B changes into a 3D view by selecting the 3D Montage tab on the bottom left of the display.



The views can be set to **Rotate** automatically or you can rotate them manually by clicking and dragging them with the mouse.

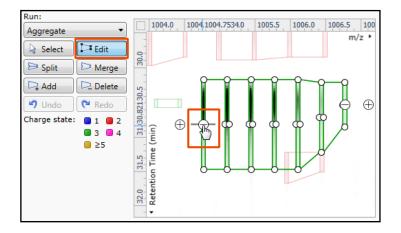
Editing of peptide ions in the View Results stage

As an example of using the editing tools which are located on the left of the **Run** view, we will remove and add back the 'monoisotopic peak' for the detected peptide ion selected below. A peptide ion can be selected from the 'Peptide ions' list or located using the various views.

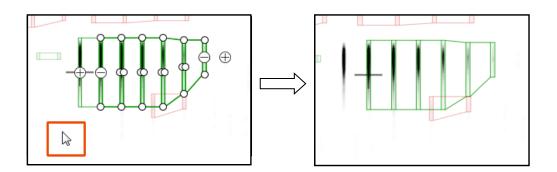
1. Locate the peptide ion at approx 1005.7 m/z and 30.8 min using the **Go To Location** tool (at the intersection of the m/z and RT axis).



2. Select the Edit tool and click on the peptide ion to reveal the 'edit handles'

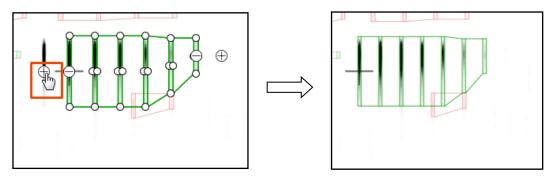


3. Click on the 'minus' handle over the monoisotopic peak to remove it.

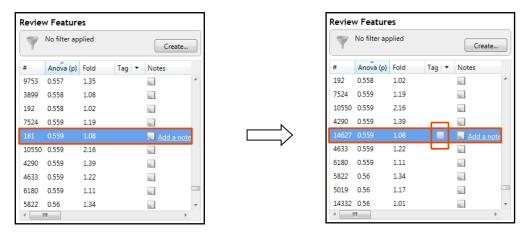


4. Click outside the boundary of the peptide ion to update the view.

5. To add a peak to an existing peptide ion, ensure that **Edit** is selected then click inside the peptide ion to reveal the handles.



- 6. Click on the 'plus' handle on the peak to add it.
- 7. Then click outside the peptide ion to update the view.
- 8. Note: If you are not satisfied with the editing use the Undo button and retry.
- 9. **Note**: that a tag is automatically added to the edited peptide ion in the table and the peptide ions id number is changed to the next available one at the end of the list.



The other tools: split, merge, add and delete behave in a similar fashion and their use can be combined to achieve the desired results.

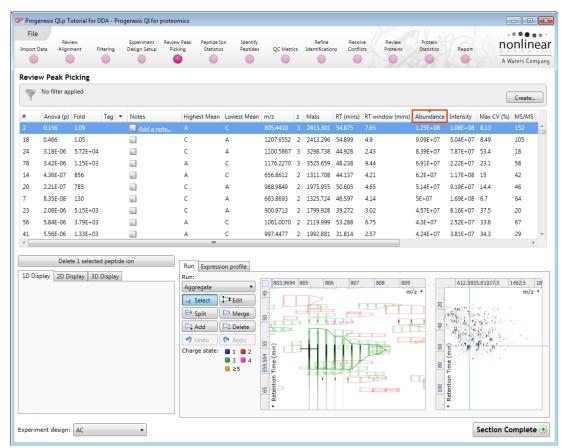
Selecting and tagging peptide ions for Peptide Ion Statistics

There are a number of ways to 'refine' your 'Ranked List' of analysed peptide ions before examining them with the Statistical tools in **Peptide Ion Statistics**. These make use of simple 'Selection' and 'Tagging' tools that can be applied to the various groupings created in Stage 6 (page 25). An example is described below.

First expand the 'Peptide ions' table to show all the details by clicking on the 'Splitter Control' to the right of the Review Peptide ions table.

Reviev	v Peak Pi	cking			
Y	No filter ap	plied		Create	
#	Anova (p)	Fold	Tag 💌	Notes	
8761	9.76E-11	Infinity		10	*
8045	1.01E-10	Infinity		10	
1391	1.04E-10	Infinity		Add a not	
1142	1.21E-10	Infinity		10	
14023	1.26E-10	Infinity		10	
11985	1.38E-10	Infinity		10	
7057	1.44E-10	Infinity		10	
8028	1.56E-10	Infinity		10	
5390	1.92E-10	Infinity		10	
2579	1.95E-10	Infinity		10.	
7921	2E-10	Infinity		19.	
	2.06E-10	Infinity		in	Ŧ
<	11			+	

Then order on **Abundance** and select all peptide ions with an Abundance of 1E+05 and greater, (the exact number is not important).



With the peptide ions still highlighted right click on them and select 'New Tag'.

Review Vesk Picking Notes Higher Man Lowest Man n/2 Modes Lowest Man Notes Notes Higher Man Lowest Man Notes RT (min) RT (min) RT (min) RT (min) Additanotes C A 1999 20 A 1999 A State A 1999 A 1999 A 1999 A A 1999 A Notes: tossing 2 2 A A Notes: tossing 2 <th c<="" th=""><th>File</th><th>Revi</th><th></th><th>iltering</th><th>Experiment Design Setup</th><th>Review Per Picking</th><th>ek Peptide I Statistic</th><th></th><th>QC Metri</th><th>cs</th><th>Refine Identification</th><th>Resolv s Conflic</th><th></th><th>Protein Statistics</th><th>Report</th><th></th><th>A Waters Cor</th><th>1 0</th></th>	<th>File</th> <th>Revi</th> <th></th> <th>iltering</th> <th>Experiment Design Setup</th> <th>Review Per Picking</th> <th>ek Peptide I Statistic</th> <th></th> <th>QC Metri</th> <th>cs</th> <th>Refine Identification</th> <th>Resolv s Conflic</th> <th></th> <th>Protein Statistics</th> <th>Report</th> <th></th> <th>A Waters Cor</th> <th>1 0</th>	File	Revi		iltering	Experiment Design Setup	Review Per Picking	ek Peptide I Statistic		QC Metri	cs	Refine Identification	Resolv s Conflic		Protein Statistics	Report		A Waters Cor	1 0
4035 0.0203 1.74 ▲ Add a note C A 1196.9362 3 3587.787 39.300 0.921 1E+05 2.57E+05 20.8 0 1686 0.0799 2.62 ▲ Add a note C A 706.6327 4 2822.502 63.027 1.26 1E+05 5.39E+05 62.6 1.2 5464 2.83E+08 Infinity Add a note C A 1585.5178 3 4753.532 46.597 0.399 1E+05 1.7E+05 16.9 0 2941 0.0744 1.49 Add a note A No tags to assign 7 2 2160.051 36.226 0.754 1E+05 1.96E+05 25.4 6 391 0.000567 3.03 A No tags to assign 7 2 1260.051 36.226 0.754 1.665 1.76 6 0 391 0.000366 2.08 C A New tag																	Create		
666 0.0799 2.62 Add a note:: C A 706.6327 4 2822.502 63.027 1.26 1.605 5.391 6.26 1.2 444 2.83E-08 Infinity Add a note:: C A 1585.5178 3 4753.532 46.597 0.359 1.6-05 1.7E+05 1.69 0 941 0.0744 1.49 Add a note:: A No tags to assign 7 2 2 160.051 3.626 0.754 1.6-05 1.964+05 2.54 6 391 0.000567 3.03 A No tags to assign 7 2 2 1405.675 45.667 1.94 9.99E+04 2.37E+06 1.69 0 1681 0.000148 6.8 A A Quick Tags 2 1.246.668 18.939 1.32 9.99E+04 2.37E+05 1.69 0 1681 0.00366 2.0.8 C A 814.8726 4 325.5461 37.560 0.71 9.99E+04 2.87E+05 2.24 6 1000867 4.5		Anova (p)	Fold	Tag 💌	Notes	Н	ighest Mean	Lowest Mean	m/z	z	Mass	RT (mins)	RT window (mins)	Abundance	Intensity	Max C	V (%) MS/MS		
464 2.83E-08 Infinity Add a note C A 15855178 3 4753332 46.597 0.359 1E+05 1.7E+05 1.69 0 771 0.0177 540 Add a note C A 1157.6035 3 3499.789 46.561 0.808 1E+05 1.2E+05 1.26 0 941 0.0744 1.49 Add a note A No tags to assign 7 2 2160.051 36.226 0.754 1E+05 1.96E+05 25.4 6 391 0.000567 3.03 A New tag 2 1405.675 45.667 1.94 9.99E+04 2.37E+06 1.69 0 681 0.00366 20.8 C Affinity Add a note C A 8179225 1633.83 3.9467 0.743 9.98E+04 2.87E+05 2.24 6 381 0.000887 4.5 A C 8179225 2 1633.83 3.9467 0.743 9.98E+04 2.87E+05 2.24 6 9 9 C					Add a no													-	
Add a note C A 1985 238-08 18-03 12-05 12-05 12-05 0.039 12-05 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.039 12-05 0.059 0.005					Add a no														
941 0.0774 1.49 Add a note A No tags to assign ?? 2 2400031 36.226 0.774 1.605 1.96E+05 2.54 6 391 0.000567 3.03 A New tag 2 2460575 45.667 1.94 9.99E+04 2.3E+05 1.7.6 8 312 0.000148 6.8 A Quick Tags 2 1284.668 18.939 1.32 9.99E+04 2.3TE+06 16.9 0 661 0.00366 20.8 C A C 817.92Z5 2 1633.83 39.467 0.743 9.96E+04 2.8TE+05 2.24 6 379 0.0448 1.50 C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 4.7E+05 11.8 4 - Delete 2336 selected peptide ions Run: Expression profile Nevression profile OLisplay 3D Display 612.5825.41037.5 1462.5 18 Gei gi					Add a no													G	
391 0.000567 3.03 A New tag 2 1405.675 45.667 1.94 9.99E+04 2.53E+05 17.6 8 312 0.000148 6.8 A A Quick Tags 2 1284.668 18.939 1.32 9.99E+04 2.37E+06 16.9 0 681 0.00366 20.8 C A C 8179225 2 1633.830 39.467 0.743 9.99E+04 2.87E+05 22.4 6 379 0.0448 150 C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 2.87E+05 12.8 4 Delete 2336 selected peptide ions To belete 2336 selected peptide ions D Display 2D Display 3D Display Outdoot @ Reco Quick Tags 1.62 3.2 3.255.461 3.7.560 0.71 9.98E+04 2.87E+05 12.8 4 Outdoot @ Reco Run: Run: Run: Run: Run: Run: Run: Run: R					Add a no														
312 0.000148 6.8 A C C C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 2.87E+05 22.4 6 9.3 1 1 0.00887 4.5 C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 4.7E+05 118 4 T 1.9225 2 1633.830 39.467 0.743 9.98E+04 2.87E+05 22.4 6 9.3 1 1 0.0048 150 C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 4.7E+05 118 4 T 1.9225 2 1.633.830 39.467 0.743 9.98E+04 4.7E+05 118 4 T 1.9225 2 1.633.830 39.467 0.743 9.98E+04 4.7E+05 118 4 T 1.9225 2 1.633.830 39.467 0.743 9.98E+04 4.7E+05 118 4 T 1.9225 2 1.633.830 39.467 0.743 9.98E+04 4.7E+05 118 4 T 1.9225 2 1.633.830 39.467 0.743 9.98E+04 4.7E+05 118 4 T 1.9225 2 1.633.830 39.467 0.743 9.98E+04 4.7E+05 118 4 T 1.925 1.926	941	0.0744	1.49		Add a no	te A		No tags to assi	gn 27	2	2160.051	36.226	0.754	1E+05	1.96E+05	25.4	6		
661 0.00366 20.8 C C Edit tags 2 3 3055.314 53.762 0.925 9.99E-04 1.94E-05 69.3 1 381 0.000867 4.5 A C 817.9222 2 1633.830 39.467 0.743 9.98E-04 2.87E+05 22.4 6 379 0.0448 150 C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 4.7E+05 11.8 4 • Delete 2336 selected peptide ions Run Expression profile Run: Aggregate •	391	0.000567	3.03		4	А		New tag		2	1405.675	45.667	1.94	9.99E+04	2.53E+05	17.6	8		
381 0.000887 4.5 A C 817.9222 2 1633.830 39.467 0.743 9.98E+04 2.87E+05 22.4 6 379 0.0448 150 C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 2.87E+05 118 4 + Toletet 2336 selected peptide ions Run Expression profile Run: Aggregate Image: Colspan="4">Image: Colspan="4" Image: Colspan="4" Image: Co	312	0.000148	6.8		10	А		Quick Tags	•	2	1284.668	18.939	1.32	9.99E+04	2.37E+06	16.9	0		
379 0.0448 150 C A 814.8726 4 3255.461 37.560 0.71 9.98E+04 4.7E+05 118 4 Delete 2336 selected peptide ions Run Expression profile Run: Aggregate 903.9694 805 806 807 809 612.5825.01037/5 1462/5 18 Split Merge 9	681	0.00366	20.8		10	C	~	Edit tags	52	3	3055.314	53.762	0.925	9.99E+04	1.94E+05	69.3	1		
Delete 2336 selected peptide ions Run Expression profile D Display 2D Display 3D Display Run: Aggregate 9803.9694 Select 1 Edit Split Merge Undo Redo Split 1 0 2 Sig 1 0 2 Sig <td>381</td> <td>0.000887</td> <td>4.5</td> <td></td> <td>12</td> <td>А</td> <td></td> <td>С</td> <td>817.9225</td> <td>2</td> <td>1633.830</td> <td>39.467</td> <td>0.743</td> <td>9.98E+04</td> <td>2.87E+05</td> <td>22.4</td> <td>6</td> <td></td>	381	0.000887	4.5		12	А		С	817.9225	2	1633.830	39.467	0.743	9.98E+04	2.87E+05	22.4	6		
Delete 2336 selected peptide ions Run Expression profile Run: Aggregate Select I Edit Split Merge Add Delete Split Merge Split Merge Split	379	0.0448	150		12	C		Α	814.8726	4	3255.461	37.560	0.71	9.98E+04	4.7E+05	118	4	Ŧ	
	D Disp				e ions		n: ggregate ≩ Select ≩ Split ≩ Add 7 Undo		tion Time (min) 50 45 to	8		807		80 60 40 20	(min)	5.01037			

Give the Tag a name. i.e. 'Most abundant'.

ОК	Cancel
	ОК

On clicking **OK** the Tag is added to the peptide ions highlighted in the table (signified by a coloured square).

Review Peak Picking								
Vo filter applied								
#	Anova (p)	Fold	Tag 💌	Notes				
4035	0.0203	1.74	-	Add a note				
1686	0.0799	2.62	-	🔜 Add a note				
5464	2.83E-08	Infinity	-	🔜 Add a note				
4771	0.0177	540	-	🔜 Add a note				
2941	0.0744	1.49	-	🔜 Add a note				
3391	0.000567	3.03		10				
8312	0.000148	6.8		1				
5681	0.00366	20.8		1				
2381	0.000887	4.5		1				
2379	0.0448	150		2				
•								
	Delete 2336 selected peptide ions							

Now right click on any peptide ion in the table and select **Quick Tags** this will offer you a number of standard tag options. Select **Anova p-value....** Then set the threshold as required and adjust default name as required and click **Create Tag**.

OP New Quick Tag	
Where a feature has:	
Anova p-value: ≤ 🔻 0.05	
Apply the following tag:	
Anova p-value ≤ 0.05	
	_
Create tag Cancel	

Revie	Review Features							
Y	No filter a	applied						
#	Anova (o) Fold	Tag 💌	Note	s Highest Mean			
1686	0.0799	2.62		2	С			
5464	2.83E-08	Infinity		a.	С			
4771	0.(****	Most abunda	nt	- A	<u>.dd a note</u> C			
2941	0.0			-	А			
3391	0.	New tag		-	٨			
8312	0.	Quick Tags		•	Anova p-value			
5681	0.0	Edit tags			Max fold change			
2381	0.0	Add to Clip G	allery		No MS/MS data			
				-	No protein ID			
2379	0.0448	150		4	C C			
1720	0.322	1.32		a.	А			

Once this tag appears against peptide ions in the table right click on the table again and create another Quick Tag, this time for peptide ions with a **Max fold change** ≥ 2

OP New Quick Tag
Where a feature has: Max fold change: 2
Apply the following tag:
Max fold change ≥ 2
Create tag Cancel

The table now displays peptide ions with multiple tags.

Review Features							
Vo filter applied							
#	Anova (p)	Fold	Tag 💌	Notes			
2154	0.000674	63.5		A			
5011	2.72E-08	Infinity		10.			
1833	0.0187	3.03		a .			
2929	0.00372	6.8		10.			
4035	0.0203	1.74		10.			
1686	0.0799	2.62		1 2			
5464	2.83E-08	Infinity		10.			
4771	0.0177	540		Add a note			
2941	0.0744	1.49		10.			
3391	0.000567	3.03		10.			
8312	0.000148	6.8		10.			

The tags can be used to quickly focus the table on those peptide ions that display similar properties.

For example: to focus the table on displaying those peptide ions that have an **Anova p-value** \leq **0.05** click on **Create** on the filter panel above the table.



Drag the tag on to the panel Show peptide ions that have all of these tags and press OK.

QP Filter the peptide ions	×
Create a filter Show or hide peptide ions based on a selection to create the filter. For more guidance, please s	of their tags. Move tags to the appropriate boxes ee the <u>online reference</u> .
Available tags:	Show peptide ions that have all of these tags:
Most abundant (2336 peptide ions) Anova p-value ≤ 0.05 (7971 peptide ions) Max fold change ≥ 2 (9880 peptide ions)	Show peptide ions that have at least one of these tags:
	Hide peptide ions that have any of these tags:
Clear the filter	OK Cancel

Now order the current peptide ions in the table by the **Highest mean** so that all the peptide ions showing the highest mean for **Condition C** are at the top of the list.

Then highlight all the peptide ions with the highest mean for **condition C** and create a new Tag for them.

#	Anova (p)	Fold	Tag 🔻	Notes	Highest Mean	Lowest Mean
12806	4.58E-08	Infinity		🔜 Add a note		
12714	3.36E-05	Infinity		Add a note		
12706	0.00567	31.6		Add a note		
12803	0.014			🔜 Add a note		
	0.00402			🔜 Add a note		
2520	0.000314	51.9		Add a note		А
9576	4.31E-07	Infinity		2	А	с
14182	0.00267	1.72E+03			А	с
14183	0.000601	Infinity			А	С
11696	0.00584	3.42			А	С
10687	0.0413	2.06		12	А	с

	#	Anova (p)	Fold	Tag 💌	Notes		Highest Mean	Lowest Mean
	12806	4.58E-08	Infinity		Add a			А
	12714	3.36E-05	Infinity		🔜 Add a			А
	12706	0.00567	31.6		Add a	note		А
	12803	0.014		bundant		note		А
·	2708	0.00402	_	p-value ≤		note		А
	2520	0.00031	IVIAX TO	d change	22	note		А
	9576	4.31E-07	New ta	5			A	С
	14182	0.00267	Quick	ags	•		A	с
	14183	0.00060	P Edit tag	s			A	с
	11696	0.00584	Add to	Clip Galle	ry		A	с
	10687	0.0413	2.06		10		А	С

Create a tag for them called Significantly up in C, tagging 3959 peptide ions.

Finally view the tags you have just created by clicking on Edit in the Tag filter panel, above the table.

Tag filter applied peptide ions may be hidden	UP Filter the peptide ions Create a filter Show or hide peptide ions based on a selection to create the filter. For more guidance, please so	of their tags. Move tags to the appropriate boxes ee the <u>online reference</u> .
Make sure that only the tag for the Most abundant peptide ions is shown and press OK.	Available tags: Max fold change ≥ 2 (9880 peptide ions) Anova p-value ≤ 0.05 (7971 peptide ions) Signifidantly up in C (3959 peptide ions) Clear the filter	Show peptide ions that have all of these tags: Most abundant (2336 peptide ions) Show peptide ions that have at least one of these tags: Hide peptide ions that have any of these tags: OK Cancel

To move to the next stage in the workflow, Peptide Statistics, click Section Complete.

Stage 8: Peptide Ion Statistics on selected peptide ions

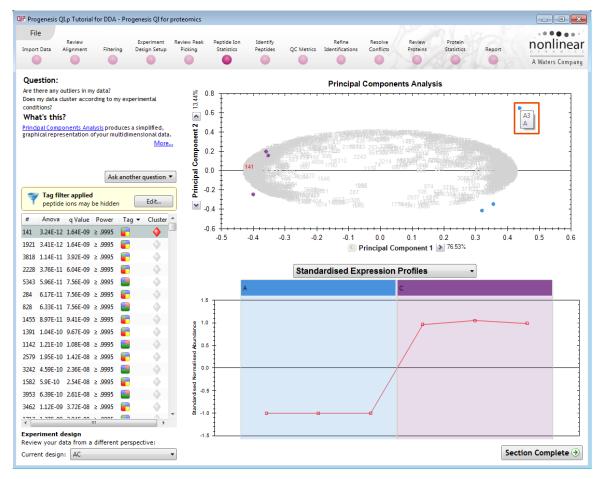
The user guide now describes the functionality of the Multivariate Statistics.



Peptide Statistics opens calculating the Principal Components Analysis (PCA) for the active 'tag' in this case the **Most abundant** peptide ions.

As an example we will start by examining the behaviour of the **Most abundant** peptide ions from the previous stage, **Review Peak Picking**.

The statistical analysis of the selected data is presented to you in the form of interactive graphical representation of answers to questions asked of the analysed data.



Note: the LC-MS runs (samples) are displayed as solid coloured circles on the plot. To identify the runs, a tooltip is displayed when the cursor is held over each circle.

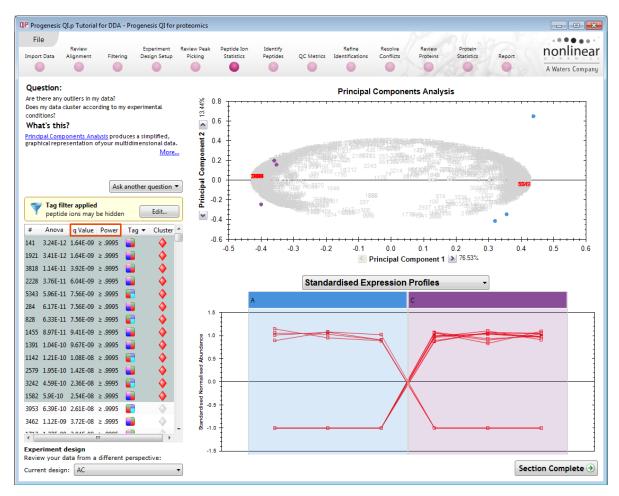
Principal Component Analysis (PCA)

In **Peptide Stats** the first statistically based question asked of the data takes the form of a Quality Control assessment:

Are there any outliers in my data? And does my data cluster according to my experimental conditions? It answers this question by:

'Using Principal Components Analysis (PCA) to produce a simplified graphical representation of your multidimensional data'.

PCA can be used to determine whether there are any outliers in the data and also look at how well the samples group. The groupings that can be observed on the 2D PCA plot can be compared to your experimental conditions and conclusions can be drawn regarding possible outliers in your data. Selecting peptide ions in the table will highlight the peptide ions on the 'Biplot' and their expression profiles will appear in the lower panel.



Note: the Table in the Stats view contains additional columns:

q value: tells us the expected proportion of false positives if that peptide ion's p-value is chosen as the significance threshold.

Power: can be defined as the probability of finding a real difference if it exists. 80% or 0.8 is considered an acceptable value for power. The Power Analysis is performed independently for each peptide ion, using the expression variance, sample size and difference between the means.

Also, for a given power of 80% we can determine how many samples are required to ensure we find a difference if it actually exists.

Note: Power analysis is discussed in Appendix 7 (page 83)

Correlation Analysis

Use the tags, created in Review Peak Picking, to filter the peptide ions in the table.

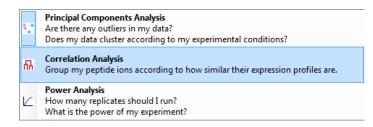
We are going to explore the Correlation Analysis for all the peptide ions that were tagged at the view results stage with an **Anova p-value≤0.05**.

On pressing OK the PCA will recalculate using these peptide ions, you can (to save time) stop this calculation by pressing **Cancel calculation** and then set up Correlation Analysis for the 7971 peptide ions.

Create a filter Show or hide peptide ions based on a selection poxes to create the filter. For more guidance, p	
Available tags: Max fold change ≥ 2 (9880 peptide ions) Signifidantly up in C (3959 peptide ions) Most abundant (2336 peptide ions)	Show peptide ions that have all of these tags: Anova p-value ≤ 0.05 (7971 peptide ion Show peptide ions that have at least one o these tags: Hide peptide ions that have any of these tags:

To set up the **Correlation Analysis** using this filtered data set click on **Ask another question** (above the table)

A selection of 3 tools will appear in the form of questions.



Select the second option to explore 'peptide ion correlation based on similarity of expression profiles'



This time the statistically based question(s) being asked is:

'Group my (selected) peptide ions according to how similar their expression profiles are'

The question is answered by:

'Using Correlation analysis to evaluate the relationships between the (selected) peptide ions' expression profiles'.

The answer is displayed graphically in the form of an interactive dendrogram where the vertical distance, between each peptide ion, can be taken as indicative of how similar the expression profiles of each cluster of peptide ions are to each other.



Correlation Analysis enables the grouping of peptide ions together according to how similar their expression profiles are.

To highlight all the peptide ions demonstrating **Increased expression in the C** group click on a 'node' for a branch of the Dendrogram (as shown above). As before, right click on the highlighted peptide ions in the table and create a Tag for these peptide ions (Up regulated in C).

Create new tag	—
Up regulated in C	
	OK Cancel

Also create a tag for those peptide ions showing **Increased expression in A** by first clicking on the other 'main' node then right click on the highlighted peptide ions in the table and creating the New tag (Up regulated in A).

Create new tag	—
Up regulated in A	
	OK Cancel

Comment: When you review the tags using **Edit** you can see that the Magenta and Yellow tags have been assigned to the same number of peptide ions. This shows how tabulated information about peptide ions can be used alongside interactive graphical plots of multivariate statistical analysis to explore your data.

Note: two groups is a special case, for more groups this will not be the case, and additional tagging will be required.

QP Filter the peptide ions	×
Create a filter Show or hide peptide ions based on a selection of to create the filter. For more guidance, please so	of their tags. Move tags to the appropriate boxes se the <u>online reference</u> .
Available tags:	Show peptide ions that have all of these tags:
Most abundant (2336 peptide ions) Max fold change ≥ 2 (9880 peptide ions) Signifidantly up in C (3959 peptide ions)	Anova p-value ≤ 0.05 (7971 peptide ions)
Up regulated in A (4012 peptide ions)	Show peptide ions that have at least one of these tags:
	Hide peptide ions that have any of these tags:
Clear the filter	OK Cancel

Tip: when reviewing the tags (see above) if you are not applying a new filter then use the **Cancel** button to return to the main view, this prevents unnecessary recalculation of your data.

To move to the next stage in the workflow, Identify Peptides, click Section Complete.

Stage 9: Identify peptides

Progenesis QI for proteomics does not perform peptide identifications itself for DDA data. Instead it supports identifications by allowing you to export MS/MS peak lists in formats 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.

File	Data A	Review Nignment Filte	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics		ntify tides	QC Metrics Id	Refine lentifications	Resolve Conflicts Proteins		Report	0	onlinea Waters Compa
	fy Pep		orting peak list files	MS/MS S										
a pro		ntification progra				1		ting export list of						-
-		r applied		Expo			Run	Scan number	Exported	Peptide ion intensity	Precursor intensity	(%)	Charge	Precursor m
Υ.	NO IIIC	applied	Create		51		A1	2686	No	7.6e+007	5.2e+005	0.7	2	
-	v				12	-	A1	2726	No	7.6e+007	5.1e+007	66.5	2	
	MS/MS	Proteins	Tag ▼ Scc		8		A1	2767	No	7.6e+007	6.3e+007	82.2	2	539.30
4	231	0			13		A1	2806	No	7.6e+007	3.9e+007	50.6	2	539.30
	152	0			19		A1	2855	No	7.6e+007	1.5e+007	20.2	2	539.30
2	109	0			26	-	A1	2906	No	7.6e+007	2.9e+006	3.8	2	539.30
В	105	0		V	33	-	A1	2948	No	7.6e+007	1.5e+006	1.9	2	539.30
5	95	0		V	43		A1	2990	No	7.6e+007	8.5e+005	1.1	2	539.30
4	88	0		V	47		A1	3040	No	7.6e+007	7.3e+005	1.0	2	539.30
5	86	0		V	32		A2	2846	No	7.7e+007	1.5e+006	2.0	2	539.30
	85	0		v	6		A2	2890	No	7.7e+007	6.6e+007	85.5	2	539.30
7	80	0		V	10		A2	2931	No	7.7e+007	6.1e+007	79.6	2	539.30
	75	0		V	17	1	A2	2979	No	7.7e+007	2.5e+007	33.1	2	539.30
31	72	0		V	21		A2	3027	No	7.7e+007	7.4e+006	9.7	2	539.30
8	72	0		V	30	1	A2	3077	No	7.7e+007	1.9e+006	2.4	2	539.30
				4										÷
arfo	rming t	the Search		Peptid	e ion nu	mber 1	, m/	z 539.3004, i	etention	time 29.63 min, ch	narge +2			
	-	h program you're	using:	Run:A1	Scan nu	mber:26	86		1			1		
/lasco lelp			•						15000 -					
E	xport 38	393 ms/ms spectra	a	Betention time 30.	987 -	ø		Intensity	10000 -					
	IS Prepro	-		on tír				트	5000-					
	imit frag	ment ion count	40	ju 30.	994 -				5000-					
	Deisot	oping and charge	deconvolution	č					0	200 400			1000	
	Impor	t search results					mz		U	200 400	600 <i>m/z</i>	000	1000	

Note: ensure that NO tag filters are applied and that Mascot is the selected search engine.

Determining protein identification is dependent on the availability of MS/MS data for the LC-MS runs. This data may be available but limited if the LC-MS was performed in a data dependant MS/MS detection mode due to under sampling. Under these conditions MS/MS data acquisition is dependent on thresholds and parameters set prior to the acquisition of the LC-MS run.

For this example we are using LC-MS runs containing MS/MS data where the data was acquired in a data dependant mode (DDA).

The Peptide Search page shows the number MS/MS that have been matched to each peptide ion in the Peptide ion list (see above). MS/MS scans are matched to a peptide ion if their precursor m/z and aligned retention time fall within the area of one of the isotopes of the peptide ion. The MS/MS scans which are matched to the displayed peptide ions are shown in the MS/MS spectra list on the right.

The first step is to decide which MS/MS scans you wish to export to be identified. By default this is all the available spectra for the peptide ions displayed in the Peptide ions list. This number is visible on the Export button.

The set can be targeted using the tags and also refined with respect to quantity and quality of the spectra being sent to the search engine.

Note: by default the table is ordered on the number of MS/MS spectra available for each peptide ion.

The total number of spectra included in this set is **38248** as shown on the Export button.

Before exporting the spectra, the set can be further refined.

Note: many of the abundant peptide ions have a large number of spectra associated with them.

To control the number of spectra for each peptide ion, expand the **Batch inclusion options**.

Progenesis	QI.p Tutorial for	DDA - Prog	enesis QI for pro	teomics										
File port Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identificatior	Resolve s Conflicts	Review Prot Proteins Stati		Report	nonli	ne
									•			0	A Waters C	Comp
entify P	eptides			MS/MS Sp	ectra									
in ms/ms i	ion searches by identification pro	exporting p	eak list files	 Batch in 	nclusion optic	ons for creating	export list of r	ns/ms spectra	1					
ptide ions		yrann.			Rank	greater than	• 5		Peptide i	on intensity less that	n 🔻			
y No f	ilter applied	ſ		Pa	ptide ion #	ess than	•		Precure	or intensity less tha	n 🔻			
1		l	Create	Fe			-			,				
MS/I	MS Proteins	Т	ag 🔻 Scc		Charge	ess than	•		Precurs	or intensity (%)	n 🔻			
231	0		📮 📩	Sc	an number 🛛	ess than	•			Run name contain	s 🔹			
152	0				Exported									_
109	0		•		exported	equal to	•	•	Peptid	e sequence contain	s 🔻			
105	0		•		Isotope	ess than	•		Protei	n accession contain	s 🔻]		
95	0		•		ID score	ess than	•		Protein	description contain	s 🔹			
88	0				10 00010 []	coo man								
86	0		•							Include in export	Exclude fro	om export	Clear all filter	rs
85	0		•											_
80	0		•		_									
75	0		•	Export	Rank #	Run S	can number	Exported P	eptide ion intensity	Precursor intensit	y (%)	Charge	Precursor m/z	I
72	0		•		5 1	827 C1	5230	No	4.2e+00	5 3.0e+00	5 70.2	2	732.8827	
1 72	0		-		5 1	828 C3	1931	No	3.6e+00	5 2.4e+00	65.1	2	592.7861	
71	0		•			603 C1	6285	No	2.9e+00			3	599.6564	
67	0		- v	-	6 2	602 C2	6470	No	3.7e+00	5 1.2e+00	5 31.3	2	631.3630	
	III		+											
rformin	ng the Search						539.3004, re	etention tin	ne 29.63 min,	charge +2				
lect the se	earch program yo	u're using:		Run:A1	Scan numbe	r:2686	1	5000-						
lascot		-												
elp				=			1	- 0000						
Export	18225 ms/ms sp	ectra		·E 28.9	°′] 🤇		Intensity							
MSMS Pre	processing			Ē						li -				
📃 Limit f	ragment ion cour	nt 400	A V	Betention time 30.9	94 -			5000 -						
📃 Dei	isotoping and cha	arge deconv	olution	B	l			0-				l. he i		
Jm	port search result	s				_1_m/2		0	200	400 600 <i>m/z</i>	800	10	00	
				<u> </u>										_
C	ar all identificatio												tion Comple	_

For example: We will make use of the 'Rank' value to reduce the number of Spectra being used for each peptide ion to a maximum of 5.

The 'Rank' of each MS/MS spectra is determined by comparing its % value against all other spectra matched to the same peptide ion.

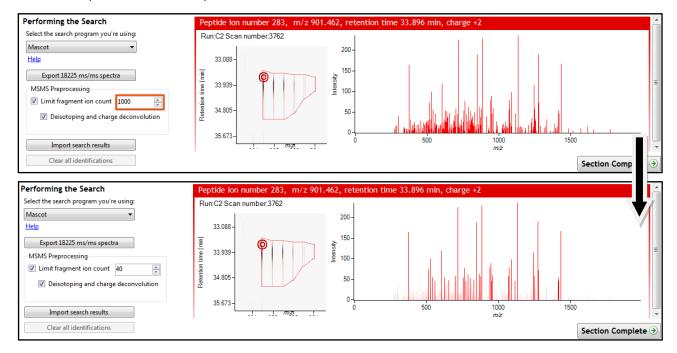
Export E	Ran	k #	Run	Scan number	Exported	Feature intensity	Precursor intensity	(%)	Charge	Precursor m/z	Isotope Id so	ore
	23	0	A 1	4000	M	1.0000	1.4000	10	1	050.0010	•	_
		The ran	k of each	MS/MS spect	trum found	d by comparing it	s '%' values against	all other	spectra n	natched to the	same feature.	
	24	J	-		NU	1.401000	1.701000	1.9	4	000.0010		
	25	9	A3	5042	No	9.3e+007	8.8e+005	1.0	2	656.8614	1	
	26	9	A1	5379	No	1.2e+008	1.0e+006	0.9	2	656.8610	1	
	27	9	A3	5374	No	9.3e+007	7.7e+005	0.8	2	656.8615	1	
4												Þ

Note: the % value for each spectra is the Precursor intensity as a percentage of the Peptide ion intensity

Set the Rank filter to 'greater than' 5 and click **Exclude from export** this reduces the number to spectra to export to **18225**

Limiting the 'fragment ion count' (FIC) for the spectra being exported can improve the quality of the spectral data being used in the search by removing noisy peaks.

For example for the current spectra, reduce the FIC from 1000 to 40.



Note: the effect this has on the number of peaks in the spectra. This 'limitation' is applied to all the spectra being exported; hence the export file size will be reduced.

For this example we will not limit the fragment count, so leave it un-ticked (the default setting).

Performing an MS/MS Ion Search

Having chosen 18225 spectra to export, as described above:

- 1. Select appropriate search engine i.e. Mascot
- 2. Click 'Export current query set' to save search as file
- 3. Perform search on appropriate search engine and save results file
- 4. Click 'Import search results', locate results file and open

Please refer to Appendix 9 (page 86) for details of the 'Search Engine' parameters used in this example

Note: the blue link provides you with details on the appropriate formats for exporting search results and access to additional formats

Note: an example Search Results file, from a MS/MS Ion search, is available in the folder you restored the Archive to (Search Results.xml). Select the 'Mascot' method and import this file to see results like those below.

On importing the Search results the Peptide ions table updates to reflect the identified proteins and the relevant score for each searched peptide ion.

OF Progenesis (QI.p Tutorial for DDA	- Progenesis QI for p	roteomics									• 🕺
File Import Data	Review Alignment Filte	Experiment Design Setup	Review Peak Peptide I Picking Statistic		QC Metrics	Refine Identifications	Resolve Conflicts		rotein atistics	Report	nonli	near
			• •				•	- 18		98	A Waters	Company
Identify Pe			MS/MS Spectra									
	on searches by expo dentification program		 Batch inclusion of 	ptions for creating	g export list of	ms/ms spectra	1	_				
Features:			Ra	nk greater than	▼ 5		Fea	ature intensity le	s than	•		
Wo filt	ter applied	Create	Feature	ID less than	•			ursor intensity le		•		
# MS/MS	Proteins	Tag 💌 Scor	Char	ge less than	•		Preci	ursor intensity (%)	is than	•		
94 231	0	– –	Scan numb	er less than	•			Run name co	ntains	•		
2 152	2 gi 254976387,gi		Export	ed equal to	•	•	Pep	tide sequence co	ntains	•		
52 109 18 105	11 gi 112181139,gi 2 gi 254976387,gi						Pro	tein accession co	ntains	•		
75 95	2 gil234970367,gil	23. 120.	QIP Import s	e less than	•			ein description		•		
44 88	- 3 gi 21702505,gi 2	549 🛑 39.80						ein description [co	ntains	·		
36 86	0			348 search hits hav	e been import	ted and assigne	ed to features	Include in exp	ort Exclud	le from exp	Clear all f	ilters
9 85	2 gi 254976387,gi	25! 🛑 66.8										
27 80	0		Ex					Precursor intens	ity (%)	Charge	Precursor m/z	Isot
4 75 68 72	2 gi 254976387,gi : 0	25: 89.7				L	OK	2.9e+			1176.2256	*
131 72	c 2 gi 254976387,gi	25: 🗧 81.4: 🗸	6	245 C1	867	No	2.0e+006				610.6482	
*		+	6	348 A3	7695	No	2.1e+005	1.0e+	005 49.3	3	941.8418	+ ·
Performing	the Search		Feature numbe	er 1, m/z 539.	.3004, rete	ntion time 2	29.63 min, d	harge +2				-
	arch program you're	using:	Run:A1 Scan nu	mber:2686		15000-				1		
Mascot Help		•				15000-						
	18225 ms/ms spectra		=			10000 -						
MSMS Prep			·E 28.987-		Intensity	10000-						E
· · · ·	agment ion count 4	.00	E 28.987 -		르	5000-						
Deis	otoping and charge (30.994 -									
						0		يدار البدانيلا بيد		يا باليا ب	. I.,	_
Imp	ort search results			<i>m/z</i>		Ó	200	400 60		300	1000	-
Clear	all identifications									Se	ection Comple	ete 🦻

In order to review, and refine the quality of the **Peptide Search** results click on the next stage in the workflow, **Refine Identifications**.

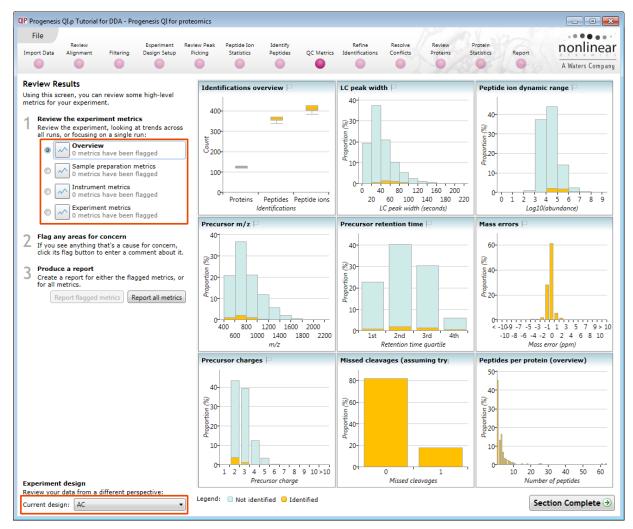
Performing the search Select the search program you're using:	
Mascot 🔹	
Mascot	
PLGS (*.xml) SEQUEST (dta & out files) SEQUEST (dta & pepXml files) Phenyx	
MS ^E Search	<u>A</u>
Deisotoping and charge deconv	volution
Import search results	
Clear all identifications	

Performing the search										
Select the search program you're using:										
Mascot 💌										
Help										
Export 18225 ms/ms spectra										
MSMS Preprocessing										
Limit fragment ion count										
Deisotoping and charge deconvolution										
Import search results										
Clear all identifications										

Stage 10: QC Metrics

Progenesis QI for proteomics includes a number of batch-based QC metrics which you can examine to increase confidence in, or identify issues with, your data. These views are presented at the "QC Metrics" page. By setting up experimental groupings that correspond to your batch metadata, you are able to investigate whether there are any systematic effects associated with your processing. This gives you confidence that your conclusions are not affected by technical biases within the course of the experiment.

The charts are updated with changes in your active experimental design, allowing you to examine the effects of all stages of your processing if you wish through using different batch divisions.



Metrics

There are two sets of metrics. Firstly, the overview page provides nine at-a-glance summary measurements which cover the experiment as a whole. Then, the remainder of the pages provide more detailed information and bring in the batch-by-batch detail, some of which builds on the overview charts:

- Sample preparation metrics highlighting issues or problems with the preparation of your samples: Missed cleavages, modifications and abundance dynamic range
- Instrument metrics highlighting whether your chromatography column and mass spectrometer are configured and performing correctly: Mass accuracy, abundance dynamic range (again), precursor charges, MS1 scan rates
- Experiment metrics concerning the identified proteins and peptides in your experiment, allowing you to pick out any outlying runs or conditions:
 Proteins, peptides, peptides per protein, % of peptide ions identified, proteins per condition

More detailed information on the QC metrics is available on the <u>FAQ</u> pages.

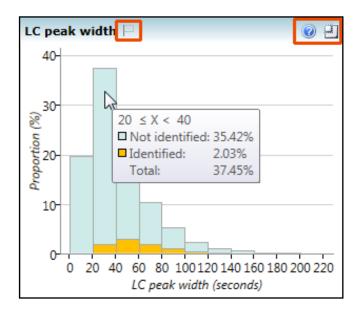
Interpretation and use

It's worth noting that there is no simple 'right' or 'wrong' answer as to whether your data are of high enough quality.

Note: QC measurements are designed to flag up potential issues for investigation, hence there must be an assignable cause to the variation observed to render QC practical.

Furthermore, the QC metrics will be rebuilt if you delete and re-do any stages leading up to them (for example, clearing identifications and re-searching with changed identification parameters, or altering the alignment). Hence, if you archive your experiment before and after your changes, or export the charts before and after, you can see the effects of your changes upon the quality measurements – a very useful method for assessing your interventions.

Note: that the overview metrics can each be expanded by clicking on the top-right icon in the sub-window. Additionally, hovering over a column will bring up a tooltip containing quantitative information on the results.



You can also tag metrics of interest or concern with a comment for your records, which is saved with the experiment. To do this, click the empty flag icon in the tab header for the given metric (or in the tile header on the overview screen):

	Missed cleavages 🏳	PTMs 🏳 Abundance dynamic range 🏳	Identifications overview	\square	0 🛛
1					

This will pop up a dialog allowing you to enter a comment describing why this metric has been flagged.

Reporting

You can export a report for your own records, or to pass on to another member of your team:

Report all metrics:

Generates a report containing all metrics shown in the application (overview, sample preparation metrics, instrument metrics and experiment metrics). This may be useful for documentation purposes, or to verify the quality of your experiment.

Report flagged metrics:

Generates a report of only metrics you have flagged, along with the message you provided. This may be useful for giving to a technician or other team member, to highlight areas of the experiment that need improvement.

In order to review, and refine the quality of the **Peptide Search** results click on the next stage in the workflow, **Refine Identifications**.

3	Crea	duce a report ite a report for either the ill metrics.	flagged metrics, or
		Report flagged metrics	Report all metrics

Stage 11: Refine Identifications

In this example the organism under study is Clostridium difficile

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 40
- Remove identifications where less than 2 hits were returned
- Remove all identifications where the Protein Description Contains 'hypothetical'
- Remove all identifications where the Protein Description **Contains** the following: 'Like', 'Putative', 'Probable', 'Potential', 'Predicted' and 'Partial'
- Remove all identifications where the Protein Description Doesn't contain 'Clostridium difficile'

IP Progen	nesis QI.p Ti	utorial fo	r DDA - Pr	rogenesis QI for pro	oteomics											
File																
		view .		Experiment	Review Pea						fine	Resolve	Review	Protein	21	nonlinea
Import Da	ata Align	ment	Filtering	Design Setup	Picking	Statistics	Pept	ides C	C Metrics	Identit	fications	Conflicts	Proteins	Statistics	Report	DYNAMIC
													1	8798 V.	1.2	A Waters Compan
	Identific				Batch	deletion criter	ia				1					
	eptide ider nt results, y			e unwanted or iem here.		Score	e less than	n •	40		J	s	equence Length	less than	•	
	ecify a set					Hit	s less than	n •					Charge	less than	•	
valu	ues for a se			ter the property s you want to		Mas	less than	n •					Sequence	contains	•	
dele					Mas	s error (ppm	less than	n •]				Accession	contains	•	
	identificat hlighted in		tching the	criteria will be		m/:	less thar	n •					Description	contains	•	
Del	ete the ur	wanted	l identifi	cations	R	etention Time	e less thar	ı •					Modifications	contains	•	
	delete the i			don't want, click			((
		tching S	earch Res	sults, to delete						Delet	te matchi	ng search i	results	ete non-matching se	earch results	Reset the criteria
	the highlig Delete No			h Results, to		# Score	e Hits	m/z	RT(mins)	Charge	Mass	Mass err	Sequence	Accession	Modifications	ه
				highlighted	V 1		5	539.30	29.63	2	1076.59	-0.37	LLFTQVDNK	🎯 gi 87239956		s-layer protein,
Res	set the cri	teria to	start ag	ain	✓ 1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🎯 gi 146325984		S-layer protein,
) To s	specify ano	ther bat	ch of iden	tifications to	▼ 1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🎯 gi 92380869		s-layer protein,
	ete, click Ri p 1 above.	eset the	Criteria a	nd then return to	▼ 1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🔮 gi 206725031		S-layer protein .
					1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🎯 gi 87239952		s-layer protein,
- T	No filter ap	plied		Create	1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🎯 gi 112181139		s-layer protein,
				Create	1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🎯 gi 255101963		cell surface prot
# 1	Total Hits	m/z	RT (min	Charge Tag	V 1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🔮 gi 206725029		S-layer protein
1 6	60	539.30(29.63	2	▼ 1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🔮 gi 21702505		S-layer protein
2 1	10	805.441	54.88	3	1		5	539.30	29.63	2	1076.59		LLFTQVDNK	🔮 gi 92380871		s-layer protein,
3 5	55	598.321	30.51	2	▼ 1		5	539.30	29.63	2	1076.59		LLFTQVDNK	- 51		S-layer protein
4 1	10	624.291	27.63	2	▼ 1		5	539.30	29.63	2	1076.59		LLFTQVDNK	- 51		cell surface prot
		604.337		2	2	75.17	5	805.44	54.88	3	2413.3(2.48	LVSPAPIVLA	1 🌑 gi 254976387		cell surface prot
		626.314		2	2	75.17	5	805.44	54.88	3	2413.30	2.48	LVSPAPIVLA	1 💿 gi 255101963		cell surface prot
				2	V 3	51.72	5	598.32	30.51	2	1194.63	-0.21	YQVVLYPEG	k 💿 gi 254976387		cell surface prot
· · ·			46.60	-	✓ 3		5	598.32	30.51	2	1194.63		YQVVLYPEG	- 51		S-layer protein .
		462.27(2	V 3	51.72	5	598.32	30.51	2	1194.63	-0.21	YQVVLYPEG	k 🔇 gi 255101963		cell surface prot
		753.828		2	V 3	51.72	5	598.32	30.51	2	1194.63	-0.21	YQVVLYPEG	k 🔞 gi 112181139		s-layer protein,
10 2		595.319	36.92	2 🧃	V 3	51.72	5	598.32	30.51	2	1194.63	-0.21	YQVVLYPEG			S-layer protein
11 1	18	573.803	24.19	2	V 3	51.72	5	598.32	30.51	2	1194.63	-0.21	YQVVLYPEG	k 💿 gi 87239952		s-layer protein,
12 8	84	573.324	41.74	2	V 3	51.72	5	598.32	30.51	2	1194.63	-0.21	YQVVLYPEG	k 💿 gi 92380869		s-layer protein,
14 3	30	656.861	44.14	2 🥤	*					1						•
	10	543.293	21.96	3 📮 🕶	3486 se	arch results. 1	137 matchi	ng batch c	lelete opt	ions.					Section	on Complete 🥱
٠								-							Sectio	in complete 🤄

On the Batch detection options panel, set the Score to less than 40, 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 (1137 matching out of 3486)

OIP Delete 1137 search results?

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

Now click Reset the criteria to clear the previous threshold then apply the next filter 'Hits: less than 2'.

Now in the 'Description' field enter '**hypothetical**' and delete matching search results. Then also in Description field enter the 'regular expression': **regex: like|puta|prob|pote|pred|part** and delete matching search results.

		atonarro	or DDA - Pr	rogenesis	Qi tor pr	oteomics										
File	Re	view		Exc	eriment	Review Peak	Peptide Ion	Identify		Refine	Resolv	ve R	Review	Protein		
nport Data		nment	Filtering		gn Setup	Picking	Statistics	Peptides	QC Metrics	Identifications			roteins	Statistics	Report	nonline
•			•		•	•	•	•		•	•		9/	0	9.5	A Waters Comp
efine lo	dentific	ations				Batch d	eletion criteria									
			ns include remove th				Score	less than	•		Seque	ence Leng	th less th	an 🔻		
Spec	ifv a set	of dele	tion crite	eria			Hits	less than	•			Charg	ge less th	an 🔹		
In the	batch d	eletion o	riteria, er itifications	nter the			Mass	less than	•			Sequen	ce contair	ns 🔻		
delete	e.					Mass	error (ppm)	less than	•			Accessio	on contain	ns 🔻		
	dentificat ghted in		tching the	e criteria	will be		m/z	less than	•			Descriptio	on contain	ns 🔻 🔽	egex: like puta p	rob pote pred par
Delet			d identifi			Dei	tention Time	(lass these	•			Iodificatio			-	
			a identifi ations you				tention time	less than			м	logification	ns contai	ns •		
either									(Delete matcl	hing searc	h results	🗆 Delet	e non-matching	search results	Reset the criteri
	elete Ma he highli		earch Res	sults, to	delete											
• D	elete No	n-match	ing Searc			OP D	elete 728 searc	h results?				23	lence	Accession	Modification	IS
d	elete the	IDs tha	t are not	highligh	ted								QVDNK	🕥 gi 8723995		s-layer protein,
							A	ire very want to	oormononthy	delete 728 pept	tida saarch	n coculte?	QVDNK	gi 1463259	84	S-layer protein
Reset	t the cri	teria to	start ag	ain			Are you su	ine you want to	permanentity	sciete 720 pept	noc scurer	riesuits:				
To sp	ecify and	ther bat	ch of iden	tification			Are you so	ire you want to	permanentiy	sciete 720 pept	lioc scorer	r results:	QVDNK	gi 9238086		s-layer protein,
To spi delete	ecify and	ther bat		tification			Are you st	ine you want to	permanentiy	selete 720 pept		rresults:	QVDNK	🔮 gi 2067250	31	S-layer protein
To sp delete step 1	ecify and e, click R L above.	ther bat eset the	ch of iden	tification			Are you su	ine you want to	permanentiy	Jereie 720 pepi		Tresuits:	QVDNK QVDNK	 gi 2067250 gi 8723995 	131 12	S-layer protein s-layer protein,
To sp delete step 1	ecify and e, click R	ther bat eset the	ch of iden	tification nd then	return to		Are you si	ine you want to	permanentiy	Yes		No	QVDNK QVDNK QVDNK	 gi 2067250 gi 8723995 gi 1121811 	131 12 39	S-layer protein s-layer protein, s-layer protein,
To sp delete step 1	ecify and e, click R L above.	ther bat eset the	ch of iden	tification nd then						Yes		No	QVDNK QVDNK QVDNK QVDNK	 gi 2067250 gi 8723995 gi 1121811 gi 2551019 	131 12 139 163	S-layer protein s-layer protein, s-layer protein, cell surface pro
To spi delete step 1	ecify and e, click R L above.	ther bat eset the plied	ch of iden	tification nd then	eate		59.57	5 539.	30 29.63	Yes 2 1076.5	55 -0.37	No	QVDNK QVDNK QVDNK QVDNK TQVDNK	 gi 2067250 gi 8723995 gi 1121811 gi 2551019 gi 2067250 	131 12 139 163 129	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein
To spi delete step 1	ecify and a, click R L above. o filter ap stal Hits	ther bat eset the plied	ch of iden Criteria a	tification nd then	eate		59.57 59.57	5 539. 5 539.	30 29.63 30 29.63	Yes 2 1076.5 2 1076.5	55 -0.37 55 -0.37	No S LLF	QVDNK QVDNK QVDNK QVDNK TQVDNK	 gi 2067250 gi 8723995 gi 1121811 gi 2551019 gi 2067250 gi 2170250 	131 12 135 165 125	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein
To spi delete step 1 W No To 60	ecify and e, click R L above. o filter ap otal Hits	ther bat eset the plied m/z	ch of iden Criteria a RT (min	tification nd then Cre Charge	eate	₹ ₹ ₹ 1 ₹ 1	59.57 59.57 59.57	5 539. 5 539. 5 539.	30 29.63 30 29.63 30 29.63	Yes 2 1076. 2 1076. 2 1076.	55 -0.37 55 -0.37 55 -0.37	No ULF ULF	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK	 gi 2067250 gi 8723995 gi 1121811 gi 2551019 gi 2067250 gi 2170250 gi 2170250 gi 9238087 	131 12 139 165 129 15 11	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein s-layer protein,
To spi delete step 1 W No To 60	ecify and e, click R L above. o filter ap otal Hits	plied m/z 805.441	ch of iden Criteria a RT (min 29.63 54.88	Cre Charge 2 3	eate	₹ ₹ ₹ 1 ₹ 1 ₹ 1	59.57 59.57 59.57 59.57 59.57	5 539. 5 539. 5 539. 5 539. 5 539.	30 29.63 30 29.63 30 29.63 30 29.63	Yes 2 1076.2 2 1076.2 2 1076.2 2 1076.5 2	55 -0.37 55 -0.37 55 -0.37 55 -0.37	No S LLF S LLF LLF LLF	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK	 gi 2067250 gi 8723995 gi 1121811 gi 2551019 gi 2067250 gi 2170250 gi 2170250 gi 9238087 gi 7173294 	131 12 139 163 129 15 15 11 14	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein s-layer protein, S-layer protein
To spi delete step 1 Vo To 60 10 55	ecify and e, click R L above. o filter ap otal Hits	ther bat eset the plied m/z 539.30(805.441 598.321	ch of iden Criteria a RT (min 29.63 54.88 30.51	Cre Charge 2 3 2	eate	₹ ₹ ₹ ₹ ₹ 1 ₹ 1 ₹ 1	59.57 59.57 59.57 59.57 59.57 59.57	5 539. 5 539. 5 539. 5 539. 5 539. 5 539.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63	Yes 2 1076. 2 1076. 2 1076. 2 1076. 2 1076. 2 1076.	55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37	No ULF ULF ULF ULF ULF ULF	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK	 gil2067250 gil8723995 gil121811 gil2551019 gil2067250 gil2170250 gil2170250 gil238087 gil7173294 gil2549763 	133 122 136 162 125 15 11 14 187	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein S-layer protein S-layer protein cell surface pro
To spi delete step 1 To 60 10 55 10	ecify and e, click R L above. o filter ap	ther bat eset the plied m/z 539.30(805.441 598.321 624.291	ch of iden Criteria a RT (min 29.63 54.88 30.51 27.63	Cre Charge 2 3 2 2	eate	₹ ₹ ₹ ₹ 1 ₹ 1 ₹ 1 ₹ 1 ₹ 2	59.57 59.57 59.57 59.57 59.57 59.57 75.17	5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 805.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63	Yes 2 1076. 2 1076. 2 1076. 2 1076. 2 1076. 3 2413.	55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 36 2.48	No ULF ULF ULF ULF ULF	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK	 gil2067250 gil8723995 gil1121811 gil2551019 gil2067250 gil2170250 gil2170250 gil9238087 gil7173294 gil2549763 gil2549763 gil2549763 	331 32 35 465 425 45 44 487 487	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein S-layer protein, S-layer protein cell surface pro cell surface pro
To spudelete step 1 To To 60 10 55 10 35	ecify anc a, click R L above. o filter ap	ther bat eset the plied m/z 539.30(805.441 598.321 624.291 604.337	ch of iden Criteria a RT (min 29.63 54.88 30.51 27.63 26.33	Cre Charge 2 3 2 2 2 2	eate	▼ 1 ▼ 1 ▼ 1 ▼ 1 ▼ 1 ▼ 1 ▼ 1 ▼ 2 ▼ 2	59.57 59.57 59.57 59.57 59.57 59.57 75.17 75.17	5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 805. 5 805.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 44 54.88	Yes 2 1076.3 2 1076.3 2 1076.3 2 1076.3 3 2413.3 3 2413.3	55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 30 2.48	No ULF ULF ULF ULF ULF ULF UVS UVS	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK SPAPIVLAT	 gil2067250 gil8723995 gil1121811 gil2551019 gil2067250 gil2170250 gil2540763 gil2551012 	331 32 35 465 425 45 44 487 487 465	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein s-layer protein s-layer protein cell surface pro cell surface pro cell surface pro
To spidelete step 1 V No To 60 10 55 10 35 15	ecify anc e, click R L above. o filter ap	ther bat eset the plied m/z 539.30(805.441 598.321 624.291 604.337 626.314	ch of iden Criteria a RT (min 29.63 54.88 30.51 27.63 26.33 38.94	Charge Charge 2 3 2 2 2 2 2	return to	I I <td< td=""><td>59.57 59.57 59.57 59.57 59.57 75.17 75.17 51.72</td><td>5 539. 5 539. 5 539. 5 539. 5 539. 5 805. 5 805. 5 805. 5 598.</td><td>30 29.63 30 30.51</td><td>Yes 2 1076.3 2 1076.3 2 1076.3 2 1076.3 2 1076.3 3 2413.3 3 2413.3 2 1194.6</td><td>55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 30 2.48 6: -0.21</td><td>No ULF ULF ULF ULF ULF ULF UVS UVS VS VS</td><td>QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK</td><td> gil2067250 gil2067250 gil723995 gil1121811 gil2551019 gil2067250 gil2170250 gil2170250 gil2170250 gil2170250 gil22549763 gil2551019 gil2551019 gil2549763 </td><td>331 32 335 665 225 55 11 44 887 887 865 887</td><td>S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein S-layer protein s-layer protein cell surface pro cell surface pro cell surface pro</td></td<>	59.57 59.57 59.57 59.57 59.57 75.17 75.17 51.72	5 539. 5 539. 5 539. 5 539. 5 539. 5 805. 5 805. 5 805. 5 598.	30 29.63 30 30.51	Yes 2 1076.3 2 1076.3 2 1076.3 2 1076.3 2 1076.3 3 2413.3 3 2413.3 2 1194.6	55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 30 2.48 6: -0.21	No ULF ULF ULF ULF ULF ULF UVS UVS VS VS	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK	 gil2067250 gil2067250 gil723995 gil1121811 gil2551019 gil2067250 gil2170250 gil2170250 gil2170250 gil2170250 gil22549763 gil2551019 gil2551019 gil2549763 	331 32 335 665 225 55 11 44 887 887 865 887	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein S-layer protein s-layer protein cell surface pro cell surface pro cell surface pro
To spidelete step 1 V No To 60 10 55 10 35 15 30	ecify ance , click R (above. o filter ap (tal Hits	ther bat eset the plied m/z 539.30(805.441 598.321 624.291 604.337 626.314 663.86§	ch of iden Criteria a 29.63 54.88 30.51 27.63 26.33 38.94 46.60	Charge Charge 2 2 2 2 2 2 2 2	eate	I I <td< td=""><td>59.57 59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72</td><td>5 539. 5 539. 5 539. 5 539. 5 539. 5 805. 5 805. 5 598. 5 598.</td><td>30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.53 30 29.53 30 29.53 30 29.53 30 51</td><td>Yes 2 1076.3 2 1076.3 2 1076.3 2 1076.3 3 2413.3 3 2413.3 2 1194.0 2 1194.0</td><td>-0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 32 2.48 34 -0.21</td><td>No ULF ULF ULF ULF UVS UVS VQ VQ</td><td>QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK SPAPIVLAT VVLYPEGK VVLYPEGK</td><td> gil2067250 gil8723995 gil121811 gil255019 gil2067250 gil2067250 gil2238087 gil2249763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 </td><td>331 32 335 462 425 45 44 487 487 463 487 487 487 487 487 487 487 487</td><td>S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein s-layer protein s-layer protein cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro s-layer protein</td></td<>	59.57 59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72	5 539. 5 539. 5 539. 5 539. 5 539. 5 805. 5 805. 5 598. 5 598.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.53 30 29.53 30 29.53 30 29.53 30 51	Yes 2 1076.3 2 1076.3 2 1076.3 2 1076.3 3 2413.3 3 2413.3 2 1194.0 2 1194.0	-0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 32 2.48 34 -0.21	No ULF ULF ULF ULF UVS UVS VQ VQ	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK SPAPIVLAT VVLYPEGK VVLYPEGK	 gil2067250 gil8723995 gil121811 gil255019 gil2067250 gil2067250 gil2238087 gil2249763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 	331 32 335 462 425 45 44 487 487 463 487 487 487 487 487 487 487 487	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein s-layer protein s-layer protein cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro s-layer protein
To spidelete step 1 No 10 55 10 35 15 30 10	ecify anc , click R L above. o filter ap tal Hits	ther bat eset the plied m/z 539.30(805.441 598.321 604.337 626.314 663.865 462.27(ch of iden Criteria a 29.63 30.51 27.63 26.33 38.94 46.60 34.33	Charge Charge 2 2 2 2 2 2 2 2 2 2 2 2	return to	I I <td< td=""><td>59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72 51.72</td><td>5 539, 5 539, 5 539, 5 539, 5 539, 5 539, 5 805, 5 805, 5 598, 5 598, 5 598,</td><td>30 29,63 30 20,51</td><td>Yes 2 1076.5 2 1076.5 2 1076.5 2 1076.5 2 1076.5 3 2413.3 3 2413.3 2 1194.4 2 1194.4</td><td>-0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 36 2.48 36 2.48 66 -0.21 66 -0.21 66 -0.21</td><td>No ULF ULF ULF ULF ULF UVS UVS VQ VQ VQ VQ VQ</td><td>QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK SPAPIVLAT VVLYPEGK VVLYPEGK</td><td> gil2067250 gil2067250 gil723995 gil121811 gil2551019 gil2067250 gil2067250 gil2234087 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil251019 gil251019 </td><td>331 32 335 662 125 15 14 487 487 465 877 465 877 465 877 477 477 477 477 477 477 477</td><td>S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein S-layer protein s-layer protein cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro</td></td<>	59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72 51.72	5 539, 5 539, 5 539, 5 539, 5 539, 5 539, 5 805, 5 805, 5 598, 5 598, 5 598,	30 29,63 30 20,51	Yes 2 1076.5 2 1076.5 2 1076.5 2 1076.5 2 1076.5 3 2413.3 3 2413.3 2 1194.4 2 1194.4	-0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 36 2.48 36 2.48 66 -0.21 66 -0.21 66 -0.21	No ULF ULF ULF ULF ULF UVS UVS VQ VQ VQ VQ VQ	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK SPAPIVLAT VVLYPEGK VVLYPEGK	 gil2067250 gil2067250 gil723995 gil121811 gil2551019 gil2067250 gil2067250 gil2234087 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil251019 gil251019 	331 32 335 662 125 15 14 487 487 465 877 465 877 465 877 477 477 477 477 477 477 477	S-layer protein s-layer protein, s-layer protein, cell surface pro S-layer protein S-layer protein S-layer protein s-layer protein cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro
To spidelete step 1 VC To 60 10 55 10 35 15 30 10 12	ecify anc , click R L above. o filter ap atal Hits	ther bat eset the plied m/z 539.30(805.441 598.321 624.291 604.337 626.314 663.865 462.27(753.828	RT (min 29.63 54.88 30.51 27.63 26.33 38.94 46.60 34.33 30.46	Charge Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Tag	I I <td< td=""><td>59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72 51.72 51.72</td><td>5 539. 5 539. 5 539. 5 539. 5 805. 5 805. 5 598. 5 598. 5 598. 5 598.</td><td>30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 30.51 30 30.51</td><td>Yes 2 1076:5 2 1076:5 2 1076:5 2 1076:3 2 1076:3 2 1076:3 2 1076:4 2 1076:4 2 1076:4 2 1076:4 2 1094:4 2 1194:4 2 1194:4</td><td>55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 30 2.48 31 2.48 32 -0.21 33 -0.21</td><td>No ULF ULF LLF LLF LLF LLF LVS LVS VQ VQ VQ VQ VQ VQ VQ VQ VQ</td><td>QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK VVLYPEGK VVLYPEGK VVLYPEGK</td><td> gil2067250 gil8723995 gil121811 gil2551012 gil21067250 gil2067250 gil2067250 gil2107250 gil2107250 gil2249763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2551012 </td><td>33 22 36 56 55 11 44 88 7 88 7 88 7 88 7 88 7 88 7 88</td><td>S-layer protein s-layer protein, s-layer protein, s-layer protein S-layer protein S-layer protein S-layer protein s-layer protein cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro s-layer protein S-layer protein,</td></td<>	59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72 51.72 51.72	5 539. 5 539. 5 539. 5 539. 5 805. 5 805. 5 598. 5 598. 5 598. 5 598.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 30.51 30 30.51	Yes 2 1076:5 2 1076:5 2 1076:5 2 1076:3 2 1076:3 2 1076:3 2 1076:4 2 1076:4 2 1076:4 2 1076:4 2 1094:4 2 1194:4 2 1194:4	55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 30 2.48 31 2.48 32 -0.21 33 -0.21	No ULF ULF LLF LLF LLF LLF LVS LVS VQ VQ VQ VQ VQ VQ VQ VQ VQ	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK VVLYPEGK VVLYPEGK VVLYPEGK	 gil2067250 gil8723995 gil121811 gil2551012 gil21067250 gil2067250 gil2067250 gil2107250 gil2107250 gil2249763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2549763 gil2551012 	33 22 36 56 55 11 44 88 7 88 7 88 7 88 7 88 7 88 7 88	S-layer protein s-layer protein, s-layer protein, s-layer protein S-layer protein S-layer protein S-layer protein s-layer protein cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro s-layer protein S-layer protein,
To spidelete step 1 No To 60 10 55 10 35 15 30 10 12 0 20	ecify anc , click R L above. o filter ap	ther bat eset the plied m/z 539.30(805.44) 598.321 624.291 604.337 626.314 663.865 462.27(753.828 595.315	RT (min 29.63 54.88 30.51 27.63 26.33 38.94 46.60 34.33 30.46 36.92	Charge Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	return to	I I	59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72 51.72 51.72 51.72 51.72	5 539. 5 539. 5 539. 5 539. 5 539. 5 805. 5 586. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30.51 30.51 32 30.51 32 30.51 32 30.51	Ves 2 1076.1 2 1076.5 2 1076.5 2 1076.5 2 1076.5 3 2413.3 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4	55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 53 2.48 62 2.48 63 2.41 64 -0.21 65 -0.21 66 -0.21 66 -0.21	No ULF ULF LLF LLF LLF LLS LVS VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK VVLYPEGK VVLYPEGK VVLYPEGK VVLYPEGK	gl206725C gl872395 gl872395 gl972395 gl972395 gl972302 gl920725C gl92249763 gl925549 gl925549 g	33 22 33 46 45 45 44 48 48 48 48 48 48 48 48 48	S-layer protein s-layer protein, s-layer protein, s-layer protein S-layer protein S-layer protein S-layer protein cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro cell surface pro s-layer protein S-layer protein S-layer protein
To spidelete step 1 Nc To 2 10 3 55 4 10 5 5 15 7 30 8 10 9 12 10 20 11 18	ecify anc , click R L above. o filter ap	ther bat eset the plied m/z 539.30(805.44) 598.321 624.291 604.337 626.314 663.866 462.27(753.828 595.315 573.805	ch of idem Criteria a 29.63 54.88 30.51 27.63 26.33 38.94 46.60 34.33 30.46 36.92 24.19	Charge Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Tag	I I	59,57 59,57 59,57 59,57 75,17 75,17 51,72 51,72 51,72 51,72 51,72 51,72 51,72	5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30.51 30.51 32 30.51 32 30.51 32 30.51	Yes 2 1076.5 2 1076.5 2 1076.5 2 1076.5 2 1076.4 2 1076.4 3 2413.3 3 2413.3 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4	55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 30 2.48 31 2.48 32 2.48 33 2.48 34 -0.21 35 -0.21 36 -0.21 36 -0.21 36 -0.21	No LLF LLF LLF LLF LLF LVS LVS VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK PAPIVLAT VVLYPEGK VVLYPEGK VVLYPEGK VVLYPEGK	gl206725C gl872399 gl112811 gl251012 gl251012 gl251012 gl251012 gl2551012 gl2551012 gl2551012 gl2551012 gl254763 gl2551012 gl254763 gl2551012 gl254763 gl2551012 gl212705725C gl2551012 gl212705725C gl2551012 gl21705725C gl2551012 gl21705725C gl2551012 gl21705725C gl275725C gl2551012 gl21705725C gl27575C gl2757	131 12 13 14 15 15 11 14 18 17 18 18 18 18 18 18 18 19 19 19 19 19 19 19 19 19 19 19 19 19	S-layer protein s-layer protein, s-layer protein, s-layer protein, S-layer protein s-layer protein cell surface prot cell surface prot cell surface prot cell surface prot s-layer protein cell surface prot s-layer protein s-layer protein s-layer protein s-layer protein
To spidelete step 1 Nc To 2 10 3 55 4 10 5 5 15 7 30 8 10 9 12 10 20 11 18 12 84	ecify anc , click R Labove. o filter ap	m/z 539.30(805.44) 598.32) 624.29) 604.337 626.314 663.865 462.27(753.828 595.315 573.805 573.324	ch of idem Criteria a 29.63 54.88 30.51 27.63 26.33 38.94 46.60 34.33 30.46 36.92 24.19 41.74	Charge Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	return to	I I	59.57 59.57 59.57 59.57 75.17 75.17 51.72 51.72 51.72 51.72 51.72 51.72	5 539. 5 539. 5 539. 5 539. 5 539. 5 805. 5 586. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598.	30 29.63 30 20.51 30 20.51 30 30.51	Yes 2 1076.5 2 1076.5 2 1076.5 2 1076.5 2 1076.5 3 2413.5 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4	55 -0.37 55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 53 2.48 62 2.48 63 2.41 64 -0.21 65 -0.21 66 -0.21 66 -0.21	No LLF LLF LLF LLF LLF LVS LVS VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK VVLYPEGK VVLYPEGK VVLYPEGK	gl206725C gl872399 gl112811 gl251012 gl251012 gl251012 gl251012 gl2551012 gl2551012 gl2551012 gl2551012 gl254763 gl2551012 gl254763 gl2551012 gl254763 gl2551012 gl212705725C gl2551012 gl212705725C gl2551012 gl21705725C gl2551012 gl21705725C gl2551012 gl21705725C gl275725C gl2551012 gl21705725C gl27575C gl2757	131 12 13 14 15 15 11 14 18 17 18 18 18 18 18 18 18 19 19 19 19 19 19 19 19 19 19 19 19 19	S-layer protein s-layer protein, s-layer protein, s-layer protein S-layer protein S-layer protein cell surface prot cell surface prot cell surface prot cell surface prot cell surface prot s-layer protein, cell surface prot s-layer protein, S-layer protein, S-layer protein
To spidelete step 1 Nc To 2 10 3 55 4 10 5 5 15 7 30 8 10 9 12 10 20 11 18	ecify anc , click R Labove. o filter ap	ther bat eset the plied m/z 539.30(805.44) 598.321 624.291 604.337 626.314 663.865 462.27(753.828 595.315 573.805	ch of idem Criteria a 29.63 54.88 30.51 27.63 26.33 38.94 46.60 34.33 30.46 36.92 24.19 41.74	Charge Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Tag	I I	59,57 59,57 59,57 59,57 75,17 75,17 51,72 51,72 51,72 51,72 51,72 51,72 51,72	5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 539. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598. 5 598.	30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30 29.63 30.51 30.51 32 30.51 32 30.51 32 30.51	Yes 2 1076.5 2 1076.5 2 1076.5 2 1076.5 2 1076.5 3 2413.5 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4 2 1194.4	55 -0.37 55 -0.37 55 -0.37 55 -0.37 56 -0.37 30 2.48 30 2.48 31 2.48 32 2.48 33 2.48 34 -0.21 35 -0.21 36 -0.21 36 -0.21 36 -0.21	No LLF LLF LLF LLF LLF LVS LVS VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ VQ	QVDNK QVDNK QVDNK QVDNK TQVDNK TQVDNK TQVDNK TQVDNK TQVDNK PAPIVLAT VVLYPEGK VVLYPEGK VVLYPEGK VVLYPEGK	gl206725C gl872399 gl112811 gl251012 gl251012 gl251012 gl251012 gl2551012 gl2551012 gl2551012 gl2551012 gl254763 gl2551012 gl254763 gl2551012 gl254763 gl2551012 gl212705725C gl2551012 gl212705725C gl2551012 gl21705725C gl2551012 gl21705725C gl2551012 gl21705725C gl275725C gl2551012 gl21705725C gl27575C gl2757	131 12 13 14 15 15 11 14 18 17 18 18 18 18 18 18 18 19 19 19 19 19 19 19 19 19 19 19 19 19	S-layer protein s-layer protein, s-layer protein, s-layer protein, S-layer protein, S-layer protein cell surface prot cell surface prot cell surface prot cell surface prot cell surface prot s-layer protein, cell surface prot s-layer protein, S-layer protein s-layer protein s-layer protein

Finally alter the Description to 'doesn't contain Clostridium difficile' and delete the matching search results.

QIP Prog	jenesis QI.p 1	Futorial fo	or DDA - P	rogenesi	s QI for pr	oteomics													23
File		view		-	periment	Review P	eak Peptide		Identify			Refine	Resolve	- P	eview	Protein			• • • *
Import		inment	Filtering		ign Setup	Pickin			Peptides	QC Metrics	Ide	ntifications	Conflict		oteins	Statistics	Report	nonlir	near
•			•		•	•	•		•			•	•			0	9.0	A Waters Co	ompany
Refin	e Identifi	cations				Bate	ch deletion cri	teria											
	r peptide ide						Sc	ore le	ess than	•				Sequence	e Length	less than	•		
	ant results,				е.			lits	ess than	•					Charge	less than	•		
	pecify a se the batch				property					•							•		
	alues for a s	et of ider	ntification	ns you wa	ant to			6	ess than					5	equence	contains	•		
	ny identifica		a shi sa ah			N	lass error (pp	m) le	ess than	•				A	ccession	contains	•		
	ighlighted in		tening th	e criteria	will be			n/z le	ess than	•				De	scription	doesn't contain	 clostrid 	ium difficile	
D	elete the u	inwante	d identif	ications			Retention Ti	me le	ess than	•				Modi	fications	contains	•		_
L τ	o delete the							6											
ei	 Delete M 	atching S	earch Pe	eulte to	delete					l	De	elete matchi	ing search	results	Dele	te non-matching	search results	Reset the crit	teria
	the highl	ighted ID)s						1						vence	Accession	Modificati	one	*
	 Delete N delete th 	on-match e IDs tha					P Delete 192 s	earch	results?					23	QVDNK	qi 2551019		cell surface (prof
							A		e you want to p		delet	102	da saarsh	reaulte?	QVDNK	gi 2170250		S-layer prote	
	eset the cr o specify an				ns to	8	• Aleyo	u sure	e you want to p	bermanentiy	ueleti	i 192 pepud	Je search	results:	QVDNK	gi[71732944		S-layer prote	
d	elete, click F	Reset the				E									QVDNK	gi 25497638	37	cell surface	prot
st	tep 1 above.	·													APIVLAT	gi 2549763	87	cell surface p	prot
	No filter a	oplied									1	Yes		0	PAPIVLAT	i 💿 gi 2551019	53	cell surface p	prot
				L Ch	eate						Ŀ				VLYPEG	i 🎯 gi 2549763	37	cell surface p	prot
#	Total Hits	m/z	RT (min	Charge	Tag		3 51.	72	5 598.3	32 30.51	2	1194.63	-0.21		VLYPEG	- 51	53	cell surface p	prot
1	60	539.30(29.63	2	•		3 51.		5 598.3		2	1194.63			VLYPEG			S-layer prote	
2	10	805.441	54.88	3			4 89.		5 624.2		2	1246.57			NAIDDA			cell surface	
3	55	598.321	30.51	2			4 89.		5 624.2		2	1246.57		-	NAIDDA	- 51		cell surface p	
4	10	624.291	27.63	2					5 604.3		2	1206.66		-	AGGVNS	- 51		S-layer prote	
5	35	604.337	26.33	2		V	5 76. 5 76.		5 604.3 5 604.3		2 2	1206.6(1206.6(-	AGGVNS AGGVNS	- 51		cell surface p	·
6	15	626.314	38.94	2			5 76.		5 604.3		2	1206.66		-	AGGVINS			precursor of SlpA [[Clostr	
7	30	663.869	46.60	2			-		5 604.		2	1206.66			AGGVINS			SipA [[Clostr S-layer prote	
8	10	462.27(34.33	2			5 76.		5 604.3		2	1206.66		-	AGGVNS	- 51		cell surface i	
9	12	753.828	30.46	2					5 604.3		2	1206.66		-	AGGVNS	- 51		cell surface i	
10	20	595.319	36.92	2			6 47.		5 626.3		2	1250.61		-	DYVDDL	- 31		cell surface (
11	18	573.803	24.19	2			6 47.	91	5 626.	31 38.94	2	1250.61	0.21	DLK	DYVDDL	gi 2551019	53	cell surface	
12	84	573.324	41.74	2			6 47.	91	5 626.3	31 38.94	2	1250.61	0.21	S DLK	DYVDDL	gi 2170250	5	S-layer prote	ein 👻
14	30	656.861	44.14	2		-					1								- F
15	10	543.293	21.96	3	•	1404	search results	192 r	matching batch	delete optio	ins.						5	tion Complet	
4			_		+				-								Sec	complet	

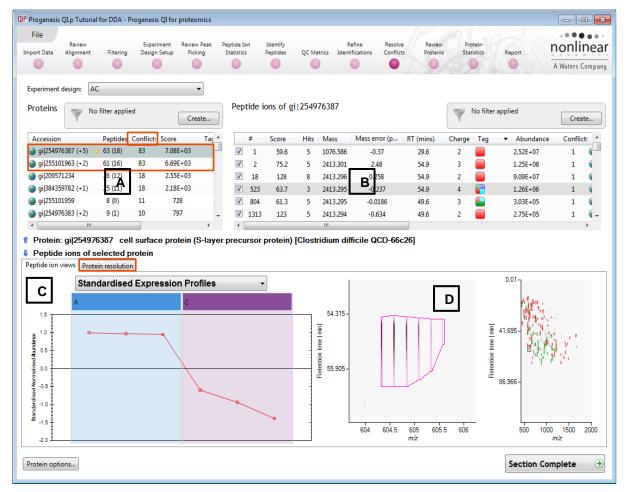
Having applied all the filters there will be 1212 search results remaining

To validate the Peptide search results at the protein level select the next stage in the workflow by clicking on **Resolve Conflicts**.

Stage 12: Resolve Conflicts

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

The **Resolve Conflicts** stage provides a number of interrelated graphical and tabular views to assist you in the validation of the peptides that have been assigned to proteins and also to review the relevance of the data returned from the search.



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.

Note: the default Protein options for protein grouping and Protein quantitation are set as shown

QIP Protein quantitation options	x
Quantitation method:	ר
Relative Quantitation using Hi-N	•
Number of peptides to measure per protein (N): 3	▲ ▼
Employ protein grouping, i.e. hide proteins whose peptides are a subset of another protein s.	
OK Cancel	

This means that if you choose **not** to resolve the conflicts then proteins, to be considered for quantitation, require at least one unique peptide (number in brackets after peptide count).

For more details on Protein Grouping page 53 and Protein Quantitation go to page 54

Open **Resolve Conflicts** and order the data in the Proteins table (A) on the basis of **Conflicts**.

Note: the look of the tables (with regards to ordering) in the following section may vary slightly.

Depending on the ordering, make '**cell surface protein (S-layer precursor etc '** the current protein by clicking on it in Window A (a circular orange symbol indicates current protein). This protein has 63 peptides assigned (window B) which have a total of 83 conflicts. To view the conflicting assignments click on the **Protein Resolution** tab (window C).

File Review port Data Alignmer	nt Filtering	Experi Design	Setup Pick	w Peak king	Peptic Stati	de Ion istics	Identi Peptid		Metrics Ide	Refine Reso ntifications Confi			rotein atistics	Report	non A Waters	M I
Experiment design:	AC			-						•	1. 1.	101.4			A mater:	comp
	No filter applie	d		eate) P	epti	de ions o	of gi 254	1976387			Y	No filte	r applied	Cn	eate
Accession	Peptides	Conflict:	Score	Taç 1	- E	4	¢ Sco	re Hit	s Mass	Mass error (p	RT (mins)	Charge	Tag	+ Abundance	e Conf	lict:
🅥 gi 254976387 (+5)	0 63 (18)	83	7.08E+03		[V	1 5	9.6 5	1076.586	5 -0.37	29.6	2		2.52E+07	1	6
🎯 gi 255101963 (+2)	61 (16)	83	69E+03		[V	2 7	5.2 5	2413.301	2.48	B 54.9	3		1.25E+08	1	6
🎯 gi 209571234	26 (12)	18	1.55E+03		[V	18 1	28 8	2413.29	5 0.258	54.9	2		9.09E+07	1	6
🎯 gi 384359782 (+1)	25 (11)	18	2.18E+03		[V	523 6	3.7 3	2413.29	5 -0.237	54.9	4		1.26E+06	1	6
gi 255101959	8 (0)	11	728		[V	804 63	1.3 5	2413.29	5 -0.0186	49.6	3		3.03E+05	1	6
9/200101000																
🎯 gi 255101555 🎯 gi 254976383 (+2)	9 (1)	10	797		- [V 1	313 1	23 5	2413.294	4 -0.634	49.6	2		2.75E+05	1	4
 gi 254976383 (+2) Protein: gi 254 Protein: gi 255 	976387 cel	l surfac I surfac	e protein (er pre	<	sor prote	ein) [Clo	m stridium d	lifficile QCD-66	5c26]	2	-	2.75E+05	1	•
 gi 254976383 (+2) Protein: gi 254 Protein: gi 255 	976387 cel 101963 cel rotein resolution	l surfac I surfac	e protein (e protein (er pre	<	sor prote sor prote	ein) [Clo	" stridium d	lifficile QCD-66	5c26]	2		2.75E+05	1	- P
gi 254976383 (+2) Protein: gi 254 Protein: gi 254 Protein: gi 255 Peptide ion views Pr	976387 cel 101963 cel rotein resolution teins for pep	I surfac I surfac n otide ior	e protein (e protein (S-laye	er pre er pre Pep	<	sor prote sor prote	ein) [Clo ein) [Clo	m stridium d stridium d 01963	lifficile QCD-66	6c26] 3q42]		Tag	2.75E+05	Conflict	• •
gi 254976383 (+2) Protein: gi 254 Protein: gi 255 Protein: gi 255	976387 cel 101963 cel rotein resolution teins for pep Peptides	I surfac I surfac n otide ior	e protein (e protein (n 523	S-laye	er pre er pre Pep	<	sor prote sor prote ions of Score	ein) [Clo ein) [Clo gi 2551	m stridium d stridium d 01963	lifficile QCD-66	6c26] 3q42]	Charge	-		_	•
gi 254976383 (+2) Protein: gi 254 Protein: gi 254 Protein: gi 255 Protein: gi 255 Conflicting prot Accession	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (1 523 e Protein Sco 7.08E+03	S-laye	er pre er pre Pep	<	sor prote sor prote ions of Score	ein) [Clo ein) [Clo gi 2551 Hits	iii stridium d stridium d 01963 Mass	lifficile QCD-66 lifficile QCD-65 Mass error (p	6c26] 3q42] RT (mins) C	Charge	-	✓ Abundance	Conflict	• • •
gi 254976383 (+2) Protein: gi 254 Protein: gi 254 Protein: gi 255 Protein: gi 255 Conflicting prot Accession gi 254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac I surfac n otide ior Conflict	e protein (e protein (1 523 e Protein Sco 7.08E+03	S-laye	er pre er pre Pep	ecurs	sor prote sor prote ions of Score 63.7 61.3	ein) [Clo ein) [Clo gi 2551 Hits 3	mi stridium d stridium d 01963 Mass 2413.295	ifficile QCD-60 ifficile QCD-60 Mass error (p -0.237	5c26] 3q42] RT (mins) C 54.9	Charge	-	 Abundance 1.26E+06 	Conflict 1	Pi A
gi[254976383 (+2) Protein: gi[254 Protein: gi[254 Protein: gi[255 eptide ion views Pr Conflicting prot Accession gi[254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (523 : Protein Sco 7.08±+03 	S-laye	Pep	 curs <li< td=""><td>sor prote sor prote ions of Score 63.7 61.3 3 123</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5</td><td>mi stridium d stridium d 01963 Mass 2413.295 2413.295</td><td>ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186</td><td>5c26] 3q42] RT (mins) C 54.9 49.6 49.6 52.1</td><td>Charge 4 3 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 </td><td>Conflict 1 1</td><td>P • •</td></li<>	sor prote sor prote ions of Score 63.7 61.3 3 123	ein) [Clo ein) [Clo gi 2551 Hits 3 5	mi stridium d stridium d 01963 Mass 2413.295 2413.295	ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186	5c26] 3q42] RT (mins) C 54.9 49.6 49.6 52.1	Charge 4 3 2 2	-	 Abundance 1.26E+06 3.03E+05 	Conflict 1 1	P • •
gi[254976383 (+2) Protein: gi[254 Protein: gi[254 Protein: gi[255 eptide ion views Pr Conflicting prot Accession gi[254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (523 : Protein Sco 7.08±+03 	S-laye	Pep	 curs <li< td=""><td>e ions of Score 63.7 61.3 123 122 51.7</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5</td><td>m stridium d stridium d 01963 Mass 2413.295 2413.295 2413.294 2413.294 1194.628</td><td>Infficile QCD-66 Infficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206</td><td>RT (mins) C 54.9 49.6 49.6</td><td>Charge 4 3 2 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 </td><td>Conflict 1 1</td><td>P ^ 3 E</td></li<>	e ions of Score 63.7 61.3 123 122 51.7	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5	m stridium d stridium d 01963 Mass 2413.295 2413.295 2413.294 2413.294 1194.628	Infficile QCD-66 Infficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206	RT (mins) C 54.9 49.6 49.6	Charge 4 3 2 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 	Conflict 1 1	P ^ 3 E
gi[254976383 (+2) Protein: gi[254 Protein: gi[254 Protein: gi[255 eptide ion views Pr Conflicting prot Accession gi[254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (523 : Protein Sco 7.08±+03 	S-laye	Pep	 ecurs 	e ions of Score 63.7 61.3 3 123 3 122 51.7 89.8	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2	m stridium d stridium d 01963 Mass 2413.295 2413.295 2413.294 2413.294	Ifficile QCD-66 Ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607	Sc26] 3q42] RT (mins) C 54.9 49.6 49.6 52.1 30.5 27.6	Charge 4 3 2 2 2 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 	Conflict 1 1 1 1	
gi 254976383 (+2) Protein: gi 254 Protein: gi 254 Protein: gi 255 Protein: gi 255 Conflicting prot Accession gi 254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (523 : Protein Sco 7.08±+03 	S-laye	Pep	 curs <li< td=""><td>e ions of Score 63.7 61.3 3 123 3 122 51.7 89.8 2 50.3</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 2 5</td><td>m stridium d stridium d 01963 Mass 2413.295 2413.295 2413.294 2413.294 1194.628</td><td>ifficile QCD-66 ifficile QCD-63 0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393</td><td>Sc26] 3q42] RT (mins) C 54.9 49.6 52.1 30.5</td><td>Charge 4 3 2 2 2 2 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 </td><td>Conflict 1 1 1 1</td><td>P</td></li<>	e ions of Score 63.7 61.3 3 123 3 122 51.7 89.8 2 50.3	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 2 5	m stridium d stridium d 01963 Mass 2413.295 2413.295 2413.294 2413.294 1194.628	ifficile QCD-66 ifficile QCD-63 0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393	Sc26] 3q42] RT (mins) C 54.9 49.6 52.1 30.5	Charge 4 3 2 2 2 2 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 	Conflict 1 1 1 1	P
gi[254976383 (+2) Protein: gi[254 Protein: gi[254 Protein: gi[255 eptide ion views Pr Conflicting prot Accession gi[254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (523 : Protein Sco 7.08±+03 	S-laye	Pep V V V V V V	 curs <li< td=""><td>sor prote sor prote sor prote score 63.7 61.3 123 122 51.7 89.8 2 50.3 3 64.9</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 2 5 5 4 4 4</td><td>III iii stridium d otridium d</td><td>ifficile QCD-64 ifficile QCD-63 -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338</td><td>5c26] 3q42] RT (mins) C 54.9 49.6 49.6 52.1 27.6 47.3 53.9</td><td>Charge 4 3 2 2 2 2 2 2 2 2 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 </td><td>Conflict 1 1 1 1 1 1 1</td><td>• • • • • • • • • • • • •</td></li<>	sor prote sor prote sor prote score 63.7 61.3 123 122 51.7 89.8 2 50.3 3 64.9	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 2 5 5 4 4 4	III iii stridium d otridium d	ifficile QCD-64 ifficile QCD-63 -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338	5c26] 3q42] RT (mins) C 54.9 49.6 49.6 52.1 27.6 47.3 53.9	Charge 4 3 2 2 2 2 2 2 2 2 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 	Conflict 1 1 1 1 1 1 1	• • • • • • • • • • • • •
gi[254976383 (+2) Protein: gi[254 Protein: gi[254 Protein: gi[255 eptide ion views Pr Conflicting prot Accession gi[254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (523 : Protein Sco 7.08±+03 	S-laye	Pep V V V V V V V V V V	 curs <li< td=""><td>e ions of Score 63.7 61.3 3 123 3 122 51.7 89.8 2 50.3</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 5 5 4</td><td>III iii stridium d otridium d</td><td>ifficile QCD-66 ifficile QCD-63 0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393</td><td>Sc26] 3q42] RT (mins) C 54.9 49.6 52.1 30.9 27.5 47.3</td><td>Charge 4 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 </td><td>Conflict 1 1 1 1 1 1 1</td><td>• • • • • • • • • • • • •</td></li<>	e ions of Score 63.7 61.3 3 123 3 122 51.7 89.8 2 50.3	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 5 5 4	III iii stridium d otridium d	ifficile QCD-66 ifficile QCD-63 0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393	Sc26] 3q42] RT (mins) C 54.9 49.6 52.1 30.9 27.5 47.3	Charge 4 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 	Conflict 1 1 1 1 1 1 1	• • • • • • • • • • • • •
gi 254976383 (+2) Protein: gi 254 Protein: gi 254 Protein: gi 255 Protein: gi 255 Conflicting prot Accession gi 254976387 (+:	976387 cel 101963 cel rotein resolution teins for pep Peptides 5) • 63 (18)	I surfac	e protein (e protein (523 : Protein Sco 7.08±+03 	S-laye	Pep V V V V V V	 curs <li< td=""><td>sor prote sor prote sor prote score 63.7 61.3 123 122 51.7 89.8 2 50.3 3 64.9</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 2 5 5 4 4 4</td><td>III iii stridium d otridium d</td><td>ifficile QCD-64 ifficile QCD-63 -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338</td><td>5c26] 3q42] RT (mins) C 54.9 49.6 49.6 52.1 27.6 47.3 53.9</td><td>Charge 4 3 2 2 2 2 2 2 2 2 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 </td><td>Conflict 1 1 1 1 1 1 1</td><td></td></li<>	sor prote sor prote sor prote score 63.7 61.3 123 122 51.7 89.8 2 50.3 3 64.9	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 2 5 5 4 4 4	III iii stridium d otridium d	ifficile QCD-64 ifficile QCD-63 -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338	5c26] 3q42] RT (mins) C 54.9 49.6 49.6 52.1 27.6 47.3 53.9	Charge 4 3 2 2 2 2 2 2 2 2 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 	Conflict 1 1 1 1 1 1 1	

In this case the conflicting peptide assignments are with '**The same protein**' (from a different strain) which also contains 83 conflicts. A simple resolution to these conflicts is to right click on the conflicting protein and turn off all its peptides (based on lower number of peptides and score).

File Review Port Data Alignment	Filtering		niment Review n Setup Pick			de Ion istics	Ident Peptic		Metrics Ide	Refine Resolutions Conflik			rotein atistics	Report	A Waters	M
	AC lo filter applie	ed		•	P	eptic	de ions (of gi 25	1976387			7	No filte	er applied	Cre	eate
Accession	Peptides	Conflict	Score	Tac	*	#	Sco	re Hi	s Mass	Mass error (p	RT (mins)	Charge	e Tag	▼ Abundance	e Conf	lict:
谢 gi 254976387 (+5)	o 63 (63)	0	7.08E+03			V	1 5	9.6 5	1076.586	-0.37	29.6	2		2.52E+07	0	6
) gi 255101963 (+2)	0 (0)	0	0			V	2 7	5.2 5	2413.301	2.48	54.9	3		1.25E+08	0	6
🕽 gi 209571234	26 (12)	18	2.55E+03			V	18 1	28 8	2413.296	0.258	54.9	2		9.09E+07	0	6
) gi 384359782 (+1)	25 (11)	18	2.18E+03			V 5	623 6	3.7 3	2413.295	-0.237	54.9	4		1.26E+06	0	6
gi 255101959	8 (0)	11	728			V 8	804 6	1.3 5	2413.295	-0.0186	49.6	3		3.03E+05	0	6
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25510	76387 cel 01963 cel	l surfa			er pre	< Curs	sor prot		m stridium d	ifficile QCD-66	-	2	-	2.75E+05	0	+
gi 254976383 (+2) Protein: gi 25497 Protein: gi 2551(ptide ion views Prot	76387 cel 01963 cel tein resolution	l surfa I surfa	ce protein (ce protein (er pre er pre	< Curs	sor prot sor prot	ein) [Clo	‴ stridium d stridium d	ifficile QCD-66	c26]	2	-	2.75E+05	0	F
gi 254976383 (+2) Protein: gi 25497 Protein: gi 2551(ptide ion views Prot	76387 cel D1963 cel tein resolution ins for pep	I surfa	ce protein (ce protein (S-lay	er pre er pre	< Curs	sor prot sor prot	ein) [Clo ein) [Clo	iii stridium d stridium d 01963	ifficile QCD-66 ifficile QCD-63	c26] q42]		Tag	2.75E+05	0 Conflict:	Pi -
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 25510 ptide ion views Prot Conflicting prote Accession	76387 cel 01963 cel tein resolution ins for pep Peptides	I surfa	ce protein (ce protein (on 523	S-lay	er pre er pre	< curs	sor prote sor prote ions of	ein) [Clo ein) [Clo gi 2551	iii stridium d stridium d 01963	ifficile QCD-66 ifficile QCD-63	c26] q42]	harge	Tag			
gi 254976383 (+2) Protein: gi 25497 Protein: gi 2551(ptide ion views Prote Conflicting prote	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surface I surface otide id Conflic	ce protein (ce protein (ce protein (ce protein Sco	S-lay	er pre	< ecurs ecurs ecurs	ions of Score	ein) [Clo ein) [Clo gi 2551 Hits	stridium d stridium d 01963 Mass	ifficile QCD-66 ifficile QCD-63 Mass error (p 1	c26] q42] RT (mins) C	harge		▼ Abundance	Conflicts	
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession ≩ gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	er pre	 curs curs curs curs curs curs curs curs 	ions of Score 63.7 61.3	ain) [Clo ain) [Clo gi 2551 Hits 3	m stridium d stridium d 01963 Mass 2413.295	ifficile QCD-66 ifficile QCD-63 Mass error (p 1 -0.237	c26] q42] RT (mins) C 54.9	harge	-	 Abundance 1.26E+06 	Conflict	3 E 3 3
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession ≩ gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	er pre	 curs curs curs curs curs curs scurs scu	ions of Score 63.7 61.3 123	ein) [Clo ein) [Clo gi 2551 Hits 3 5	m stridium d stridium d 01963 Mass 2413.295 2413.295	ifficile QCD-66 ifficile QCD-63 Mass error (p 1 -0.237 -0.0186	c26] q42] RT (mins) C 54.9 49.6	harge 4 3	-	 ▼ Abundance 1.26E+06 3.03E+05 	Conflict: 0 0	3 3 3 3 3
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession ≩ gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	er pro	 curs <li< td=""><td>ions of Score 63.7 61.3 123</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5</td><td>m stridium d stridium d 01963 Mass 2413.295 2413.295 2413.295 2413.294</td><td>ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634</td><td>c26] q42] RT (mins) C 54.9 49.6 49.6</td><td>harge 4 3 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 </td><td>Conflict: 0 0</td><td>3 E</td></li<>	ions of Score 63.7 61.3 123	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5	m stridium d stridium d 01963 Mass 2413.295 2413.295 2413.295 2413.294	ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634	c26] q42] RT (mins) C 54.9 49.6 49.6	harge 4 3 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 	Conflict: 0 0	3 E
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession ≩ gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	Per pre	 curs <li< td=""><td>ions of Score 63.7 61.3 123 122</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2</td><td>III stridium d stridium d 01963 Mass 2413.295 2413.294 2413.294</td><td>ifficile QCD-66 ifficile QCD-63 Mass error (p 1 -0.237 -0.0186 -0.634 -0.607</td><td>c26] q42] RT (mins) C 54.9 49.6 49.6 52.1</td><td>harge 4 3 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 </td><td>Conflict: 0 0 0</td><td>3 E 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3</td></li<>	ions of Score 63.7 61.3 123 122	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2	III stridium d stridium d 01963 Mass 2413.295 2413.294 2413.294	ifficile QCD-66 ifficile QCD-63 Mass error (p 1 -0.237 -0.0186 -0.634 -0.607	c26] q42] RT (mins) C 54.9 49.6 49.6 52.1	harge 4 3 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 	Conflict: 0 0 0	3 E 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession ≩ gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	er pro	 curs <li< td=""><td>ions of Score 63.7 61.3 123 122 51.7 89.8 50.3</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 2 5</td><td>m stridium d stridium d 2413.295 2413.295 2413.294 2413.294 1194.628</td><td>ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206</td><td>c26] q42] T (mins) C 54.9 49.6 49.6 52.1 30.5 27.6 47.3</td><td>harge 4 3 2 2 2</td><td>-</td><td> Abundance 1.26±06 3.03±05 2.75±05 1.62±05 3.01±07 </td><td>Conflict: 0 0 0 0 0</td><td></td></li<>	ions of Score 63.7 61.3 123 122 51.7 89.8 50.3	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 2 5	m stridium d stridium d 2413.295 2413.295 2413.294 2413.294 1194.628	ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206	c26] q42] T (mins) C 54.9 49.6 49.6 52.1 30.5 27.6 47.3	harge 4 3 2 2 2	-	 Abundance 1.26±06 3.03±05 2.75±05 1.62±05 3.01±07 	Conflict: 0 0 0 0 0	
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	Per pre	 curs <li< td=""><td>ions of Score 63.7 61.3 123 122 51.7 89.8 50.3 64.9</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 4 4 4</td><td>III stridium d o1963 Mass 2413.295 2413.294 2413.294 2413.294 2413.294 1194.628 1246.568 1246.568</td><td>ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338</td><td>c26j q42j TT (mins) C 54.9 49.6 49.6 52.1 30.5 52.1 30.5 52.1 30.5 52.1 30.5 53.9</td><td>harge 4 3 2 2 2 2 2 2 2 2 2 2</td><td>-</td><td> ✔ Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 </td><td>Conflict:</td><td>3 E 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3</td></li<>	ions of Score 63.7 61.3 123 122 51.7 89.8 50.3 64.9	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 4 4 4	III stridium d o1963 Mass 2413.295 2413.294 2413.294 2413.294 2413.294 1194.628 1246.568 1246.568	ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338	c26j q42j TT (mins) C 54 .9 49.6 49.6 52.1 30.5 52.1 30.5 52.1 30.5 52.1 30.5 53.9	harge 4 3 2 2 2 2 2 2 2 2 2 2	-	 ✔ Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 	Conflict:	3 E 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession ≩ gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	Peq	 curs <li< td=""><td>ions of Score 63.7 61.3 123 122 51.7 89.8 50.3</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 5 4</td><td>m sstridium d sstridium d 01963 Mass 2413.295 2413.295 2413.294 2413.294 2413.294 1194.628 1246.568 1246.568</td><td>ifficile QCD-66 ifficile QCD-63 Mass error (p 1 -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393</td><td>c26] q42] T (mins) C 54.9 49.6 49.6 52.1 30.5 27.6 47.3</td><td>harge 4 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td><td>-</td><td> Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 </td><td>Conflict:</td><td></td></li<>	ions of Score 63.7 61.3 123 122 51.7 89.8 50.3	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 5 4	m sstridium d sstridium d 01963 Mass 2413.295 2413.295 2413.294 2413.294 2413.294 1194.628 1246.568 1246.568	ifficile QCD-66 ifficile QCD-63 Mass error (p 1 -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393	c26] q42] T (mins) C 54.9 49.6 49.6 52.1 30.5 27.6 47.3	harge 4 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2	-	 Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 	Conflict:	
gi 254976383 (+2) Protein: gi 25497 Protein: gi 25497 Protein: gi 2551(ptide ion views Prot Conflicting prote Accession ≩ gi 254976387 (+5)	76387 cel D1963 cel tein resolution ins for pep Peptides • 63 (63)	I surfat I surfat otide id Conflic	ce protein (ce protein (on 523 ct: Protein Sco 7.08E+03	S-lay	er pro	 curs <li< td=""><td>ions of Score 63.7 61.3 123 122 51.7 89.8 50.3 64.9</td><td>ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 4 4 4</td><td>III stridium d o1963 Mass 2413.295 2413.294 2413.294 2413.294 2413.294 1194.628 1246.568 1246.568</td><td>ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338</td><td>c26j q42j TT (mins) C 54.9 49.6 49.6 52.1 30.5 52.1 30.5 52.1 30.5 52.1 30.5 53.9</td><td>harge 4 3 2 2 2 2 2 2 2 2 2 2</td><td>-</td><td> ✔ Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 </td><td>Conflict:</td><td>3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3</td></li<>	ions of Score 63.7 61.3 123 122 51.7 89.8 50.3 64.9	ein) [Clo ein) [Clo gi 2551 Hits 3 5 5 2 5 5 4 4 4	III stridium d o1963 Mass 2413.295 2413.294 2413.294 2413.294 2413.294 1194.628 1246.568 1246.568	ifficile QCD-66 ifficile QCD-63 Mass error (p -0.237 -0.0186 -0.634 -0.607 -0.206 0.254 0.393 0.338	c26j q42j TT (mins) C 54 .9 49.6 49.6 52.1 30.5 52.1 30.5 52.1 30.5 52.1 30.5 53.9	harge 4 3 2 2 2 2 2 2 2 2 2 2	-	 ✔ Abundance 1.26E+06 3.03E+05 2.75E+05 1.62E+05 3.01E+07 2.76E+07 3.38E+04 1.48E+04 	Conflict:	3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3

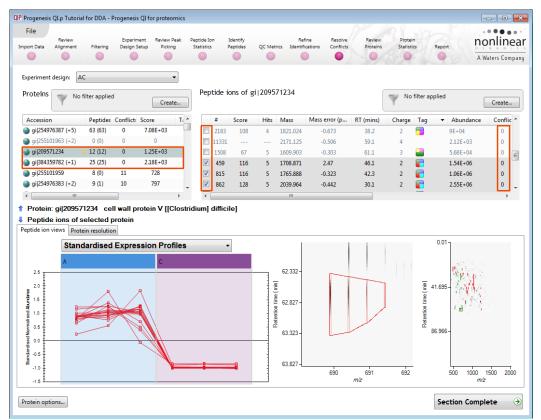
Note: as you un-assign the peptides the number of conflicts update 'on the fly' in all the windows.

In this case the conflicting peptides are unassigned from the 'precursor' protein.

In some cases you can resolve the conflicts between 2 proteins on the basis of consistent peptide expression. In the example below the proteins share 18 conflicts. For the protein showing 2 clear patterns of expression you can un-tick all the peptides with conflicts in the corresponding peptides table

Progenesis QI.p Tut	orial for DDA -	Progenesis Q	I for proteomics									E	
File Review port Data Alignmen		Experiment Design Setu		Peptide Ion Statistics	Identify Peptides	QC Metric	Ret cs Identifi		Review Proteins	Protei Statisti		port p v	Vaters Compar
Experiment design:	AC		•	Peptid	e ions of s	zi 20957	1234		ſ		<i>c</i>		
Y	No filter applie	u	Create	J .							filter appli	ed	Create
Accession	Peptides	Conflict: So	ore Ti 1	#	Score	Hits	Mass	Mass error (p	RT (mins)	Charge	Tag	 Abundance 	Conflic ^
🌍 gi 254976387 (+5	5) 63 (63)	0	7.08E+03	V 10	02 46.2	2	955.57	-0.378	24.1	2		1.87E+05	1 🗉
🔇 gi 255101963 (+2	2) 0 (0)	0	0	20	98.9	5	1502.794	-0.167	28.9	2	—	8.16E+05	1
🌛 gi 209571234	 26 (12) 	18	2.55E+03	28	30		1502.793	-0.752	28.9	3		1.38E+04	1
🌒 gi 384359782 (+1	l) 25 (11)	18	2.18E+03	22	25 41.7	5	1732.899	-0.452	29.1	3	—	6.65E+05	1
🌒 gi 255101959	8 (0)	11	728	V 46	i1 77.3	5	1732.899	-0.502	29.1	2		5.09E+05	1
🌍 gi 254976383 (+2	2) 9 (1)	10	797	28	8 52.3	5	1051.555	-0.192	23.4	2	—	4.81E+05	1 .
eptide ion views		_	ion Profiles		•		1				0	.01	
A 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4			C			Retention time (min)	62.332 - 62.827 - 63.323 -				Betention time (min) Besention time (and beta beta beta beta beta beta beta beta		
	AL	X			<u>``</u>								
-2							63.827 -	690	691 <i>m/z</i>	692		500 1000 <i>m/</i> 2	1500 2000

This leaves the peptides with the same expression pattern assigned with the appropriate protein thus resolving the conflicts.



Note: 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.

To resolve the remaining conflicts in the example shown below first order the conflicts in panel B and select the top one (which may still be selected) Panel B will display the peptides for this protein and the number of conflicts for each peptide. Panel E will also update to show the conflicting protein.

Accession	Peptides	Conflict:	Score	Ti ^		#	Score	Hits	Mass	Mass error (p	RT (mins)	Charg	e Tag	 Abundance 	e Cor	flic 🔨
婱 gi 384359782 (+1)	25 (25)	0	2.18E+03			3588	67.4	3	1791.962	0.0899	51.7	2		1.08E+05	1	
🔮 gi 255101959	0 (0)	0	0		V	2907	63	5	1198.656	0.594	28.3	2		8.54E+04	1	E
婱 gi 254976383 (+2) 🕔	9 (7)	2	797		V	954	110	4	1889.897	0.45	24.7	2		2.43E+05		
🌑 gi 126699128	7 (4)	5	860		V	1166	68.7	5	1268.666	0.259	45.	B 2		1.12E+05	Ζ (
🌑 gi 126699078	5 (2)	5	653		V	677	71.3	5	1319.646	-0.616	29.9	2		1.54E+05	(
🎱 gi 10180205 (+1)	3 (1)	2	266	-	V	1808	71.5	5	1751.977	-0.506	44	3		8.09E+04	0	
< III				•	•											•
Protein: gi 25497 Protein: gi 10180	205 Cwp	66 [[Clo				diffici	ile QCD-	66c26]								
Protein: gi 25497	205 Cwp in resolutio	66 [[Clo	ostridium] o		e]		ile QCD-									
Protein: gi 25497 Protein: gi 10180 eptide ion views Prote	205 Cwp in resolutions for per	66 [[Clo	ostridium] o	difficile	e]	de ior	ns of gi	101802	205		RT (mins)	Charge	Tag	✓ Abundance	Conflic	t: Pe
Protein: gi 25497 Protein: gi 10180 eptide ion views Prote Conflicting protei	205 Cwp in resolutions for per EPeptide	66 [[Clo	n 3588	difficile	e] Pepti	de ior	ns of gi	101802 Hits M	205		RT (mns) F 28 3	Charge 2	Tag	✓ Abundance 8.54E+04	Conflic 1	t: Pe
Protein: gi 25497 Protein: gi 10180 eptide ion views Prote Conflicting protei	205 Cwp in resolutions for per EPeptide	66 [[Clo	n 3588	difficile	₽] Pepti # 2	de ior	ns of gi core	101802 Hits M 5 11	205 1ass N					1	Conflic 1 1	_
Protein: gi 25497/ Protein: gi 10180; eptide ion views Prote Conflicting protei Accession @ gi 254976383 (+)	205 Cwp tin resolutions for per EPeptides	66 [[Clo n otide ion Conflic 2	n 3588 Protein Sco 797	difficile	Pepti # 22	de ior s 907	ns of gi core 63	101802 Hits M 5 11 3 17	205 1ass M 198.656	0.594	F ₂₈₃	2		8.54E+04	Conflic 1 1 0	١
Protein: gi[25497/ Protein: gi[10180; eptide ion views Protei Conflicting protei Accession 9 gi[254976383 (+) 9 gi[255101959	205 Cwp ein resolution s for per Pentidee 0 (0)	66 [[Clo n otide ion conflic 2 0	n 3588 Protein Sco 797	difficile	 Pepti # ✓ 2 ✓ 3 ✓ 6. 	de ior s 907 588	ns of gi core 63 67.4	101802 Hits M 5 11 3 17 4 2	205 1ass N 198.656 791.962	0.594	F ₂₈ ₃ 51.7	2 2		8.54E+04 1.08E+05	1	۲

Favouring the protein with the higher score, resolve the conflict by switching off (or un-assigning) the peptide in panel F for the protein with the lower score. By doing this, the other 3 panels update to show the change in conflicts.

Accession	Peptides	Conflict:	Score	Ti ^		#	Score	Hits Mas	s Mass error (p	RT (mins) Char	ge Tag	 Abundance 	e Coi	nflic
婱 gi 384359782 (+1)	25 (25)	0	2.18E+03		1	3588	67.4	3 179	.962 0.0899	51.7	2		1.08E+05		2
🗿 gi 255101959	0 (0)	0	0			2907	63	5 119	.656 0.594	28.3	2		8.54E-04) =
🌛 gi 254976383 (+2) 🔇	9 (9)	0	797		1	954	110	4 188	.897 0.45	24.7	2		2.43E+05		
gi 126699128	7 (4)	5	860		1	1166	68.7	5 126	.666 0.259	45.1	2		1.12E+05		
🗿 gi 126699078	5 (2)	5	653		1	677	71.3	5 131	.646 -0.616	29.9	2		1.54E+05		
🗿 gi 10180205 (+1)	1 (1)	0	136	-	1	1808	71.5	5 175	.977 -0.506	44	3		8.09E+04		, ,
•				P.					II						•
Protein: gi 25497 Protein: gi 10180 eptide ion views Prote	205 Cwp	66 [[Clo	• •	•		diffici	le QCD-6	6c26]							
Protein: gi 10180	205 Cwp in resolutio	966 [[Clo	stridium] o	•	e]		le QCD-6	-							
Protein: gi 10180	205 Cwp in resolutions for pe	o 66 [[Clo n ptide ior	stridium] o	difficil	e]	ide ior	ns of gi∣1	-	Mass error (p	RT (mins)	Charge	Tag	✓ Abundance	Confli	t: Pe
Protein: gi 101802 eptide ion views Prote Conflicting protein	205 Cwp in resolutions for pep Peptide	o 66 [[Clo n ptide ior	stridium] o	difficil	e] Pept	ide ior	is of gi 1 core H	0180205		RT (mins) 28.3	-	Tag	 Abundance 8.54E+04 	Confli	tt: Pe
Protein: gi 101802 eptide ion views Protein Conflicting protein Accession	205 Cwp in resolutions for pep Peptide	o 66 [[Clo n ptide ior s Conflict	stridium] o 2907 Protein Sco	difficil	e] Pept	ide ior ≠ s	ns of gi 1 core H 63)180205 ts Mass	6 0.594		-	-			tt: Pe
Protein: gi 10180; eptide ion views Prote Conflicting protein Accession gi 254976383 (+2)	205 Cwp in resolutions for pep Peptide 9 9 (9)	ptide ior conflict	2907 Protein Sco 797	difficil	Pept	ide ior ≠ S	Is of gi 11 core H 63 67.4	0180205 ts Mass 5 1198.65	6 0.594 2 0.0899	28.3	2	-	8.54E+04	0	t: Pe
Protein: gi 10180; eptide ion views Prote Conflicting protein Accession gi 254976383 (+2)	205 Cwp in resolutions for pep Peptide 9 9 (9)	ptide ior Conflict	2907 Protein Sco 797	difficil	Pept	ide io r ≠ S 1907	ns of gi 1 core H 63 67.4	0180205 ts Mass 5 1198.65 3 1791.96 4 2319.10	6 0.594 2 0.0899 6 0.255	28.3 51.7	2	-	8.54E+04 1.08E+05	0	tt: Pe

Note: 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.

QP Protein quantitation options
Quantitation method:
Relative Quantitation using Hi-N
Number of peptides to measure per protein (N):
Employ protein grouping, i.e. hide proteins whose peptides are a subset of another protein s.
OK Cancel

Note: the default Protein options for protein quantitation and protein grouping are set as shown

Protein Grouping

Where proteins are identified containing the same peptides then they are effectively indistinguishable aside from the score. Also where one protein contains only peptides that represent a subset of another protein's peptides, the protein with fewer peptides can be subsumed into that with the greater number.

With protein grouping switched on (default setting) protein groups and the additional members are indicated by a bracketed number located after the Accession number. Taking **flagellin subunit** as an example, when the cursor is *held over the accession number the group members (9 in total) appear in a tool tip.*

Experiment design: AC Proteins No filter applied Create	Рер	tide io	ons of gi	2549	73900			V No	o filter app	plied	Create.	
Accession Peptides Conflict: Score Ti ^		#	Score	Hits	Mass	Mass error (p	RT (mins)	Charge	Tag	 Abundance 	Confli	c ^
🎯 gi 254973900 (+9) 💿 15 (15) 0 1.95E+03 📃		76	63.9	5	1669.888	-0.0985	42.4	3		4.82E+06	0	=
gi 2095712: gi 254973900 - flagellin subunit [Clostridium difficile i22005200 - Flig field to field	QCD-	66c26]	103	5	1669.887	-0.663	42.4	2		4.32E+06	0	
gil22086309 - Flic [[Clostridium] difficile] gil22086299 - Flic [[Clostridium] difficile]			101	5	1230.609	-0.407	22.9	2		3.11E+06	0	
🕥 gil12670079 gil126697810 - flagellin C [Clostridium difficile 630]			125	5	2317.115	0.167	38.9	2		5.6E+06	0	
gil12669796 gil10281485 - flagellin subunit FliC [[Clostridium] dif gil5668937 - flagellin [[Clostridium] difficile]	ticile		60.9	5	2317.115	0.157	38.9	3		3.12E+06	0	
ail12669797 ail261863741 - flagellin [[Clostridium] difficile]			107	5	1716.857	-0.445	30.5	2		1.75E+06	0	
gil25497638 gil260682017 - flagellin subunit [Clostridium difficile gil0281487 - flagellin subunit Flic [[Clostridium] dif	CD19 ficile1	5]	43.8	5	1676.838	-1.19	34.8	3		7.66E+05	0	-
gil73745732 - flagellin subunit FliC [[Clostridium] dit					111				_		Þ	
Protein: gil254973900 flagellin subunit [Clostridium	n diffi	cile Q	- CD-66c2	61								
No protein selected Peptide ion views Protein resolution Conflicting protein	Donti	do ion	s of conf	licting	protoin							
5,	Peptie	ue ion										_
Accession Peptides Conflict: Protein Score	#	So	core H	its N	lass N	lass error (p	RT (mins)	Charge T	Tag 🔻	Abundance	Conflict:	Pe
< Þ	•											Þ
Protein options									(Section Comp	lete	•

Having performed the conflict resolution with **Employ Protein Grouping** now switch off the protein grouping to examine the effect of the process.

As grouping is switched off the grouped proteins appear with conflicts to the other group members and the number of unique peptides that are used for quantitation appear in brackets after the peptide number.

Note: flagellin has **no unique** peptides (brackets after the peptides field) as they are all present in flagellin subunit protein hence the reason for grouping. As a result all the conflicts are internal to the group.

Proteins				Y	No filter	applied		Creat		epti	ide ions o	f gi 2549	73900	Y	No filter applie	d		Create.
Accession P	Peptides C	Conflict:	Score	Tag	- Abu	indance	Mass		^	4	# Scor	e Hits	Mass	Mass error (p	RT (mins)	Charge	Tag	
🌛 gi 254973900 🜼 🗄	15 (0)	127	1.95E+03	0			30901			V 1	1845 9	4	1407.656	0.593	38.7	2		1
🍯 gi 260682017 🛛 🗄	15 (0)	127	1.95E+03				34354			V	888 10	9 5	1692.833	-1.02	20.7	2		4
🄰 gi 5668937 🛛 🔅	14 (0)	123	1.9E+03				30973			V	3358 84	7 4	1423.649	-0.431	22.7	2		7
) gi 261863741	14 (0)	121	1.76E+03				30916			V	477 47	4 5	1692.835	-0.238	20.7	3		5
gi 209571234 1	12 (12)	0	1.25E+03		3.15	+06	105305		-	V	300 43	85	1676.838	-1.19	34.8	3		7
gi 126698450	11 (4)	10	1.16E+03		8.64	+05	36159		*	•		III						•
Protein: gi 2606	82017 f	lagellin ition				fficile CD	196]											
Protein: gi 2606 ptide ion views Pro Conflicting prote	82017 f otein resolu eins for p	lagellin ition peptide	n subunit [C ion 1845	lostrio	dium di	fficile CD	196] ions of g	i 2600										
Protein: gi 2606 ptide ion views Pro Conflicting prote	82017 f otein resolu eins for p	lagellin ition peptide	n subunit (C	lostrio	dium di Pi ^	Peptide	196]			Ma	ass error (p.	RT (min	s) Charg		Abundance	Conflict	e Pep	otide Sequ
Protein: gi 2606i ptide ion views Pro Conflicting prote Accession	82017 f otein resolu eins for p	flagellin Ition Deptide Conflict:	n subunit [C ion 1845	lostric e	dium di P, ^ 99	Peptide # 1845	196] ions of g Score	i 2600		Ma	ass error (p. 0.593	RT (min 38.7	s) Chargi 2		Abundance 1.28E+05	Conflict 7		· · ·
Protein: gi 2606 ptide ion views Pro Conflicting prote Accession 9 gi 260682017 9 gi 254973900 •	82017 f otein resolu eins for p Peptides	flagellin ttion coeptide Conflict: 127 127	ion 1845 Protein Score 1.95E+03 1.95E+03	e Iostric	Pi ^ 99 99	Peptide # V 1845 888	196] ions of g Score	i 2600 Hits	Mass	Ma		•	· ·		1.28E+05 4.67E+05			otide Sequ DTDVA IRDTD
Protein: gi 2606 ptide ion views Pro Conflicting prote Accession 9 gi 260682017 9 gi 254973900 •	82017 f otein resolu eins for p Peptides 15 (0)	flagellin ttion coeptide Conflict: 127 127	ion 1845 Protein Score 1.95E+03	e V	dium di ₽ ▲ 99 99	Ficile CD Peptide 7 1845 888 3358	196] ions of g Score 99	i 2600 Hits 4	Mass 1407.656	Ma	0.593	38.7	2		1.28E+05	7	() ()	DTDVA
Protein: gi[2606: ptide ion views Pro Conflicting prote Accession gi[260682017 gi[254973900 gi[254973900 Gilder (1998) gi[261863741 gil261863741	82017 f otein resolute eins for p Peptides 15 (0) 15 (0) 14 (0) 14 (0)	flagellin tion coeptide Conflict: 127 123 121	ion 1845 Protein Score 1.95E+03 1.95E+03 1.9E+03 1.76E+03	elostric V V	dium di 99 99 99 99 ≡	Ficile CD Peptide # 1845 888 3358 \$ 477	196] ions of g Score 99 109 84.7 47.4	i 2600 Hits 4 5	Mass 1407.656 1692.833 1423.649 1692.835	Ma	0.593 -1.02 -0.431 -0.238	38.7 20.7 22.7 20.7	2 2 2 3		1.28E+05 4.67E+05 7.25E+04 5.61E+05	7	3	DTDVA IRDTD
Protein: gil2606; ptide ion views Pro Conflicting protect Accession agil260682017 gil264973900 agil25668937 gil254973900 agil261863741 gil261863741 agil216697810 Gil26697810	82017 f stein resolution Peptides 15 (0) 15 (0) 14 (0) 14 (0) 10 (0)	conflict: 127 127 123 121 91	ion 1845 Protein Score 1.95E+03 1.95E+03 1.9E+03 1.76E+03 1.19E+03	e v v	dium di 99 99 99 99 99 99 99	Ficile CD Peptide # 1845 888 3358 477 300	196] ions of g Score 99 109 84.7 47.4 43.8	i 2600 Hits 4 5 4	Mass 1407.656 1692.833 1423.649 1692.835 1676.838	Ma	0.593 -1.02 -0.431 -0.238 -1.19	38.7 20.7 22.7 20.7 34.8	2 2 2 3 3		1.28E+05 4.67E+05 7.25E+04 5.61E+05 7.66E+05	7 7 7	• • • • •	DTDVA IRDTD DTDVA
Protein: gil2606; ptide ion views Pro Conflicting prote Accession gil260682017 gil260682017 gil254973900 Gil2669837 gil2668937 gil261863741 gil261863741 gil266997810	82017 f otein resolute eins for p Peptides 15 (0) 15 (0) 14 (0) 14 (0)	conflict: 127 127 123 121 91	ion 1845 Protein Score 1.95E+03 1.95E+03 1.9E+03 1.76E+03		99 99 99 99 99 99 99 99 99 99	# # ♥ 1845 ♥ 1845 ♥ 888 ♥ 3358 ♥ 477 ♥ 300 ♥ 449	196] ions of g Score 99 109 84.7 47.4	i 2600 Hits 4 5 4 5	Mass 1407.656 1692.833 1423.649 1692.835	Ma	0.593 -1.02 -0.431 -0.238	38.7 20.7 22.7 20.7	2 2 2 3		1.28E+05 4.67E+05 7.25E+04 5.61E+05	7 7 7	3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	DTDVA IRDTD DTDVA IRDTD
Conflicting prote Accession	82017 f stein resolution Peptides 15 (0) 15 (0) 14 (0) 14 (0) 10 (0)	conflict: 127 127 123 121 91 94	ion 1845 Protein Score 1.95E+03 1.95E+03 1.9E+03 1.76E+03 1.19E+03		dium di 99 99 99 99 99 99 99	Ficile CD Peptide # 1845 888 3358 477 300	196] ions of g Score 99 109 84.7 47.4 43.8	i 2600 Hits 4 5 4 5 5 5 5	Mass 1407.656 1692.833 1423.649 1692.835 1676.838	Ma	0.593 -1.02 -0.431 -0.238 -1.19	38.7 20.7 22.7 20.7 34.8	2 2 2 3 3		1.28E+05 4.67E+05 7.25E+04 5.61E+05 7.66E+05	7 7 7 7 7		DTDVA IRDTD DTDVA IRDTD IRDTD

Now set the Protein Options back to Employ protein grouping

Protein Quantitation options

There are 4 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 1) then this option will be applied.

V	P Protein quantitation options
(Quantitation method:
	Relative Quantitation using Hi-N
	Absolute Quantitation using Hi-N Requires a calibrant protein to calculate absolute amounts Uses the most abundant N peptides Allows comparison between proteins within a run
	Relative Quantitation using Hi-N Uses the most abundant N peptides Allows comparison between proteins within a run
	Relative Quantitation using non-conflicting peptides • Uses only peptides which have no conflicting protein identifications • Allows comparison of a single protein across runs
	Relative Quantitation using all peptides • Uses all peptides identified as part of a protein • Allows comparison of a single protein across runs

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.

QIP Protein quantitation options	×
Quantitation method:	
Relative Quantitation using Hi-N	•
Number of peptides to measure per protein (N):	•
Employ protein grouping, i.e. hide proteins whose peptides are a subset of another protein's.	
OK Cance	el 🚽

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.

QP Protein quantitation options	QP Protein quantitation options	×
Quantitation method: Absolute Quantitation using Hi-N	Quantitation method:	
Number of peptides to measure per protein (N): 3	Absolute Quantitation using Hi-N	•
Calibrant accession:	Number of peptides to measure per protein (N):	3 🖍
Please enter the accession for the calibrant protein	Calibrant accession:	gi 126700794
Amount (fmol):	Amount (fmol):	50
 Please enter the amount for the calibrant protein Employ protein grouping, i.e. hide proteins whose peptides are a subset of another protein's. 	<u>Employ protein grouping</u> , i.e. hide proteins whose subset of another protein's.	peptides are a
OK Cancel	01	K Cancel

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.

Review Proteins Using this screen, you can find the proteins of interest in your experiment.	W No filter a	pplied Crea		@ H	lelp ▼		
1 Set the quantitation options	Max fold change	Highest Mean	Lowest Mean	Description	Amount (fmol) - A	Amount (fmol) - C	2
If you've not already done so, choose between relative and absolute	4.64	C A		30S ribosomal protein S16 [Clostridium difficile 630]	101	467	*
quantitation, use of Hi-N, protein	2.53			pyruvate kinase [Clostridium difficile 630]		51.8	
grouping and more.	1.7	С	A	electron transfer flavoprotein subunit alpha [Clostridium difficile 630]	197	335	
Protein options	1.88	Α	С	30S ribosomal protein S6 [Clostridium difficile 630]	1.91E+03	1.02E+03	

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.

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

QIP Protein quantitation options	3							
Quantitation method:								
Relative Quantitation using non-conflicting peptides	•							
Employ protein grouping, i.e. hide proteins whose peptides are a subset of another protein's.								
OK Cancel								

The relative Quantitation can also be performed using all peptides.

QIP Protein quantitation options	×
Quantitation method:	
Relative Quantitation using all p	eptides 🔹
Employ protein grouping, i.e. hi subset of another protein's.	de proteins whose peptides are a
	OK Cancel

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.

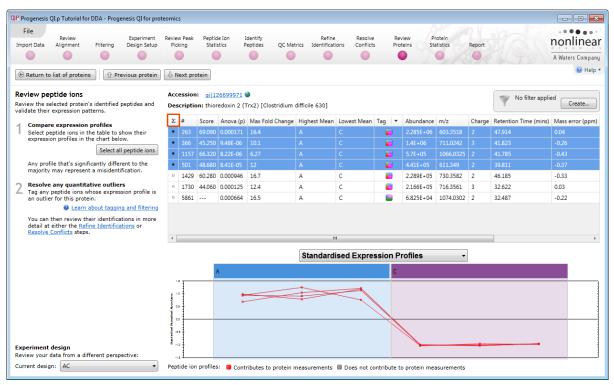
Stage 13: Review Proteins

The **Review Proteins** stage opens displaying details for all the proteins. You can now create tags at the protein level for proteins with an Anova p value ≤ 0.05 and Max Fold change ≥ 2 by right clicking on the table and selecting **Quick Tags...**

IP Progenesis QI.p Tutorial for DDA - Progenesis QI for p	proteomics										
File Review Experimen Import Data Alignment Filtering Design Setu		tide Ion Ident atistics Pepti		Refin Identifica		lesolve Revie onflicts Protei		s Report	nonlinea		
		• •		•		• •			A Waters Company		
Review Proteins Using this screen, you can find the proteins of interest in your experiment.	No filter appl	Create	Search		Q				@ Help		
 Set the quantitation options 	Accession	Peptide count	Confidence score	Anova (p)	Tag 🔹	Max fold change	Highest Mean	Lowest Mean	Description		
If you've not already done so, choose between relative and absolute quantitation,	gi 126699939	3 (3)	236	3.52E-06	4	8.66	A	с	transketolase, central and C-terminal (Sedo		
use of Hi-N, protein grouping and more.	🎯 gi 126701179	3 (3)	235	3.86E-06	4	5.64	A	С	transcription elongation factor GreA [Clostr =		
Protein options	gi[126701103	1 (1)	51.7	7.19E-06	4	6	A	с	ribose-5-phosphate isomerase 2 [Clostridiu		
	🔇 gi 126699971	5 (5)	334	9.36E-06	4	11.6	А	с	thioredoxin 2 (Trx2) [Clostridium difficile 63		
2 Create a shortlist to review In the table, sort and filter the proteins	🎯 gi 54781345 (+:	Anova p-val	ue ≤ 0.05	1.02E-05		11	A	с	(R)-2-hydroxyisocaproate dehydrogenase [(
based on their measurements, to generate a	🎯 gi 126700634	Max fold cha	ange≥2	1.63E-05	4	6.42	Α	С	PTS system mannose-specfic transporter su		
shortlist for further review.	🔇 gi 126697690	New tag		1.64E-05 🌒 5		5.88	Α	С	ferredoxin/flavodoxin oxidoreductase subur		
	gi[126699140	•	Anova p-value			Α	С	ferredoxin-NADP(+) reductase subunit alph			
To sort the table by a given value, simply click the relevant column header.	🔇 gi 126699940	P Edit tags		Max fold change			A C		transketolase, N-terminal (Sedoheptulose-7		
Review the proteins	🔮 gi 126697752	Add to Clip	Gallery	Sequen	ce		A C		NAD-specific glutamate dehydrogenase [Cl		
5 For each protein of interest, inspect the ion	gi 126698450 (+1) 11 (11)	1.16E+03	Modification Peptide tags contain			с	A	ABC transporter sugar-family extracellular s		
measurements for its peptides:	gi[126697684	3 (3)	158			ain	Α	с	phosphate butyryltransferase [Clostridium c		
View peptide measurements	🕥 gi 260682017 (+9) 15 (15)	1.95E+03	4.2E-05	•	3.72	Α	с	flagellin subunit [Clostridium difficile CD196 -		
You can also double-click to review a protein.	٠						1		Þ		
Export data for further processing By exporting your data to external tools, there's no limit to your analysis. Export to pathways tool Export protein measurements Export peptide measurements	Selected prote View peptide measure "4 15:0 1 14:0 1 14:0 1 13:0 1 12:5 1 12:5 1		loxin 2 (Trx) A M	2) [Clost	ridium	i difficile 63	0]		c 速		
Experiment design Review your data from a different perspective: Current design: AC •	Quantifiable protei	ns displayed: 1	59						Section Complete		

As an example let us explore Thioredoxin 2.

The table indicates that this protein is most highly expressed in Condition A by 11.6 fold over the lowest condition (C).

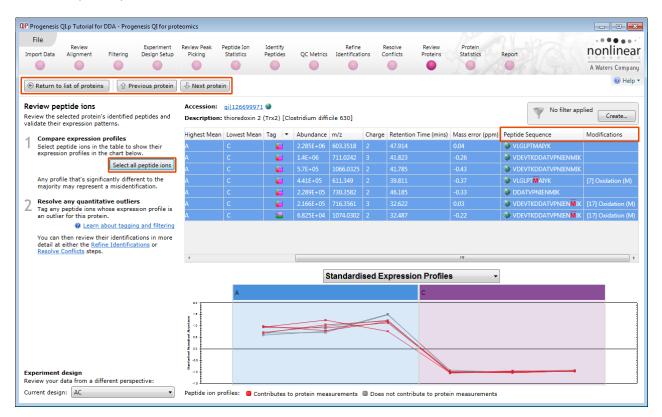


To view the corresponding peptide measurements for the current protein either double click on the protein in the table or use the **View peptide measurements** below the table.

The solid icon in Σ column indicates that the peptide contributes to protein measurements.

By selecting all the peptide ions you can compare the pattern of expression across all the samples allowing you to identify any 'atypical' behaviour assigned to the current protein.

Scroll to the right on the table for the sequence and modification details. Use the navigation buttons on the top left either to review each protein's peptides by stepping through the list or return to the protein list and select a specific protein to review.



With protein grouping switched on (default setting) protein groups and the additional members are indicated by a bracketed number located after the Accession number. The additional members can be seen by holding the cursor over the accession number.

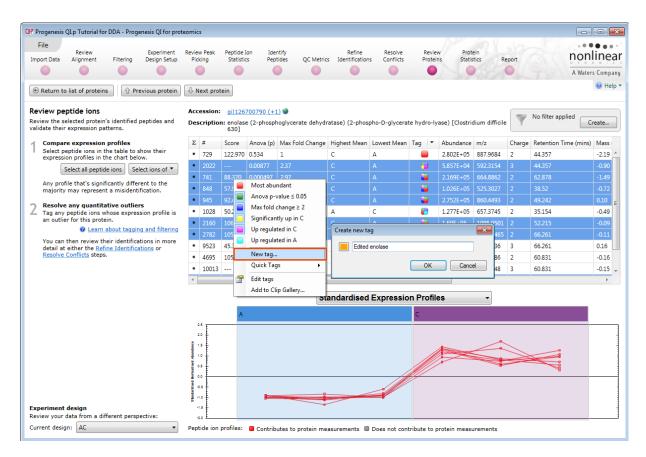
File Import Data	Review Alignment Filteri	Experiment Design Setup	Review Peak Peptid Picking Statis		QC Metrics I	Refine dentifications	Resolve Conflict		Protein Statistics	Report		A Waters Compar	
Review Prot Using this scre interest in you	en, you can find the	proteins of	No filter ap	plied Create	Search		2	•	1. 181		1.2	 Water's company Welp 	
1 Set the quantitation options If you've not already done so, choose between relative and absolute quantitation, use of Hi-N,		Accession	Peptide count	Confidence score	Anova (p)	Tag 🔹	Max fold change	Highest Mean	Lowest Mean	Description			
		🎯 gi 126698915	1 (1)	106	0.000187	4	9.04	С	Α	30S ribosomal p	rotein S15 [Clostridi *		
	ouping and more.	,	🎯 gi 126700129	3 (3)	267	0.000193	٩	2.39	Α	С	translation inhib	itor endoribonuclea	
	(Protein options	🎯 gi 126700198	1 (1)	46.6	0.000214	۷	9.55	Α	С	phosphatase, 20	family [Clostridium	
			🎯 gi 126697654	3 (3)	215	0.000221	٩	2.53	Α	С	30S ribosomal p	rotein S8 [Clostridiu	
	shortlist to review le, sort and filter the	proteins based on	🔇 gi 254973854 (-	⊧4) 6 (6)	474	0.000259	۵	2.96	С	A	60 kDa chaperor	nin [Clostridium diffi	
their mea	surements, to genera		🎯 gi 126701091 (-	-12-24072854	60 kDa shararari	0.0000cc		2 20	<u>^</u>	Α	F0F1 ATP syntha	se subunit beta [Clc	
further re	view. How are the measure	monte calculatod?	gi[254973854 - 60 kDa chaperonin [Clostridium difficile QCD-66c26] gi[8886080 - heat shock protein GroEL [[Clostridium] difficile] C							с	cell surface protein [Clostridium diff		
			🔇 gi 126698631 (-		60 kDa chaperonir					A	cell wall-binding	protein [Clostridiur	
	e table by a given va int column header.	lue, simply click	gi 126698842		60 kDa chaperonin heat shock protein				um difficile 630	c	C elongation factor P [Clostridium dif		
Deview t	he proteins		gi 126699299	2 (2)	311	0.000301		3.5	A	с	dinitrogenase in	on-molybdenum col	
For each p	protein of interest, in		gi 126700297	2 (2)	164	0.000328	4	2.1	Α	С	propanediol util	ization phosphotran	
measurem	nents for its peptides		gi 126697687	2 (2)	118	0.000351	4	3.36	Α	с	4Fe-4S ferredox	in, iron-sulfur bindin	
	View pepti	de measurements	1										
4 Export da By export	lso double-click to re ata for further proc ing your data to exte o your analysis.	essing	Selected pro		chaperonin A	[Clostri	idium d	ifficile QCD	-66c26]		C		
	Export prot	e pathways tool ein measurements ide measurements	13.2813 12.8563								₽ ₽ E		
E xperiment d Review your da Current design	lesign ata from a different p		Quantifiable prot	eins displayed: 1	159						Sect	tion Complete 🤆	

To view members peptide ions click on View peptide measurements.

Click on **Select ions of** to show the list of additional group members. Then as you click on one of the member proteins its peptide ions are highlighted in the table.

010 -																
	is QI.p Tutorial for	DDA - Pr	rogenesis QI for pro	teomics												
File	Review		Experiment	Review Peak	Peptide I	ion Ide	entify		Refine	Resolve	Revie	w Prot	ain			
Import Data		Filtering		Picking	Statistic		otides	QC Metric			Proteir			eport	n n	onlinear
											•			0	A	Waters Company
													10 A 10 A			🕢 Help 🔻
(E) Return	to list of proteins	Ŷ	Previous protein	I Next prot	tein											
Review p	Review peptide ions Accession: gi 254973854 (+4)													ſ	No. Character Paul	
Review the	selected protein's		ed peptides and					stridium diffi	cile QCD-66c26]					No filter applied	Create
validate the	eir expression patt	terns.		Σ #	C	A		Cold Channel	Highest Mean	1	Tag 💌	Abundance	(Channel	Retention Time (mins)	Mass error (ppn
	are expression p				Score 58.830	4.59E-05	3,4	Fold Change	C C		Tag 🔹	2.858E+05	m/z 766.9192	2	40.285	-0.47
	peptide ions in th sion profiles in th			015	66.390		1.99			A	-		619.8642		31.611	-0.47
	Select all peptide	e ions	Select ions of 🔻	• 799 • 996		0.00426			c	A		1.729E+05 1.383E+05	535.8258	2	35.105	-0.23
Any pr	ofile that's signific	cantly d	gi 2549738		na 400	000045	201		c	A		2.832E+05	696.7028	3	53.726	0.26
majori	ty may represent	a misid		eronin [Clostri	dium diffi	cile				A		2.832E+05 2.125E+05	1044.5501		53.762	-0.13
C Resol	ve any quantitat	tive ou								A	-	3.887E+04	775.4021		42.35	2.46
📕 📕 Tag ar	ny peptide ions wh	nose exp	gi 8886080	rotein GroEL [Clostridiu	uml			c	A		3939	717.079	3	71,787	-0.08
an out	lier for this protein Learn a		difficile]		-				C	A		3939	/1/.0/9	5	/1./0/	-0.06
You ca	in then review the		gi 25530519													
detail	at either the <u>Refin</u> re Conflicts steps.		ATCC 43255	eronin [Clostri 	dium diffi	cile										
Kesoly	e connicts steps.		gi 12669776	7												÷.
				eronin (Protein stridium diffici		(GroEL		Standardised Expression Profiles								
					0000]				Standardis	ea Express	sion Pro	mes	•			_
				rotein GroEL [[Clostridiu	um]					С					
			difficile]													
				1									/	~	_	1
				1.0									\neq			
				-								8				
											t -					
				-1.0												-
Experimer Review you	nt design Ir data from a diffi	erent ne	rspective:	-1.8												
Current des		er ente per	•	Peptide ion	profiles:	Contrib	utes t	nrotein me	asurements	Does not con	tribute to r	notein meas	rements			
	-			. opside for		Contri	ates t	protein me	asarements u	Does not con	chouse to p	notem medsi	a ementa			

You can tag all the peptide ions for a protein or edit and tag accordingly for 'atypical behaviour'. Then remove these peptides at the **Refine identification** stage in the Workflow.



Modified proteins can be located by specifically searching for proteins containing modified peptides. Click the **Return to list of proteins** button to return to the proteins list and right click on it and select **Modification** from the list of **Quick Tags**.

Accession	Peptide count Confidence score			Anova (p)	Tag 🛛 🕶	Max fold change	Highest Mean	Lowest Mean
🎯 gi 126701103	1 (1)	51.7	7.19E-06	4	6	Α	С
🎯 gi 126698450 (+1)	11 (11)		1.16E+03	8.08E-06	4	12.8	С	Α
🔇 gi 54781345 (+1)				1E-05	4	8.3		С
🎯 gi 126699971		Anova p-va Max fold cl		1.08E-05	4	11.8	Α	С
🎯 gi 126700634	-		nange 2 z	1.63E-05	4	6.42	Α	С
🔇 gi 126699140	_	New tag		2.22F-05		10	Α	С
🔇 gi 126699940	_	Quick Tage	5 🕨		p-value		Α	С
🚳 gi 126697752	<u></u>	Edit tags			old change.		Α	С
🎯 gi 126697684		Add to Clip	o Gallery	Seque			Α	С
🎯 gi 126698435	1 (1)	48.9		ication		С	Α
🎯 gi 126697583	1 (1)	79.3	Peptic	le tags cont	ain	Α	С
🎯 gi 126697690	6 (6)	578	5.49E-05	4	5.39	Α	С

To find those proteins containing peptides with Oxidation on Methionine residues enter **Oxidation (M)**. This will automatically provide a named tag when you click **Create tag**.

OP New Quick Tag		×
Where any peptide of a protein has Modification with: Oxidation M		
Can I use wildcards?		
Apply the following tag: Modification with Oxidation M		
	Create tag Ca	incel

To reduce the table to displaying only these proteins with modified peptides (Oxidation on methionine) use the tag filter to focus on these proteins.

QP Filter the proteins										
Create a filter Show or hide proteins based on a selection of their tags. Move tags to the appropriate boxes to create the filter. For more guidance, please see the <u>online reference</u> .										
Available tags:	Show proteins that have all of these tags:									
 Anova p-value ≤ 0.05 (116 proteins) Max fold change ≥ 2 (91 proteins) 	Modification with Oxidation M (13 proteins) Show proteins that have at least one of these tags: Hide proteins that have any of these tags:									
Clear the filter	OK Cancel									

The proteins table will now only display those proteins containing modified peptides.

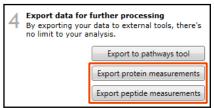
	utorial for DDA	. Trogenesis quito	protec									
File		Experiment	Review		Identify	Refine	Resolve	Revie			nonli	nea
mport Data Align	ment Filterir	ng Design Setup	Pick	ng Statistics F	Peptides QC N	Aetrics Identifications	s Conflicts	Prote	ins Statistics	Report	DYNA	M
		-			•				1812	1.8	A Waters	Compa
eview Proteir	ns			🚭 Tag filter appl	ied							🕖 Hel
sing this screen, iterest in your ex		he proteins of		proteins may b		lit Search		Q				
	ntitation optio			Accession	Peptide count	Confidence score	Anova (p)	Tag 💌	Max fold change	Highest Mean	Lowest Mean	Desc
		o, choose between tation, use of Hi-N		🕲 gi 126699971	5 (5)	334	1.08E-05	٨	11.8	A	с	thio
protein group	ing and more.			🎯 gi 126697690	6 (6)	578	5.49E-05	٩	5.39	Α	С	ferre
		Protein options		🎯 gi 254973900 (+9)	15 (15)	1.95E+03	0.000148	٨	3.81	Α	С	flag
				🎯 gi 384359782 (+1)	25 (25)	2.18E+03	0.000156	٩	3.2	С	Α	hen
	ortlist to revie sort and filter t	ew the proteins based	on	🎯 gi 126700129	3 (3)	267	0.000187	۷	2.39	Α	С	tran
	ments, to gen	erate a shortlist fo		🔇 gi 126697654	3 (3)	215	0.000221	۲	2.53	Α	C	30S
		urements calculat	ed?	🎯 gi 54781347	8 (8)	685	0.00125	•	1.94	Α	С	2-h
		value, simply click		🔇 gi 254976387 (+5)	63 (63)	7.08E+03	0.00162	4	1.63	С	Α	cell
	olumn header.			🎯 gi 126700078	6 (6)	582	0.00223	4	1.31	С	Α	mol
Review the	proteins			🎯 gi 126700372	2 (2)	126	0.00373	٩	5.31	Α	С	PTS
For each prot	ein of interest,	inspect the ion		gi 126697631	7 (7)	626	0.00879	۹	1.48	Α	С	50S
measurement	s for its peptid			🕥 gi 126697969 (+1)	10 (10)	982	0.02	4	1.65	Α	С	Beta
	View pe	ptide measurement	ts	🕥 gi 126700790 (+1)	10 (10)	917	0.0571	•	1.87	С	Α	eno
You can also	double-click to	review a protein.										
	ur analysis.	xternal tools, there	e's	Selected prote		" doxin 2 (Trx2) [Clost	ridium	difficile 630)]		
	Expor	t to pathways tool				А				С		
		rotein measuremer		Actin Mormalised Aburn 16.0 15.5 15.5 15.0 14.5 14.5 13.5 13.5		H						
operiment desi		•		44.0 14.0 13.5 13.5 1						率		
eview your data f												

Note: the Sequence Quick tag can be used to locate Proteins containing peptides with specific motifs.

Stage 14: Exporting Protein Data

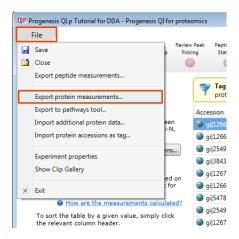
Protein data can be exported in a csv file format. You can either export the **Protein and/or peptide measurements** using the options in the File Menu or use the buttons under Step 4 both available at the **Review Proteins** stage.

As an example of Data export use the Tag filtered set from the previous section. Where you are only going to export measurements for those proteins that a have Oxidised Methionine residues.



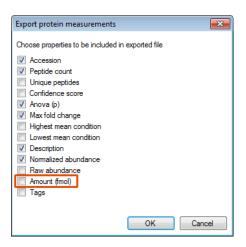
First set the tag filter as shown below. Then select Export Protein Measurements.

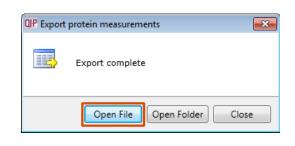
QIP Filter the proteins Create a filter	their tags. Move tags to the appropriate boxes to										
create the filter. For more guidance, please see the online reference. Available tags: Show proteins that have all of these tags:											
 ● Anova p-value ≤ 0.05 (116 proteins) ● Max fold change ≥ 2 (91 proteins) 	Modification with Oxidation M (13 proteins) Show proteins that have at least one of these tags: Hide proteins that have any of these tags:										
Clear the filter	OK Cancel										



The Export Protein Measurements dialog opens. Select the required fields and click OK. **Save** the file and then open the exported data file using the dialog that opens.

Note: if you have performed Absolute Quantification then the 'Amount' field will be available.





Excel will open displaying the exported protein measurements

A1	• E × v	f _x									
	Α	В	С	D	E	F	G	н	I	J	К
1						Normalize	d abundar	nce			
2						Α			С		
A	ccession	Peptide count	Anova (p)	Max fold change	Description	A1	A2	A3	C1	C2	C3
g	i 126699971	5	9.36E-06	11.58476058	thioredoxin 2 (Trx2) [Clostridium difficile 630]	1525647	1617593	1553317	113654.1	140964.9	150789.2
g	i 126697690	6	1.64E-05	5.879936323	ferredoxin/flavodoxin oxidoreductase subunit gamma	539615.9	622072.7	489155.7	90251.77	95359.21	95147.9
g	i 254973900;gi 10281485;	15	4.20E-05	3.724777972	flagellin subunit [Clostridium difficile QCD-66c26]	7082586	6203247	7681870	1856199	1801158	1971892
g	i 126700129	3	0.000192748	2.390273556	translation inhibitor endoribonuclease [Clostridium di	1868979	1833114	2077924	842205.4	722151.4	853783.7
g	i 126697654	3	0.000220716	2.533396933	30S ribosomal protein S8 [Clostridium difficile 630]	338209.3	383095	400934.2	153782.3	156568.4	132627.1
g	i 384359782;gi 209570719	25	0.002009624	1.884385068	hemagglutinin/adhesin [Clostridium difficile BI1]	585079.9	571830.8	706836.6	1226165	1039788	1246065
g	i 126700372	2	0.003731629	5.312860261	PTS system HPr protein [Clostridium difficile 630]	34569.83	45956.03	34245.29	4020.826	9869.877	7711.814
g	i 54781347	8	0.003987149	1.956788527	2-hydroxyisocaproate-CoA transferase [Clostridium dif	530107.9	427769.4	420032.8	232920.9	201308.1	269940.1
g	i 126700078	6	0.007662556	1.358344492	molecular chaperone DnaK [Clostridium difficile 630]	169694.5	155660.9	140698	222159.5	210880.7	200020.8
g	i 126697631	7	0.010228808	1.476686476	50S ribosomal protein L7/L12 [Clostridium difficile 630]	800237.5	686088.3	910574	559940.4	526226.6	536994
g	i 254976387;gi 11496150;	63	0.010811893	1.316557403	cell surface protein (S-layer precursor protein) [Clostri	79867266	84107432	83973903	1.1E+08	97449336	1.19E+08
5 g	i 126697969;gi 25565442;	10	0.023675339	1.842240485	Beta-subunit of electron transfer flavoprotein [Clostric	2288685	2176419	3361065	1480229	1158617	1609334
5 g	i 126700790;gi 29645204	10	0.148353122	1.752066307	enolase (2-phosphoglycerate dehydratase) (2-phospho	120504	70395.35	283290.2	282282	264544.4	283985.1
1											

Note: where there are multiple group members the other accession numbers are also exported.

At each stage in the Work flow there are a number of Export and Import options available from the **File** Menu. This includes the option to **Import Additional Protein Data** which can be used to increase the Protein meta data and also be used to sort the existing tabular data.

Exporting Protein Data to Pathways Tool(s)

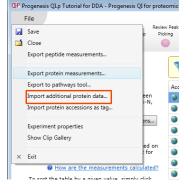
Using Progenesis, you can export protein lists to pathway analysis tools to help you understand your data in a wider biological context.

Currently Progenesis for proteomics supports the export to:

IMPaLA: which aggregates and queries many other pathway analysis tools including KEGG and Reactome)

PANTHER: classifies proteins for high-throughput analysis.

Note: Plugins for these tools are provided as standard.





First select the protein data to export to the pathways tool using tag filtering to 'focus' the set to export. Then click **Export to pathways tool**, and select the tool followed by the test to be performed

	v Peak Peptide Ion Ide king Statistics Pep		Refine ics Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	nonline	a
			•		•		9.8	A Waters Com	pany
Review Proteins Using this screen, you can find the proteins of interest in your experiment.	Tag filter appli proteins may be		Jit		<i>م</i>			🥑 Н	elp '
Set the quantitation options	Accession	Peptide count	Confidence score	Anova (p)	Tag 🔹	Max fold change	Highest Mean	Lowest Mean	D
If you've not already done so, choose between relative and absolute quantitation, use of Hi-N,	🔮 gi 126699971	5 (5)	334	9.36E-06	٩	11.6	Α	с	th
protein grouping and more.	gi 126697690	6 (6)	578	1.64E-05	٩	5.88	A	с	fei
Protein options	gi 254973900 (+9)	15 (15)	1.95E+03	4.2E-05	٩	3.72	Α	с	fla
	P Export Pathways Informa	ation			×	2.39	Α	С	tra
Create a shortlist to review			2.53	Α	с	30			
In the table, sort and <u>filter the proteins</u> based o their measurements, to generate a shortlist for	Please select a pathways		1.88	с	Α	he			
	IMPaLA: Integrated Moleo		5.31	A	с	PT			
How are the measurements calculated	IMPaLA: Integrated Molect Panther Classification Systems		1.96	Α	с	2-			
To sort the table by a given value, simply click the relevant column header.	Select the type of analysi		1.36	c	A	m			
	Pathway over-representat	ion analysis	1.48	Δ	c	50			
3 Review the proteins For each protein of interest, inspect the ion measurements for its peptides:	To perform the pathway a paste it into the genes/pr	analysis, copy t roteins section	1.32	С	A	ce			
	Open IMPaLA in my broken in my broken in my broken in the second seco	owser	1.84	Α	С	Be			
View peptide measurements						1.75	С	Α	en
You can also double-click to review a protein.		Сору	proteins to clipboa	rd Clo	se				×
4 Export data for further processing By exporting your data to external tools, there's no limit to your analysis.	Selected prote		rface proteir	n (S-lay	er prec	ursor protei	n) [Clostri	dium diff	i
Export to pathways tool			A				С		
Export protein measurements Export peptide measurements	AcSinh Normalised A 19:74 – 1 19:13 – 1 19:02 – 1 18:91 – 1 18:91 – 1		Ŧ						
Experiment design	VA 18.91		<u> P</u>						
									- b.
Review your data from a different perspective:	4								,

Select either Pathway over-representation analysis or Wilcoxon pathway enrichment analysis.

Make sure the **Open IMPaLA in my browser** is ticked and then click **Copy proteins to clipboard**

QIP Export Pathways Information											
Please select a pathways tool and type of analysis to perform.											
IMPaLA: Integrated Molecular Pathway Level Analysis											
About this plugin + Download other plugins											
Select the type of analysis to perform:											
Pathway over-representation analysis 🔹											
Pathway over-representation analysis Wilcoxon pathway enrichment analysis											
☑ Open IMPaLA in my browser											
Copy proteins to clipboard Close											

When IMPaLA opens paste in the exported values and perform the test.

pathway over-representation and enrichment an	alysis with expression and / or metabolite data
genes/proteins example input for over-representation analysis example input for enrichment analysis	metabolites - example input for over-representation analysis - example input for enrichment analysis
paste genes or proteins below	paste metabolites below
gi 124976887 gi 1394359782 gi 124973900 gi 12670790 gi 126697969 gi 126697690 gi 126697690 gi 126697631 gi 126707129 gi 12697631 gi 12670372] pr upload a file with genes or proteins Browse	or upload a file with metabolites Browse
optionally, provide genes/proteins background or over-representation analysis Browse	optionally, provide metabolites background for over-representation analysis Browse
Unigene V	specify metabolite identifier 🗸
choose analysis type:	
 pathway over-represent 	ation analysis

Note: currently the Clostridium difficile protein set being used in this user guide does not yield any pathway 'hits' with Impala or Panther.

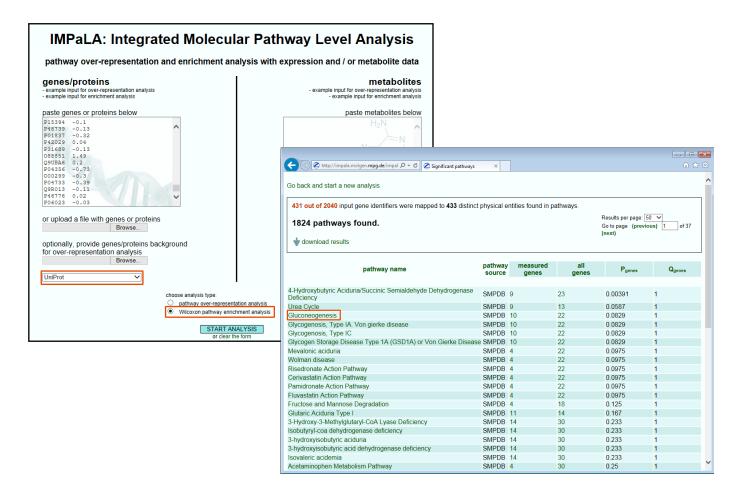
Although the previous page outlines the process of exporting data to pathway tools, a text file (**Impala_Wilcoxon_demo.txt**) has been included in the tutorial download to allow the demonstration of exporting data to Impala for pathways analysis.

In Progenesis QI for proteomics set up the **Export to pathways tool** dialog to export the data to Impala to perform a Wilcoxon pathway enrichment analysis. Click copy to clipboard to open Impala

~
QP Export Pathways Information
Please select a pathways tool and type of analysis to perform.
IMPaLA: Integrated Molecular Pathway Level Analysis 🔹
About this plugin + Download other plugins
Select the type of analysis to perform:
Wilcoxon pathway enrichment analysis 🔹
For Wilcoxon enrichment analysis, choose two experimental conditions that you would like to compare. Comparison:
Control vs. Treated V
To perform the pathway analysis, copy the protein data to the clipboard and paste it into the genes/proteins section of the IMPaLA search page.
Open IMPaLA in my browser
Copy proteins to clipboard Close

To **simulate** the **Copy proteins to clipboard** open the text file in 'Notepad', select all and copy. In the Impala window, open in your browser, right click on the genes/protein panel and paste the contents of the file Impala_Wilcoxon_demo.txt.

Select UniProt as the identifier, tick Wilcoxon pathway enrichment analysis then Start Analysis.



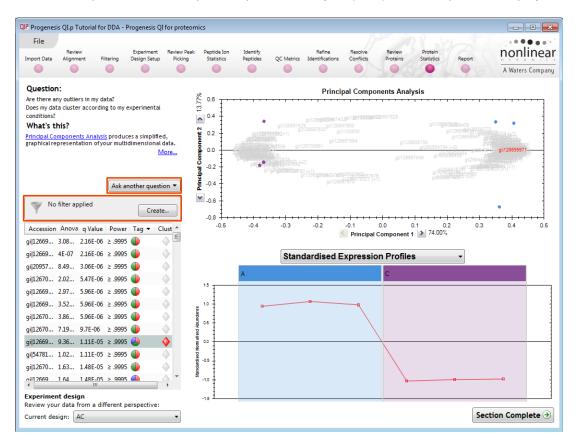
Note: if you are using your own data then pressing **Copy proteins to clipboard** will open **Impala** and allow you to paste directly into Impala, without saving to a separate file.

Note: in the case of exporting to **Panther** the normal process requires a file to be created and stored then opened in Panther to perform the pathway analysis.

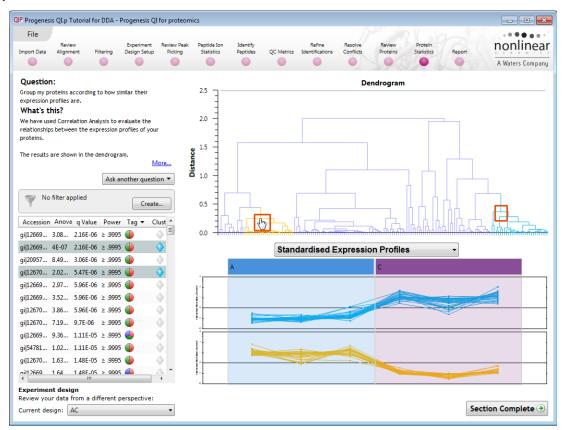
Clear any applied tag filters before moving to the **Protein Statistics** section to report on Proteins and /or peptides.

Stage 15: Protein Statistics

Protein Statistics opens with a Principal Components Analysis (PCA) for all the proteins displayed.



The Multivariate Stats can now be applied to all or subsets of proteins as determined by the current Tag filters (in this example none). Allowing you to identify similar paterns of expression using the Correlation Analysis.



Now move to the Report section to report on Proteins and /or peptides.

Stage 16: Reporting

The **Report Design** stage allows you to select what views you want to include in a report based on the list of **currently selected proteins.**

JP Filter the proteins Create a filter Show or hide proteins based on a selection of the filter. For more guidance, please see the o	their tags. Move tags to the appropriate boxes to create nline reference.
Available tags:	Show proteins that have all of these tags:
 Anova p-value ≤ 0.05 (118 proteins) Max fold change ≥ 2 (91 proteins) 	Modification with Oxidation (M) (13 proteins) Show proteins that have at least one of these tags: Hide proteins that have any of these tags:
Clear the filter	OK Cancel

As an example we will create a report for **only** the proteins containing peptides with Oxidation of Methionine residues enter **Oxidation (M)**.

- 1. First reduce the proteins to report on by selecting the '**Modification with Oxidation (M)**' tag. In this example it reduces the number of proteins in the table to 13.
- 2. Expand the various Report Design options (by default they are all selected)
- 3. Un-tick as shown below
- 4. Click Create Report

JP Progenesis QI.p Tu	utorial for DD/	A - Progenesis Q	I for prote	omics 🔤	
File				A standard and a standard and a standard a st	•••
Review Import Data Alignme		Experiment g Design Setup			inea
	•		•		s Company
Protein report Pep	tide report				
Report on your	proteins			Structure the report	
Generate a report o	ontaining the	e proteins of int	erest in	Enter a report title:	
your experiment.				Progenesis QI.p Tutorial for DDA	
1 Choose what				Select the sections to include in the report:	
		our data to sho clude in the rep			
,		out tagging and			
				🕑 🔲 Data processing methods	
2 Customise th Enter a title for		and select the	sections	👻 🔲 Experiment design	
you want to in right.	clude in it usi	ing the controls	to the		
				Include tables showing protein abundances and peptides identified for each protein	
💗 Tag filter ap		Ed	lit	✓ Protein table	
I proteins may	/ be nidden			Peptide tables	
Accession	Anova (p)	Fold Ta	ag 💌	Vertein Details	
gi 126699971	9.36E-06	11.6	٩	Reports the full details of every protein which matches your current filter	
gi 126697690	1.64E-05	5.9	٩	✓ Tags	
gi 254973900 (+9)	4.2E-05	3.7	٩	V Fags	
gi 126700129	0.000193	2.4	٩		
gi 126697654	0.000221	2.5	٩	v Peptide ion table	
gi 384359782 (+1)	0.00201	1.9	٩	📀 🔲 Peptide ion details	
gi 126700372	0.00373	5.3	٩	Create report	
gi 54781347	0.00399	2.0	•		
gi 126700078	0.00766	1.4	٩		
gi 126697631	0.0102	1.5	•		
gi 254976387 (+5)		1.3	•		
gi 126697969 (+1)		1.8	•		
gi 126700790 (+1)	0.148	1.8	•		
۰ III			÷		
Experiment design:	AC		•		

This opens a dialog to allow you to save the report, after which it will be opened in the form of a web page.

Click on the **Accession No**. in the proteins section of the Report and this will take you to the Assigned peptides for this protein

Progen	esis Q].р Т	utorial for	DDA												
Experiment:	Progen	esis QI.	p Tutorial for D	DA	1											
Report creat	_															
Proteins																
Protein building	g options															
Protein group	ing G	roup sim	ilar proteins													
Protein quantitation Relative Quantitation using Hi-3																
Accession	Accession Peptides Score Anova (p) Fold Tags Description Average Normalised Abundances															
	A C															1
gi 254976387	<u>gi 1254976387</u> 63 (63) 7080.71 <u>gi 1266999971</u>															
gi 384359782		2180.71														
gi 254973900 gi 126697969	15 (15)	1945.78 982.16	thioredoxin 2 (5 peptides	Trx2) [(Clostridium	diffic	ile 63	30]								
gi 126700790	10 (10)	917.16				-	T			-						
			Sequence		Peptide Ion	Score	Hits	Mass	Charge	Tags	Conflicts	Modifications	In quantitation	Average Normalis	ed Abundances	
gi 54781347	8 (8)	685.07	DDATVPNIENMIK		1429	60.28	5	1458.7019	2		0		no	2.29e+005	1.37e+004	
gi 126697631	7 (7)	625.77	VDEVTKDDATVPN	IENMIK	1157	66.32	5	2130.0505	2		0		yes	5.70e+005	9.09e+004	
gi 126700078 gi 126697690	6 (6) 6 (6)	582.21 578.31	VDEVTKDDATVPN		366	45.25		2130.0508		-	0		yes	1.40e+006	1.38e+005	
			VDEVTKDDATVPN		5861			2146.0459			0	[17] Oxidation (M)	no	6.82e+004	4129.52	
gi 126699971 gi 126700129	5 (5) 3 (3)	333.68 266.82	VDEVTKDDATVPN VLGLPTMAIYK	IENMIK	Accessi								1 10	/ 1/2+005	7/1 <u>9</u> ±111/1	
			VLGLPTMAIYK		-											
gi 126697654	3 (3)	214.79 125.83			-			xin 2 (Trx2)) [Clostri	idium	difficile 6	30]				
gi 126700372	2 (2)	125.83	Tags		Peptid	les 5 (5 ore 333	·									
			Most abunda		400	va 9.36		6								
			Anova p-valu		Fo	old 11.5										
			Significantly	-	- '	-		value ≤ 0.0								
			Up regulated	-	- '	-		change ≥ 2		(11)						
			Up regulated		- '	- MOG	Inicat	tion with O	xidation	(m)						
					4			А				с				
					g 15.0 ∃			H								
					ouepu 14.5											
					Abur											
					p 14.0											
					uno 13.5											
					4 13.0							+				
					ArcSinh Normalised Abbundance 1420 ArcSinh Normalised Abbundance 1320 ArcSinh Normalised Abbundance 1320 ArcSinh ArcSi							蕐				
					-	4								•		

Having closed the report it can be reopened by double clicking on the saved html file.

Note: you can also copy and paste all or selected sections of the report to Excel and/or Word.

Creating an Inclusion list

Inclusion lists can be used to try to increase the number of identified peptides you have in your experiment. They are used to control your mass spectrometer to try and concentrate the collection of MS/MS scans from specified m/z - retention time positions. Runs made using the inclusion list can then be imported into Progenesis QI for proteomics and the extra MS/MS scans added to the experiment.

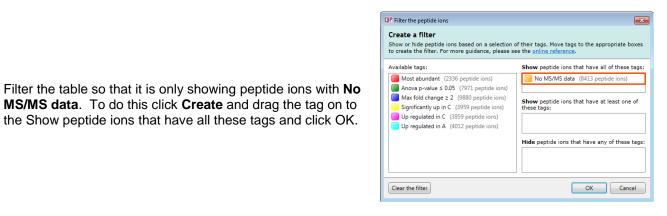
As an example we are going to create an inclusion list for all the peptide ions that show a Significant difference between groups A and C (Anova p<0.05) and have no MS/MS spectra.

Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report
	•		•		•	•	•	•	•	•	•	

First return to Review Peak Picking using the Workflow icons.

With no filters applied right click on a peptide ion in the table, select Quick tags and click on No MS/MS data.

Y	No filter	rapplied					
#	Anova	(p) Fold	Tag 💌	Note	es	Highest Mean	Lowest Mean
4022	1.11E-1	.2 Infinity	-	a.		С	Α
3631	1705-1	n <u>t-c-:</u> L.		-	Add a note	С	А
3509	2.	Most abunda		- [С	А
141	3. 📇	Anova p-valu Max fold cha		- 1		с	A
1921	3. 📩	Significantly	-			С	A
12141	1.	Up regulated				А	С
3818	1	Up regulated				С	A
7793	1.	New tag				А	С
8201	1.	Quick Tags		•	Anova p-	value	A
11083	1. 🙀	Edit tags				change	А
10123	2.758-1			14	No MS/N	1S data	А
7553	2.75E-1	1 Infinity		10	No prote	in ID	с
4446	3.5E-11	Infinity		4		С	A



Filter the table so that it is only showing peptide ions with No MS/MS data. To do this click Create and drag the tag on to

The table will now only be displaying peptide ions that have no ms/ms.

	ag filter ap eptide ions		lden												Edit	
#	Anova (p)	Fold	Tag 💌	Notes	Highest Mean	Lowest Mean	m/z	z	Mass	RT (mins)	RT window (mins)	Abundance	Intensity	Max CV (%)	MS/M	s
4022	1.11E-12	Infinity		4	С	A	1526.7795	2	3051.544	39.156	0.204	4.78E+04	2.76E+05	1.3	0	1
141	3.24E-12	Infinity		a.	С	Α	901.2218	2	1800.429	39.344	1.13	4.07E+06	1.43E+07	2.37	0	
12141	1.01E-11	Infinity		D	A	С	980.4783	2	1958.942	39.245	0.232	1.05E+04	5.4E+04	1.96	0	
3818	1.14E-11	2.06E+07		4	С	A	1441.1479	5	7200.703	39.344	0.484	2.51E+05	2.96E+05	173	0	
7793	1.42E-11	Infinity	-	2	A	С	847.7614	3	2540.262	55.142	0.202	8.84E+03	7.15E+04	2.09	0	
8201	1.71E-11	Infinity		D	С	Α	1002.0215	2	2002.028	32.829	0.21	8.64E+03	9.91E+04	2.19	0	
11083	1.72E-11	Infinity		2	С	Α	1011.0473	2	2020.080	41.307	0.22	5.45E+03	5.16E+04	2.1	0	
10123	2.75E-11	Infinity		12	С	A	1157.2779	3	3468.812	53.605	0.368	2.03E+04	9.39E+04	2.68	0	
7553	2.75E-11	Infinity		2	A	С	1207.0705	2	2412.127	34.551	0.566	3.91E+04	8.4E+04	2.86	0	
5343	5.96E-11	Infinity		D.	A	С	1372.4025	4	5485.581	32.829	0.379	2.25E+05	2.32E+05	3.96	0	
6314	6.41E-11	Infinity		12	С	A	1293.8406	4	5171.333	30.462	0.479	4.8E+04	1.62E+05	3.55	0	
10970	6.79E-11	Infinity	—	10	A	с	691.9449	3	2072.813	33.464	0.566	8.85E+03	6.09E+04	3.1	0	

Now select Export Inclusion List... from the file menu

Then select the appropriate format.

QIP Export	inclusion list	×
Select yo	our machine type and export the	inclusion list:
Format:	Thermo Finnigan inclusion list	•
	6	About this data format 🕹 Download others
Save to:	D:\Customer Data\QIP_Waters	Futorials\QIP_Non Waters Tutor Browse
		Export Cancel

Finally	export	the file	to an	appropriate	location
i intany	chpoit	the me	to un	uppropriate	looution

Note: with certain MS machines it is possible to widen the retention time windows being used, this can be controlled using the following dialog.

If you require further information on the inclusion list file formats then click Help.

Note: The new LC-MS runs can then be added to the original experiment to increase the MS/MS coverage using the **Add files** facility at the Data Import Stage.

Congratulations!

This document has taken you through a complete analysis using Progenesis QI for proteomics, from Alignment through the analysis workflow to generate lists of proteins exhibiting expression changes across biological conditions using powerful Multivariate Statistical analysis of the data.

Hopefully our example has shown you how this unique technology can deliver significant benefits with

- Speed
- Objectivity
- Statistical Power

If you would like to see the benefits of running Progenesis QI for proteomics using your own data and explore the Progenesis QI for proteomics workflow please go to Appendix 4: Licensing Runs (page 76).

QIP P	rogenesis QI.p Tutorial for DDA - Progenesis QI fo	or prot	teomi
	File		
	-	ent :tup	Revi Pi
	Close		
	Export peptide ion data		
	Import peptide ion numbers as tag		
	Export all identifications		
	Export inclusion list		
	Experiment properties		
	Show Clip Gallery		
×	Exit		

Inclusion list retention time windows									
Do you want to widen the retention time windows									
by 0.00 in minutes?									
Yes No									

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, AB SCIEX, Agilent, Bruker and Thermo) 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.

QP Create New LC-MS Experiment	
Create a new label-free experiment named:	
Progenesis QI.p_Tutorial for DDA	
Data type	
Profile data	
Centroided data	
Resolution (full width at half maximum) 50000	
Machine type	High resolution mass spectrometer
High resolution mass spectrometer	e.g. Waters SYNAPT G2/G2-S, AB SCIEX TripleTOF, Agilent QTOF, Bruker Maxis, Thermo LTQ Orbitrap
Experiment folder	Thermo LTQ Iontrap in Enhanced mode.
Save experiment in the same folder as the run data	
Choose an experiment folder	Low resolution ion trap e.g. Bruker HCT, Bruker HCT Ultra, Thermo LTQ XL
	Thermo FT-ICR
Create experiment	Cancel

Select the 'Import Data file format', in this example they are mzXML files

Then locate your data files using Import...

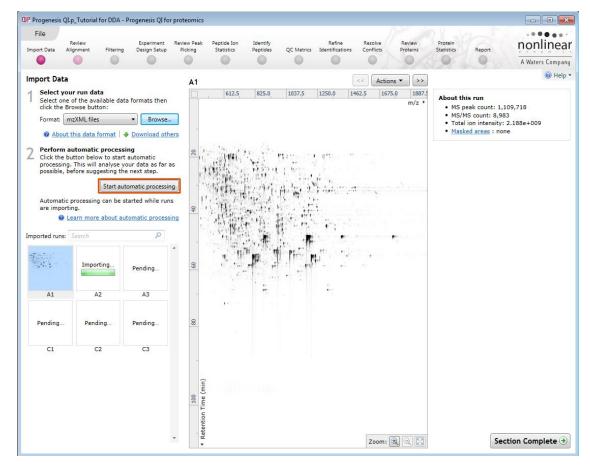
OP Progenesis	QI.p_Tutorial	for DDA - Pr	ogenesis QI fo	r proteomics						AW -			
File Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	A Waters Company
Select o	your run dat one of the ava Browse butt mzXML files	iilable data f :on: :	Browse.										🕡 Help ୟ
2 Perform Click the processi possible	Bruker Dalt	D.5304.2798 onics (.d) D.5149.2874 D.5304.2797	natic ata as far step. 75 c processi		J =	QLp_DDA Tu New folder	tor 🕨 DDA	_Raw data ►	• 4 ,	Search DL	DA_Raw data ≋≡ ▼	<mark>×</mark> م	
	NetCDF files Version: 1.0 Thermo (.ra	s D.5304.2796 aw) D.5304.2801 ICR (.raw)	ⁱ⁸ tic process	sing	 Desktop Downloads Dropbox Recent Plac ShareFile 		* III	Name Progene A1.mzxn A2.mzxn A3.mzxn	nl	rial for DDA.A	nalysis 10/0 20/0 20/0	e modified 09/2014 08:04 05/2008 08:23 05/2008 08:27 05/2008 08:29	
					Desktop Libraries Documer Music Pictures	nts		C1.mzxn C2.mzxn C3.mzxn	nl nl		20/0 20/0	05/2008 08:25 05/2008 11:50 05/2008 11:50 05/2008 08:25	εc
Na	runs have be	en imported	yet		Videos	File name	₹ "C3.mzxm	<	''' 'A2.mzxn ▼	mzXML file	es (*.mzxml)	► encel	
				Ŧ									

Locate and select all the Data files (A1 to C3).

On loading the selected runs your data set will be automatically examined and the size of each file will be reduced by a 'data reduction 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: as the loading process starts you can also 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 7.

Additional details on Selection of Alignment Reference

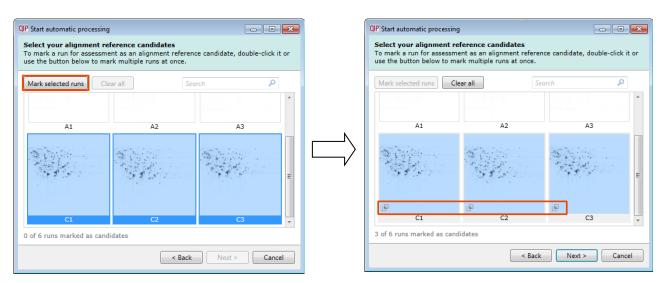
1. Assess all runs in the experiment for suitability

- This method compares every run in your experiment to every other run for similarity.
- The run with the greatest similarity to all other runs is chosen as the alignment reference.

If you have no prior knowledge about which of your runs would make a good reference, then this choice will normally produce a good alignment reference for you. This method can take a long time.

2. Use the most suitable run from candidates that I select

• This method asks you to choose a selection of reference candidates, and the automatic algorithm chooses the best reference from these runs.



Select a subset of your runs as 'alignment reference candidates' and click **Mark selected runs.** An icon appears on the bottom left of each run to indicate that it is selected as a candidate.

This method most appropriate when you have some prior knowledge of your runs suitability as references:

- runs from pooled samples
- runs for one of your experimental conditions will contain the largest set of common peptides.

Also preferred option when analysing a large number of runs

3. Use this run

• This method allows you to manually choose the reference run.

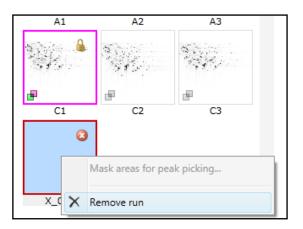
Manual selection gives you full control, but there are a couple of risks to note:

- If you choose a pending run which subsequently fails to load, alignment will not be performed.
- If you choose a run before it fully loads, and it turns out to have chromatography issues, alignment will be negatively affected (for this reason we recommend that you let your reference run fully load and assess it's chromatography before loading further runs).

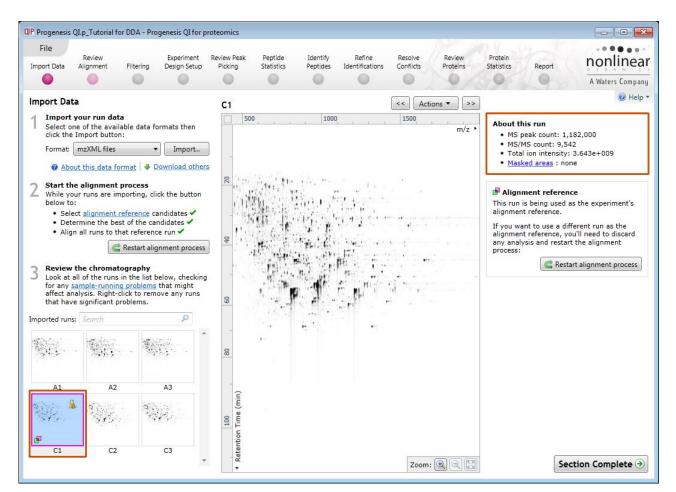
Review Chromatography

Each data file appears as a 2D representation of the run. At this stage you will be warned if any of the data files have been 'centroided' during the data acquisition and conversion process.

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



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 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 14 in this user guide) by clicking Section Complete.

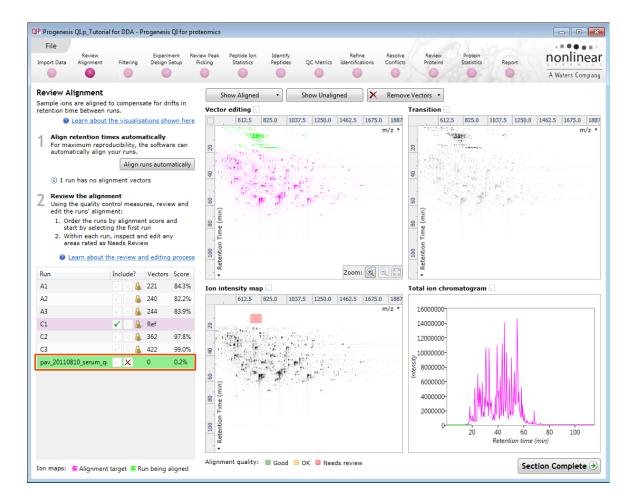
Appendix 2: Stage 1 Processing failures

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; the automatic processing dialog will prompt you to 'drop-off' at the **Review Alignment** stage on completion to investigate the problem.

QIP Processing Complete						
Automatic processing complete (with warnings). Time taken: 3 minutes 9 seconds						
 Importing runs: Selecting reference: 	7 of 7 processed C1					
Aligning runs:	6 of 6 processed A 1 run failed to align - continuing without it					
Peak picking:	14624 peaks found					
 Creating design: 	Created					
Protein quantitation:	Relative Quantitation using Hi-3					
	Close Identify Pe	ptides 📀				

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

QIP Processing Complete	IP Processing Complete						
Automatic processing complete (with warnings). Time taken: 3 minutes 40 seconds							
A Importing runs:	7 of 7 processed A 1 failed to import						
 Selecting reference: 	C1						
 Aligning runs: 	5 of 5 processed						
 Peak picking: 	14624 peaks found						
 Creating design: 	Created						
 Protein quantitation: 	Relative Quantitation us	ing Hi-3					
		Close Identify Peptides	•				

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, where in importing, you specified selection of the alignment reference 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).

1	Processing Complete		
	Automatic process Time taken: 19 seconds	ing failed.	
	▲ Importing runs:	6 of 6 processed A 1 failed to import	
	X Selecting reference:	All reference candidates failed to import	
	Aligning runs:	Unable to start.	
	Peak picking:	Unable to start.	
	Protein quantitation:	Unable to start.	
		Close	Import Data 🏈

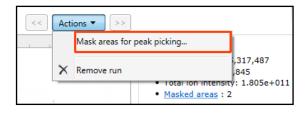
In this case, the processing dialog would halt and prompt you to select another reference.

Appendix 3: Stage 1 Data QC review and addition of exclusion areas

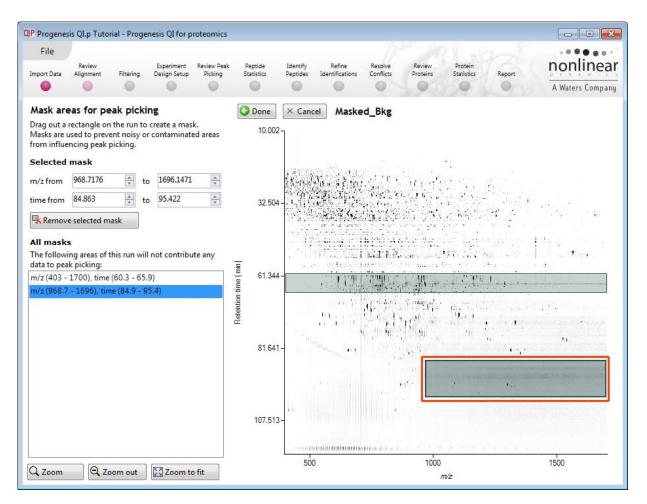
During the process of Data QC you may identify areas of the raw data for a particular run that appear 'noisy' yet still have identifiable 'isotopic patterns'.

For example if the run is part of a 'replicate set' of runs it is possible to exclude such areas on the noisy run by applying a mask to the area. By doing so this area is excluded during the initial part of the detection process in order that it does not 'interfere' with the detection of the peptide ions in the replicate group.

To do this select Masked areas from selected run on the bottom left of the screen.



Drag out an area over the noisy part of the run to create the mask.



Note: Click **Done** to return to the **Import Data** view where you can zoom into the masked where you will see the isotopic peptide ions in the noise.

Note: if the level of noise is high and affecting many of your runs a preferred approach would be to reoptimise the chromatography to improve the levels of noise in your data.

Appendix 4: Licensing runs (Stage 3)

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.

File	Licensing	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	A Waters Compa
ongle	License	Runs												
	allation is runs only	currently re	estricted to	analyse	Run nam	e							Licence state	License this run
Tolicons		ns, vou nee	ad an ova	luption	C:\Users\	andy.borthwid	k\Documen	ts\Customer	Data\Progene	sis QI.p_Tut	orials and De	mo Suites\Q	I.p Unlicensed	d 🔽
				om a sales	C:\Users\	andy.borthwid	k\Documen	ts\Customer	Data\Progene	sis QI.p_Tut	orials and De	mo Suites\Q	I.p Unlicensed	d 🔽
represen	itative.				C:\Users\	andy.borthwid	k\Documen	ts\Customer	Data\Progene	sis QI.p_Tut	orials and De	mo Suites\Q	I.p Unlicensed	d 📝
Once licensed, your runs can be analysed on					C:\Users\	andy.borthwid	k\Documen	ts\Customer	Data\Progene	sis QI.p_Tut	orials and De	mo Suites\Q	I.p Unlicensed	d 🔽
any insta	llation of t	he software	e. The lice	ence is	C:\Users\	andy.borthwid	k\Documen	ts\Customer	Data\Progene	sis QI.p_Tut	orials and De	mo Suites\Q	I.p Unlicensed	d 🔽
automati experime		ided when	archiving	an	C:\Users\	andy.borthwid	k\Documen	nts\Customer	Data\Progene	sis QI.p_Tut	orials and De	mo Suites\Q	I.p Unlicensed	d 🔽
compute available	r, <u>click he</u> on this co	een license <u>re</u> to make f omputer. ou can <u>open</u>	the licenc	es										
lf you hav	ve just ins	talled a dor	ngle, <u>click</u>	here.										
								Run licen	ce code: 🛛 🗙	->0000:->0000:->0	XX		Use Lic	ence Code

A message confirming successful installation of your licences will appear.

Installation	n complete	×
i	Successfully installed licences for Progenesis QI for proteomics.	
	ОК	

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

Appendix 5: Manual assistance of Alignment

Approach to alignment

To place manual alignment vectors on a run (C2 in this example):

- 1. Click on Run C2 in the **Runs** panel, this will be highlighted in green and the reference run (C1) will be highlighted in magenta.
- 2. You will need approximately 5 alignment vectors evenly distributed from top to bottom of the whole run.
- 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

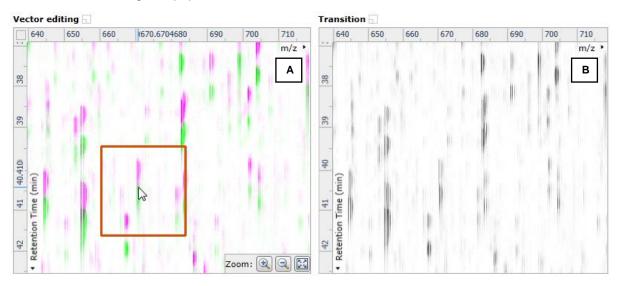


Note: the peptide ions moving back and forwards between the 2 runs in the **Transition** window (B) indicates the misalignment of the two runs.

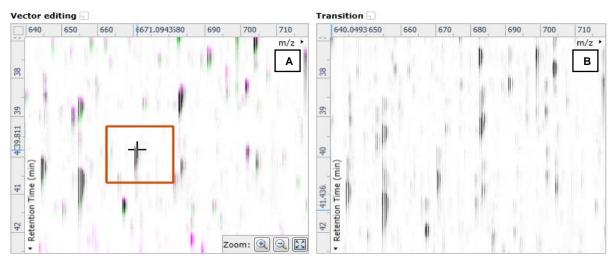
Note: the Ion Intensity Map gives you a colour metric, visually scoring the current alignment. The overall score is placed next to the Vectors column in the table. With each additional vector added this score will update to reflect the 'changing' overall quality of the alignment. The colour coding on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

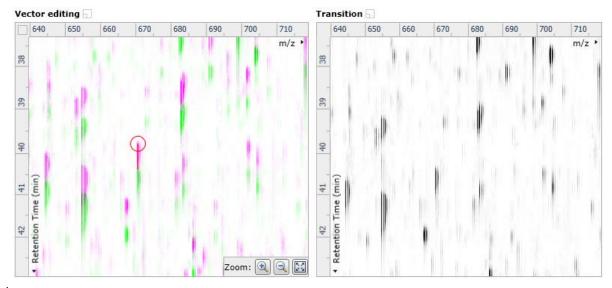


5. As you are holding down the left mouse button (depending on the severity of the misalignment), the alignment vector will automatically find the correct lock. If not, drag the green peptide ion over the corresponding magenta peptide ion of the reference run. The red box will appear as shown below indicating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

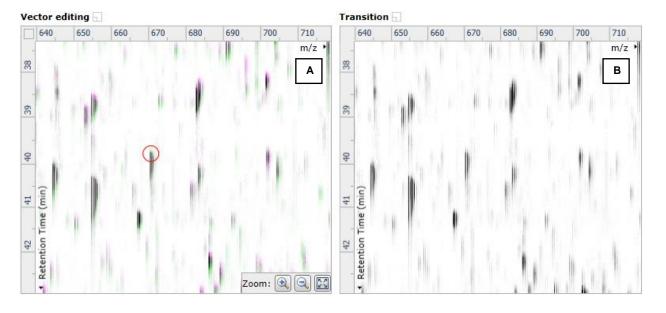
6. On releasing the left mouse button the view will 'bounce' back and a red vector, starting in the green peptide ion and finishing in the magenta peptide ion will appear.



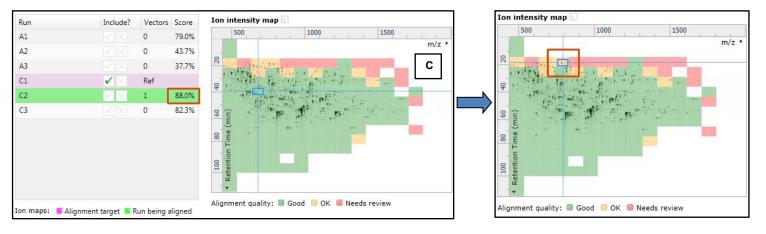
Waters

Note: an incorrectly placed vector is removed by right clicking on it in the Vector Editing window

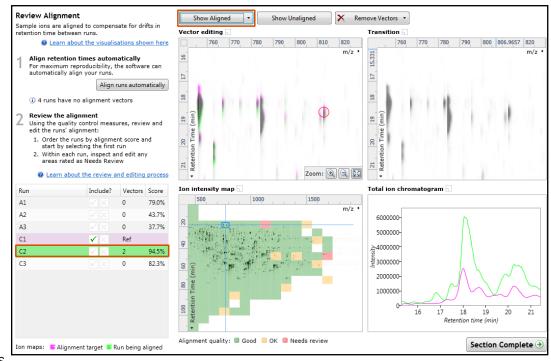
7. Now click Show Aligned on the top tool bar to see the effect of adding a single vector.



8. With the placement of a single manual vector the increase in the proportion of the **lon Intensity Map** (C) showing green is reflected in the improved alignment score in the table. Now click in the lon 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.

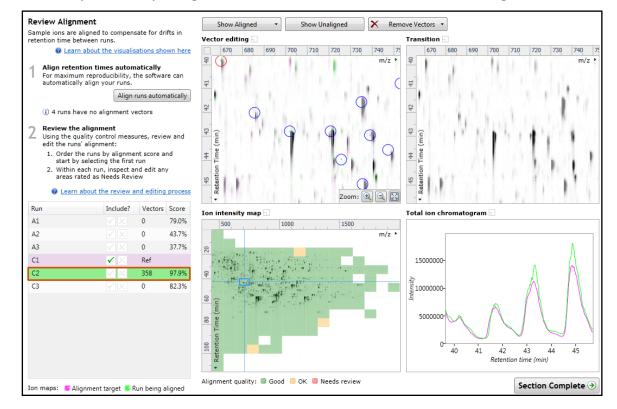


Waters

- 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 2 vectors from the top to the bottom of the run is sufficient to markedly improve the alignment (**Note**: the improvement in the score with the addition of only 2 vectors).
- 12. At this point you would redo the automatic alignment of this image by selecting **Align runs automatically**. **Note**: if you are focusing only on the alignment of one run, then un-tick the other runs in the alignment dialog.

Run	Notes	Vectors
✓ A1	This run has not been automatically aligned	
✓ A2	This run has not been automatically aligned	
✓ A3	This run has not been automatically aligned	
🗸 C2	run has user vectors	
🗸 C3	This run has not been automatically 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.

To review the vectors automatic and manual, return to page 15.

Appendix 6: 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

rogenesis QI.p Tutorial for DDA - Progenesis Q File Review Experim ort Data Alignment Filtering Design S	ent Review	Peak Peptide Ion	Identify Peptides	QC Metrics	Refine Identifications	Resolve Conflicts	Review Proteins	Protein Statistics	Report	ņ	online
						•	-	0	0	A	Waters Comp
AC-2 New											(i) He
hich experiment design type do	ou want	to use for this	experim	ent?							
OO OO Between-subject Design)	-subject	-				
Do samples from a given subject appear in only one condition? Then use the between-subject design.	A		Delete	s	ave you taken ubject under o hen use the w	lifferent co	nditions?		Before	During	After
To set up this design, you simply group the runs according to the condition (factor level) of the samples. The ANOVA calculation assumes that the			A2 <u>Remove</u> A3 <u>Remove</u>	e a	ote: you must very subject fo within-subject	r every con	dition to use	Patient X	X1	X2	X3
conditions are independent and therefore gives a statistical test of whether the means of the conditions	c	QIP Create New Exp Enter a name for Before During an	the experim	ent design:			as	Patient Y	Y1	Y2	¥3
are all equal.	Add condit	How do you want	to group the				but	Patient Z	Z1	Z2	Z3
		Copy an exis	ting design:				The				
				a	Create designs		VA				
				re di re	epeated measu ifferences can educed as a sou ondition differe reate a more p	res ANOVA i be eliminate irce of betw ences (which	ndividual ed or reen n helps to				
				ti P ci	he within-subje hought of as an aired-samples omparison betw epeated measu	extension o t-test to incl veen more t	of the lude				

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.

QIP Progenes	sis QI.p Tutorial fo	r DDA - Progenesi	is QI for prot	eomics					
File Import Data	Review Alignment Filt	Experiment Pering Design Setup			lentify ptides QC Metrics	Refine Reso Identifications Confl		Protein Statistics Report	nonlinear
			-				1981	19 198	A Waters Company
AC	AC-2	Before D	uring and	After Treatme	ent I × 🔄	New			🕜 Help 🤊
Setup the experimen	conditions and conditions and su t design on the r mples to the corr	ubjects for your ight, and then as	ssign each ition cell		Before	During	After	Add Condition	
2. Add a ro	-	ect.	location	Patient A	A1	A2	A3		
	C	98		Patient C	C1	Select Sample	Select Sample		
	C			Add Subject					
								Se	ction Complete 🏵

You can create additional Experimental Designs using the New tab

All of these Experimental Designs are available at all the following stages in the workflow.

Appendix 7: Power Analysis (Peptide Stats)

Power analysis is a statistical technique that is used to gauge how many replicates are needed to reliably see expression differences in your data. It is available through the Progenesis Stats section of the workflow.

To perform a power analysis of the data click on **Ask another question** at the top of the table in the Progenesis Stats screen. A selection of 3 tools will appear in the form of questions.

v	Principal Components Analysis Are there any outliers in my data? Does my data cluster according to my experimental conditions?
ጨ	Correlation Analysis Group my features according to how similar their expression profiles are.
	Power Analysis How many replicates should I run? What is the power of my experiment?

Select the option

'How many replicates should I run and what is the power of my experiment?'

It answers this question by informing you:

'How many replicates you need so that at least 80% of your peptide ions with a power >0.8'

Using the Significant p<0.05 peptide ions (7971), as an example, view the power analysis.



This is displayed graphically showing that 78.6% of the 7991 peptide ions have a power of 80% or that 4 replicates would give you 96.4% of your data with power > 0.8.

- The power of a statistical test reflects our confidence in the experimental data's ability to find the differences that do actually exist
- The power is expressed as a percentage, where 80% power is an accepted level, therefore allowing you to assess the number of sample replicates that would be required to achieve a power of 80%.

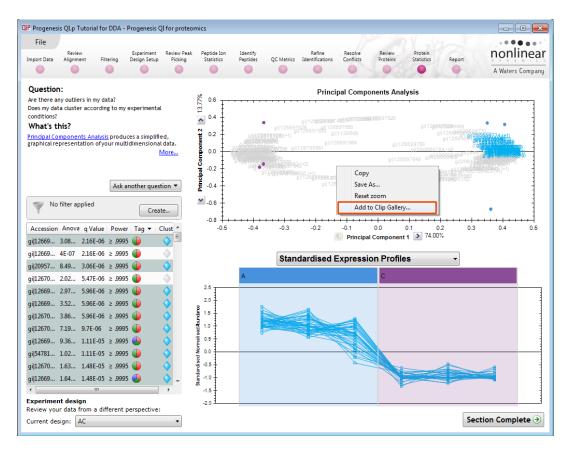
Appendix 8: Using Clip Gallery to Save and Export Pictures and Data

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

The saved images 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 **Protein Statistics** view, displaying the PCA plot.

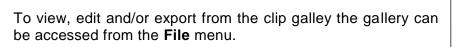
Right click on the Biplot View and select Add to clip gallery....

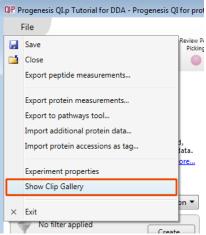


This will open a dialog displaying what is to be saved and allows you to alter the title and provides a description of the image for later reference.

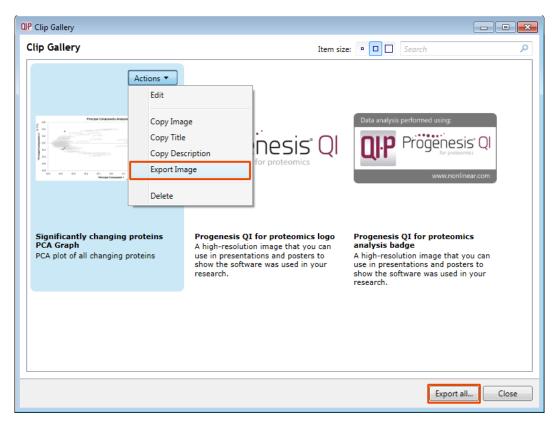
0.6	Princ	pai Componenta An	alysis	
0.4		CHE 21 120301 1011 1011		1.1
0.2				· Aller and
0.4 . 0.3 -0.2 *				
-0.6				
-05 -04	43 42	-0.1 0.0 Principal Component	0.1 0.2	e3 0.4
			74.09%	_
Significan	tly changir	ng protein	s PCA Gr	aph
CA plot o	f all changi	ng protein	s	

Enter details as required and click Add to clip gallery





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



Note: there is also the capacity to **Export all...** the images 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 image title.

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

Appendix 9 Search engine parameters (Stage 9) Mascot

The parameters applied to the Mascot search that yielded the search results used in this user guide are shown below:

маѕсот	MS/MS Ions Search								
Your name	andy.borthwick	Email	andy.borthwick@nonlinear.com						
Search title	Progenesis QI search								
Database(s)	MSDB	Enzyme	Trypsin 🔻						
	NIST_Yeast_Sigma SwissProt	Allow up to	1 🔻 missed cleavages						
	Torlo2	Quantitation	None						
Taxonomy	Firmicutes (gram-positive ba	cteria)	-						
Fixed modifications	Carbamidomethyl (C)	> <	Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term)						
	Display all modifications 📃		Ammonia-loss (N-term C) Biotin (K)						
Variable modifications	Oxidation (M)	> <	Biotin (N-term) Carbamyl (K) Carbamyl (N-term) Carboxymethyl (C)						
Peptide tol. ±	9 ppm ▼ # ¹³ C 0 ▼	MS/MS tol. ±	0.6 Da 🔻						
Peptide charge	2+ 🔻	Monoisotopic	Average						
Data file	D:\Customer Data\QIP_Waters Browse								
Data format	Mascot generic 🔹	Precursor	m/z						
Instrument	ESI-TRAP 🔻	Error tolerant							
Decoy		Report top	AUTO 🔻 hits						
	Start Search		Reset Form						

Database : NCBInr (circa 01/14) was used with the Taxonomy restriction set to Fermicutes

Fixed modifications: Carbamylation(C) and variable modification Oxidation (M)

Peptide Tol: 9ppm

Instrument: ESI-Trap

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