

Progenesis LC-MS Tutorial

for LC-MS version 2.5



- 1

Introduction

This tutorial takes you through a complete analysis of 6 LC-MS runs with 2 groups (3 replicate runs per group) using the unique Progenesis LC-MS workflow. It starts with LC-MS data file loading then Alignment, followed by Analysis that creates a list of interesting features (peptides) which are explored within Progenesis 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. However, the document covers all the stages in the LC-MS workflow, therefore if you are using your own data files refer to Appendix 1 then start at page 5.

How to use this document

You can print this tutorial to help you work hands-on with the software. The complete tutorial takes about 50 minutes 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 LC-MS?

You can freely explore the quality of your LC-MS data using Image QC and then licence your own LC-MS runs using this evaluation copy of Progenesis LC-MS. Instructions on how to do this are included in a section at the end of the tutorial document. Alternatively if you would like to arrange a demonstration in your own laboratory contact support@nonlinear.com and we will help you.

LC-MS Data used in this tutorial

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 tutorial as well as invaluable discussion on the handling of the data.

Workflow approach to LC-MS run analysis

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

LC-MS Data	Reference Run Selection	Alignment	Filtering	Group Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report
Stage		Descript	ion							Page
LC-MS Data Import		LC-MS analysi		port: Sel	ection ar	nd review	of data fi	les for		5
Reference Run Selection		Referer	nce Run	Selectio	n: Selec	t run to al	ign to.			6
Licensing			•	vs licensi ached (A	0	ividual da 3)	ıta files w	hen there)	6
Alignment		Alignm	ent: auto	omatic an	id manua	al run aligi	nment			7
Filtering			•	ng filters i d Numbe		es based opes.	on Reter	ntion Time	9,	18
Group Setup		Group aligned	-	efining o	ne or mo	re group	setups for	r analyse	d	21
View Results						e results, features f			ion,	22
Progenesis Stats		-		its: perfo cted grou	•	ultivariate	statistica	l analysis	on	31
Peptide Search						t of MS/M h engines		a to, and	import	36
Peptide Filter		Peptide	Filter: I	manage p	peptide ic	ds and filte	ers			39
Protein View						lution of p ch engine		conflicts	for	41
Report		Report:	generat	e a repoi	rt for pep	tides and	proteins			46

Restoring the LC-MS Tutorial

Open Progenesis LC-MS and downloaded the Compressed (.zip) Tutorial Archive file from the 'Download 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 LC-MS tutorial archive file. To do this, first locate the LC-MS Tutorial Archive file using the **Browse** button and press Open.

Open Experim	ent		6	
Look in: Recent Places Desktop Andy Bothwick Computer	LC-MS v2 Name	S Tutonal Date taken Tags Istorial 2.5 Progenesist cmtAn	P 🗊 •	
Network	File name: Files of type	LC-MS Tutorial_25.Progen Progenees LC-MS Experim	Open Cancel	

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 fror	n archive	
Import	LC-MS Tutorial from archive	
🔘 Rep	lace an existing experiment	
Experin	nent to replace:	~
Orea	ite a new experiment	
Name:	LC-MS Tutorial	
Folder:	C:\Users\Andy.Borthwick\Desktop\LC-MS v2.5 Tutorial	
		Import Cancel

Then press Import.

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

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

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

LC-MS Tutorial - Progenesis LC-MS		
Eile		
LC-MS Data Reference Run		nonlinear
	tering Group Setup View Results Progenesis Stats Peptide Search Peptide Filter Protein View Report	dynamics
Import Data	Data processing methods:	
mzXML files	Feature detection method: Default	
	Peak processing method: Profile data	
Include?	No problems found	<u>^</u>
No problems found	No problems found	
A2	AL	
A3 Pending		
C1 Pending		
C2 Pending C3 Pending		
	No problems found	
	The data file was imported with no problems.	
	The data appears to be in the correct format to be analysed by this software.	
✓ Include run in analysis		
X Don't include run in analysis		
Exclude areas from selected run		
	Sect	tion Complete 🧿

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: as each data file is loaded the progress is reported in the **Import Data** list. The dialog below the image reports on the QC of the imported Data files. In this case 'No problems found' with this data file.

Note: the **'Data Processing Methods'** selected, when the experiment was created, are reported next to the Add data files link (see Appendix 1, page 50).

Note: 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 2, page 52). Not required for this data set.

Once all the files have been imported move to the next stage in the workflow by clicking **Section Complete.**

Stage 2: Reference Run selection

This stage in the analysis workflow allows you to review and select the most appropriate Reference LC-MS run to align all the other runs to.

LC-MS Tutorial - Progenesis LC-MS		- • ×
Eile		
LC-MS Data Reference Run Import Selection Alignment Filtering	Oroup Setup View Results Progenesis Stats Peptide Search Peptide Filter Protein View Report	nonlinear
Choose reference run		
The reference run will be used when aligning each of the runs in your experiment.		
Choosing a good reference run will help during the alignment stage. Ideally, the reference run should show a clear and representative feature pattern, and have a minimum of distortion.		
For experiments such as time series or dose response, choosing the middle point tends to give the best results.		
Run Reference		-
A1 🔳		
A2 👘		
A3		
C1 🕑		
C2		
C3 🖷		
🗊 Use as reference run		
	Sectio	n Complete Э

To select a Reference run either click on the run in the list and then click **Use as reference run** or double click on the run in the list.

Now move to the next stage in the workflow by clicking Section Complete.

Stage 3: Licensing

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

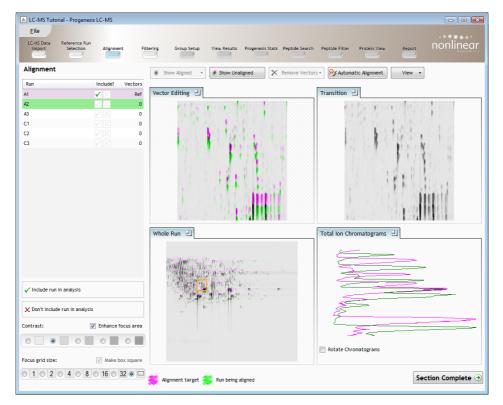
LC-MS Data	Reference Run		
Import	Selection	Licensing	Alignment

For details on how to use Licensing go to Appendix 3 (page 53)

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

Stage 4: Alignment

At this stage Progenesis LC-MS Alignment opens displaying your data.



Generation of alignment vectors

The alignment of LC-MS runs is required in the LC (retention time) direction, this is key to correcting for the variable elution of peptides during the chromatographic separation.

The Alignment algorithm will generate 'Automatic' vectors, in the retention time direction for each run, to enable the alignment of all the LC-MS runs to the 'Reference Run'.

ide Filter	Protein View	Repor
· - 1941	Automatic Alignm	ent
ansition	μ	

The alignment vectors are generated automatically for all the LC-MS runs by using the 'Automatic vector wizard' accessed by clicking on **Automatic Alignment** on the top tool bar.

Select (tick) the runs you require to generate vectors for and click OK.

		lignment Ins for automatic alignment vector generation		
Add	Run	Notes	Vectors	
	A1	this run does not need to be aligned as it is the alignment reference		-
V	A2	automatic alignment will be performed for this run		0
V	A3	automatic alignment will be performed for this run		0
V	C1	automatic alignment will be performed for this run		0
V	C2	automatic alignment will be performed for this run		0
1	C3	automatic alignment will be performed for this run		0
		ОК	Cancel	

If applying alignment automatically now move to page 16

The following pages in this tutorial explain in more detail the views and functions of the Alignment stage in the Progenesis LC-MS Alignment, with details on:

- The Program layout
- Adding vectors manually

These pages act as a useful guide and reference to the Alignment Stage that you can return to after having generated the Alignment vectors automatically and will help with the refinement of your alignment.

Taking a detailed approach to alignment

In some cases, where the misalignment is severe, using a combination of a 'few' manually placed vectors on each run and then using the Automatic vector wizard to generate the rest of the vectors for each run can give better results.

In this example try placing some manual vectors before generating the automatic vectors.

The following sections describe the layout of the Alignment Stage and how to manage the placement of manual vectors on your LC-MS runs

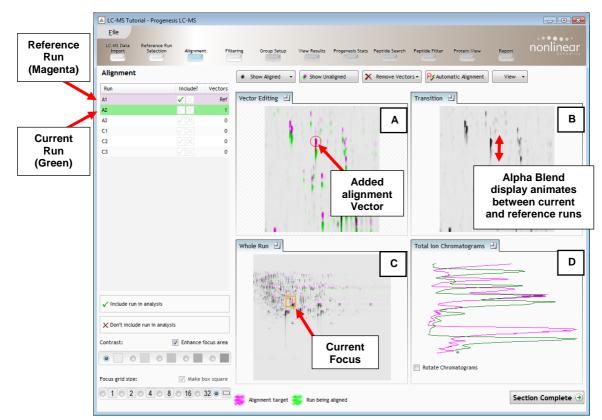
Layout of Alignment

To familiarize you with Progenesis LC-MS Alignment, this section describes the various graphical features used in the alignment of the LC-MS runs.

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

- Click on the features shown in the current focus (orange rectangle) in Window C, this will update windows A,B and D as shown below.
- In window A click and hold the left mouse button on a green feature.
- If the green and magenta features (immediately above) have not aligned automatically then **drag** the green feature over the magenta feature and **release** the mouse button.
- The image will 'bounce' back and a red vector, starting in the green feature and finishing in the circled magenta feature will now appear as shown below in window A.

The experiment structure is displayed on the left of the screen in the Run panel.



The **Runs:** panel shows the run that is currently being aligned in green, and the run it is being aligned to in magenta.

The **Ref** run for any experiment is the run that you chose, in this case **A1** highlighted in magenta.

Run	Include?	Vectors
A1	\checkmark \times	Ref
A2	√ X	1
A3		0
C1	🖌 📈	1
C2		0
C3	√ X	0

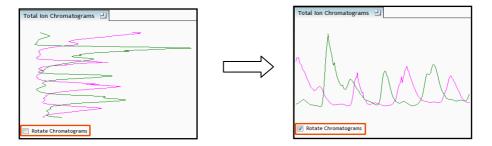
Vector Editing (Window A): is the main alignment area and displays the area defined by the current **focus** rectangle shown in Window C. The current image is displayed in green and the chosen reference image is displayed in magenta. Here is where you place the alignment vectors.

Transition (Window B): uses an **alpha blend** to animate between the current and reference runs. Before the runs are aligned, the features appear to move up and down. Once correctly aligned, they will appear to pulse. During the process of adding vectors, this view will change to show a zoomed view of the area being aligned to help accurate placement.

Whole Run (Window C): shows the focus for the other windows. When you click on the view the orange 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 using the controls in the bottom left of the screen or by clicking and dragging out a new area with the mouse.

Total Ion Chromatograms (Window D): shows the current **total ion** chromatogram (green) overlaid on the Reference chromatogram (magenta). As the features 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 **W hole Run** view (Window C).

Note: the orientation of the TIC view can be changed according to individual preference



This view assists in the verification of the feature alignment .

Note: the icon to the right of the 'Window' titles expands the view $% \left({{{\bf{N}}_{{\rm{B}}}} \right)$

Total Ion Chromatograms 🖳

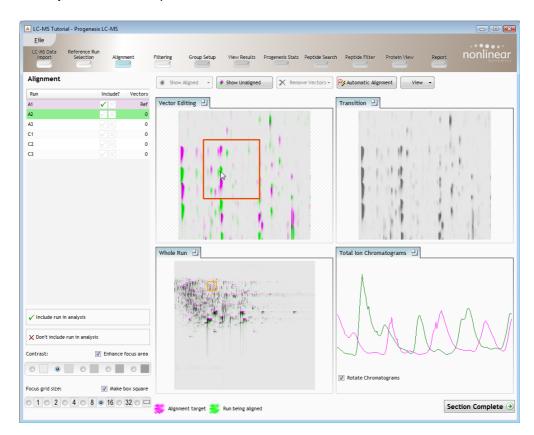
Approach to alignment

To place manual alignment vectors on a run (A2 in this example):

- 1. Click on Run A2 in the **Runs** panel, this will be highlighted in green and the reference run (A1) will be highlighted in magenta.
- 2. You will need approximately 5 10 **alignment vectors** evenly distributed from top to bottom of the whole run.
- 3. First ensure that the size of the focus area is set to **8 or 16** in the Focus grid size on the bottom left of the screen.

Contrast:	Enhance focus area
Focus grid size:	Make box square
01020408	16 ○ 32 ○ □□

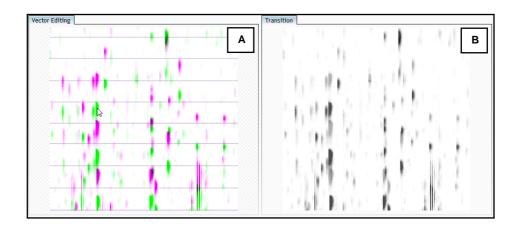
4. Click on an area (see below) in the **Whole Run** window (C) to refocus all the windows. Adjust Contrast as required.



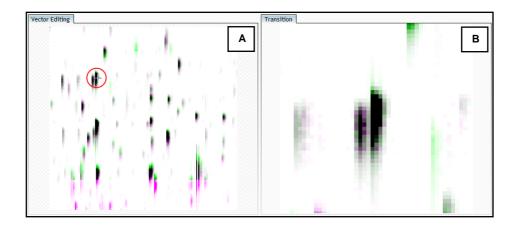
Note: the features moving back and forwards between the 2 runs in the **Transition** view indicating the misalignment of the two LC-MS runs

Note: The **Total Ion Chromatogram** view also reflects the misalignment of the 2 runs for the current Retention Time range (vertical dimension of the current Focus grid in the **Whole Run** view

5. Click and hold on a green feature in Window A as shown below.

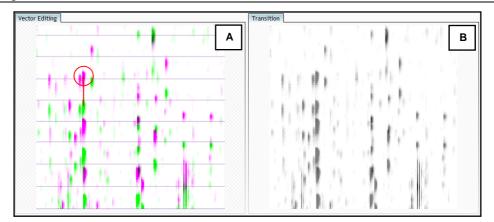


6. As you are holding down the left mouse button drag the green feature over the corresponding magenta feature of the reference image. The vector will appear as shown below as a red circle with a 'cross hair' indicating that a positional lock has been found for the overlapping features.



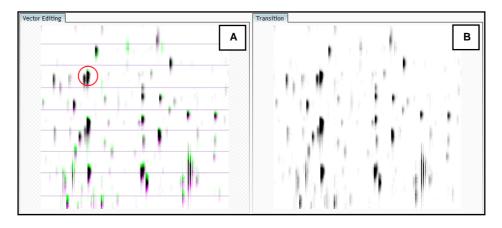
Note: as you hold down the mouse button, window B zooms in to help with the alignment.

7. On releasing the left mouse button the image will 'bounce' back and a red vector, starting in the green feature and finishing in the magenta feature will appear.

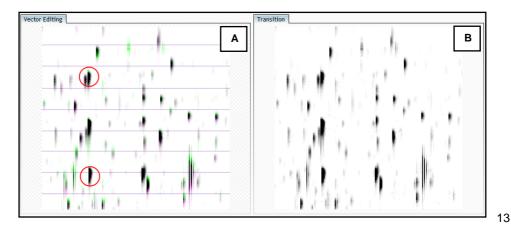


Note: an incorrectly placed vector is removed by right clicking on it in the Vector Editing window

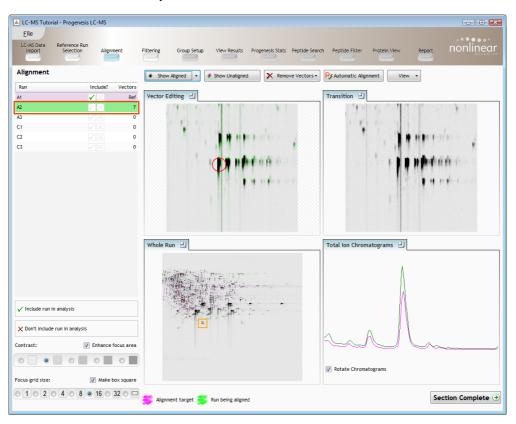
8. Now click Show Aligned on the top tool bar to see the effect of adding a single vector.



9. Additing an additional vector will improve the alignment further. **Note** this time as you click to add the vector it 'jumps' automatically to the correct position using the information from the existing alignment vector



10. Repeat this process moving the focus from top to bottom on the Whole Run view



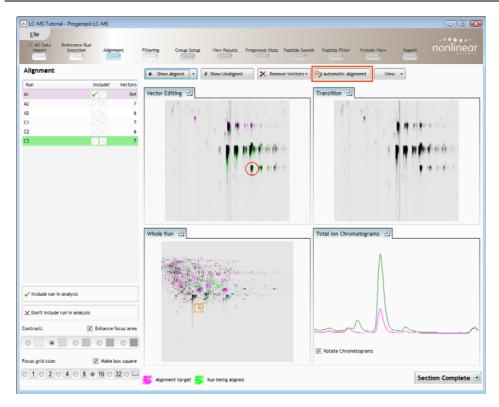
Note: the number of vectors you add is recorded in the Runs table

11. Now move on to the next run to align and repeat the addition of a few manual vectors

The number of manual vectors that you add at this stage is dependant on the misalignment between the current run and the Reference run. In many cases only using the Automatic vector wizard will achieve the alignment.

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

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



13. Then select Automatic vectors and click OK.

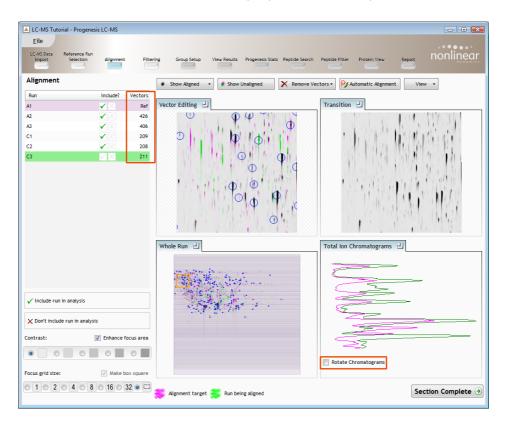
Automatic A	ignment	×
Select the n	ins for automatic alignment vector generation	
Add Run	Notes	Vectors
🔳 A1	this run does not need to be aligned as it is the alignment reference	-
🔽 A2	run has user vectors	7
🔽 A3	run has user vectors	6
🔽 C1	run has user vectors	7
🔽 C2	run has user vectors	6
🔽 C3	run has user vectors	7
	ОК	Cancel

Note: the tick boxes next to the 'Run name control' which control whether vectors will be generated for each run.

Reviewing generation of alignment vectors

After applying **Automatic alignment** the number of vectors will be updated on the **Runs** panel and the vectors will appear (in blue) on the image.

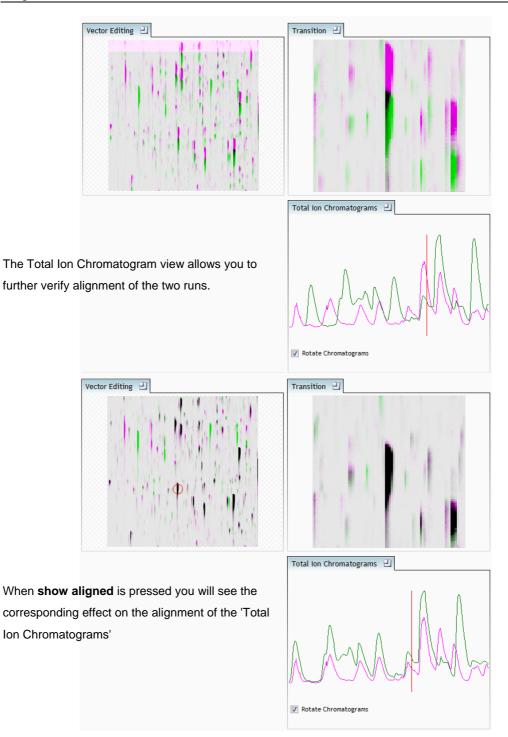
If the alignment has worked well then in Windows A and C the grid lines should show minimal distortion, Window B will show features pulsing slightly but not moving up and down.



At this point, you should check the automatically placed (blue) vectors. This will be easier with a larger grid size. Make sure the grid size is set to 4 using the '**Focus grid size**' control at the bottom left of the window.

In each square, you can, if required edit the vectors to improve the image alignment (for more information refer to section on Approach to Alignment page 11

Note: the orientation of the TIC view can be changed according to individual preference using the Rotate Chromatograms option



Stage 5: Filtering

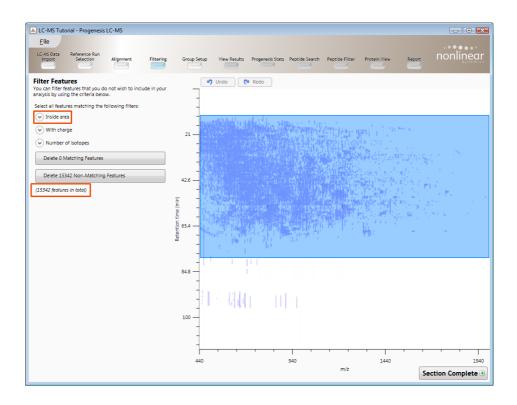
Now that you have reviewed your aligned Runs, you are ready to analyse them. Move to the **Filtering** stage, by either clicking on **Section Complete** (bottom right) or on Filtering on the workflow.



During the few minutes that the automatic analysis requires, a progress bar will appear telling you first that it is applying alignment to the Runs and then that it is Analysing.

	1	
Applying alignment to A2.		Analyzing

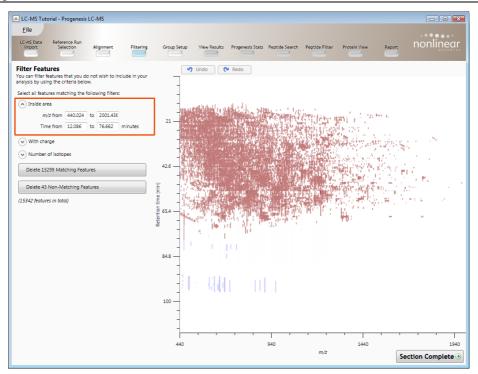
On completion of analysis the Filtering stage will open displaying the number of features detected in this example, 15342. If required you can remove features based on position, charge state, number of isotopes or combinations of these feature properties.



For example, to delete features with early and late 'Retention times' drag out an area as shown.

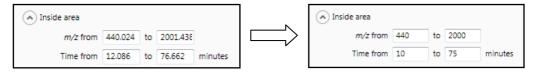
All features contained within the mask will be selected.

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As you release the mouse button the ranges for the masked area will appear on the top left

Note: the limits can be adjusted by entering the required values in the boxes

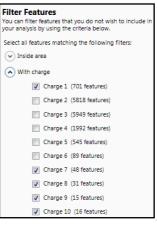


To remove the (in this case 47) features outside of the selected area, press the **Delete 47 Non-Matching Features** button

In addition to setting limits for 'Retention time and m/z', features can also be selected on the basis of charge or the number of isotopes present. Thus allowing you to refine the selection through a combination of feature properties

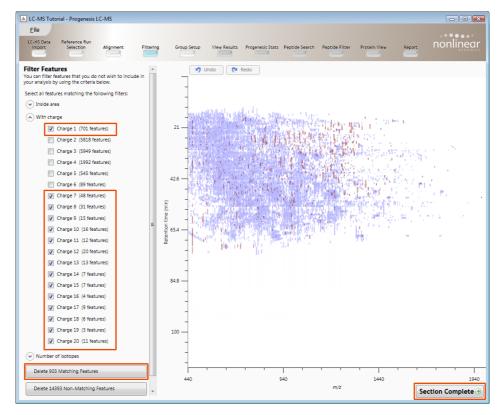
For example: when charge state is selected the number of features present at each charge state is displayed, these can be selected accordingly.

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



For this tutorial, we will filter the area as shown and remove features with a charge state of 1 and 7 and above.

We will now delete a further 903 features with a charge state of 1 and 7 and above by ticking the various options.



Hence all features with a charge state of 1 and 7 and above will appear red (see above).

To remove these features press Delete 903 Matching Features

You can use the **Undo** button to bring back deleted features, however, when you move to the next section you will lose the capacity to undo the filter.

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

Stage 6: Group Setup for Analysed LC-MS runs

At this stage in the workflow you can setup one or more groupings of your sample data.

For this example, group the analysed LC-MS runs to reflect the Biological groupings in the original study. This tutorial contains 2 groups: A and C, with 3 replicates each.

LC-MS Tutorial - Progenesis LC-MS		
Eile LC-WS Data Reference Run Import Selection Alignment Piltering Group set	up View Results Progenesis Stats Peptide Search Peptide Filter Protein View	
Groups AC Group your runs into the groups you wish to compare (e.g. Control runs vs. Treatment runs) You can create more sets of groupings (e.g. adding Male vs. Female), which you can choose between when doing comparisons.	Add Selected Runs to Group P 01 Add to new group 02	
A Delete A1 Remove A2 Remove A3 Remove Add group		
		Section Complete ④

Creating a group

- 1. Click on all of the runs you wish to include in a group
- 2. Press the 'black triangle' next to the **Add Selected Runs to Group** button on the main toolbar.
- 3. Select Add to new group... from the drop down menu.
- 4. A new group will appear in the Groups pane on the left panel
- 5. Rename the group by over typing the new group name (e.g. A)
- 6. Repeat steps 1 to 5 until all the Runs are grouped.

In the example shown the grouping has been renamed "AC" using the rename button

To create another Group Setup, for example comparing only 2 replicates for A and C groups, click on Create a new group setup (see right).

	_		
AC •	b	aje	$\left[\times\right]$
	AC 🗸	AC 🔹 🛅	

Give it a new name (i.e. AC_2). The Runs will reappear in the main window. Create the new groups as described above.

	nesis LC-MS					00
Eile LC-45 Seta Anteresce Run Selaction	Aligonest ritlering Grou	Setup View Results	Progenesis Stato Peptide Search	Reptide Mitter Pro	tain View Repor	, nonlinea
Groups AC_2 Group your AC Control run AC_2	ompare (e.g.	Runs Add Se	lected Runs to Group •	۹		
Female), which you can choo	groupings (e.g. adding Male vs. se between when doing	œ				
comparisons.		-				
	Delete	1				
	A1 Remove]				
]				
٨	A1 Remove					
A C	A1 Bessive A2 Bessive					
٨	A1 Bessure A2 Bessure Delete					
٨	A1 Instant A2 Instant Delete C1 Instant					

Note: the **Group set up** drop down will now contain both setups and the ungrouped data files (A3 and C3) will remain in the main window.

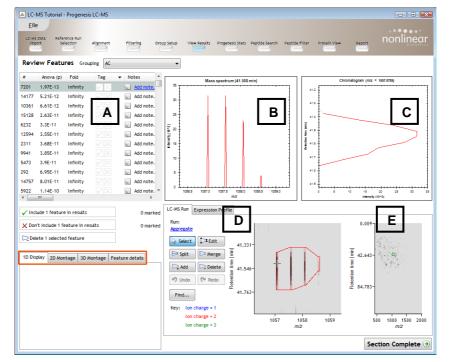
Switch back to the AC Group Setup.

Then move to the next stage in the workflow by clicking on Section Complete.

Stage 7: Validation, review and editing of results

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

The review stage has 4 display modes: 1D, 2D, 3D and Feature Details controlled by the tabs on



the bottom left of the display. Each display has multiple views to allow comparative exploration of the detected features on the aligned LC-MS runs.

Exploring analysed data using the Data displays

The 1D Display

Window A: shows the list of features 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'

To include a feature in the selection for the next section of the analysis, click on the **Include features in results** button at the bottom of the table. On clicking the button it will move on to the next feature on the list.

To select a group of features drag out a selection on the table and click

on the Include feature in results button (see right)

Window B: displays the Mass spectrum for the current feature on the selected Run (in window D).

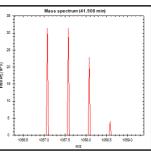
Window C: displays the Chromatogram for the current feature on the selected Run (in window D).

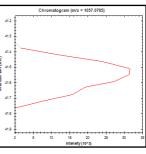
Window D: displays the details of the currently selected run. By default the selected run is an Aggregate of all the aligned runs.

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

The feature editing tools are located in this window (see page 27 for functional explanation).

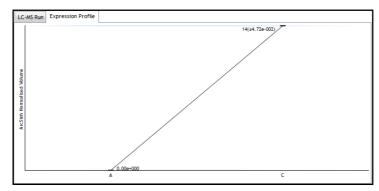
7201 14179	1.97E-13								
		Infinity	\checkmark \times	Add note.					
	4.31E-12	Infinity	\checkmark \times	Add note.					
10365	6.84E-12	Infinity	\checkmark \times	Add note.					
443	9.57E-12	Infinity	\checkmark \times	Add note.					
14762	2.15E-11	Infinity	\checkmark \times	Add note.					
15129	2.37E-11	Infinity	\checkmark \times	Add note.					
6240	3.32E-11	Infinity	\checkmark \times	Add note.					
12594	3.62E-11	Infinity	\checkmark \times	Add note.					
5479	3.78E-11	Infinity	\checkmark \times	Add note.					
9945	3.91E-11	Infinity	\checkmark \times	Add note.					
2310	6.84E-11	Infinity	\checkmark \times	Add note.					
5926	1.12E-10	Infinity	\checkmark \times	Add note.					
6790	1.21E-10	Infinity	\checkmark \times	📃 Add note. 🖕					
•	111			+					
✓ Include 1 feature in results 14393 market									





Run: Aggregate		
Seler	Aggregate	-
	A1	
🗁 Split	A2	
Add 🟳	A3	4
1) Und	C1	
	C2	8
Find	C3	
Kew lon ch	arge - 1	

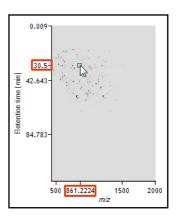
Clicking on the Expression Profile tab in Window D shows the comparative behaviour of the feature across the various biological groups based on group average normalised volume. The error bars show +/-3 standard errors.



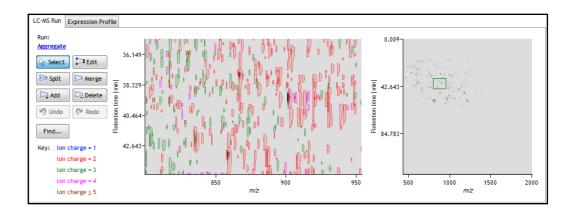
Window E: shows where the current feature is located on the LC-MS run by means of the 'Green' rectangle.

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).

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



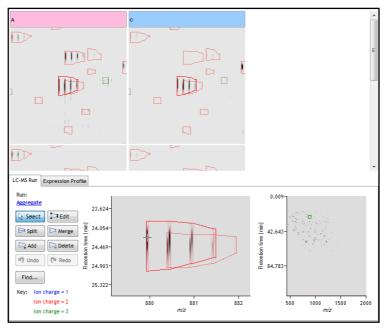
The 2D Display

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

In the 2D Montage mode, Window B displays a montage of the current feature across all the aligned LC-MS

LC-MS Tute	rial - Progenesi	s LC-MS		
Eile LC-MS Data	Reference Run			
Import	Selection	Alignment	Filtering	Group Setup View Results Progenesis Stats Peptide Search Peptide Filter Protein View Report nonlinear
Review Fe	atures Grou	ping AC		•
# Anova	(p) Fold	Tag	- Notes	A
1262 1.225		\checkmark \times	Add note.	Production of National In Market of Collegebra of
3327 1.46E		\checkmark \times	Add note.	
6562 1.55E-	10 Infinity	\checkmark \times	Add note.	말 것 같은 것 같아요. 이는 것 같아. 바람은 것 같은 가슴을 가지?
2767 1.75E-	10 Infinity	✓ ×	Add note.	tradi Deserve tradite <u>deserve de la constante de</u>
13100 1.99E-	10 Infinity	\checkmark \times	Add rote.	A HERE AN A REPORT OF THE A REPORT OF THE ANALYSIS AND A REPORT OF
3477 2E-10	Infinity	\checkmark \times	Add rote.	the of the second of the B in collection in the second of the
6780 2.03E	10 Infinity	\checkmark \times	Add rote.	
6527 2.16E	10 Infinity	\checkmark \times	Add note.	Design of the second
8982 2.44E	10 Infinity	\checkmark \times	Add note.	
7799 2.77E-	10 Infinity	\checkmark \times	Add note.	
7026 3.23E-	10 Infinity	\checkmark \times	Add note.	All to the second
10095 3.27E-	10 Infinity	\checkmark ×	Add note.	and all more than a second sec
8979 3.8E-1	0 Infinity	\checkmark \times	📃 Add note. 🛫	
<			,	
✓ Include 1 fe	ature in results		14393 marked	LC-MS Run Expression Profile
× Don't includ	e 1 feature in re	sults	0 marked	Run: Aearceate
Delete 1 se	ected feature			5.624
				Discont Discontene
				Add Dente g 24,654
1D Display 2	Montage 3D N	iontage Feat	ture details	Add Delete g
Show all ou	tines			17 Undo C1 Redo 8 24.469-
V Multiple co	umns per group	1		Adds Description g 24,654 g 44,640 g 44,783 g 44,640 g 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44,783 44
Contrast:				Key: Ion charge = 1 24,903
0				Ion charge = 2
			•	Ion charge + 3
Montage size:				Ion Charge - 4 764.5 765 765.5 766 500 1000 1500 2000 Ion charge 2.5 m2 m2
0 -		0	•	
				Section Complete 🕘

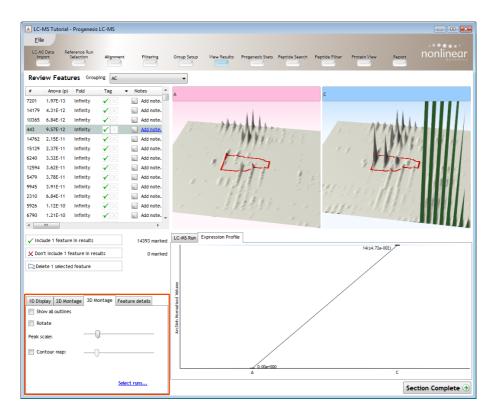
The appearance of the Montage (window B) is controlled by the panel on the bottom left of the display.



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

The 3D Display

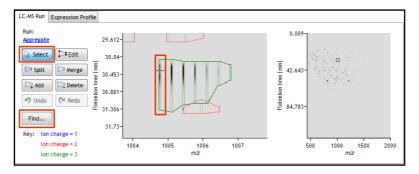
Window B changes into a 3D view by selecting the 3D Montage tab on the bottom left of the display.



The number of 3D views displayed in the montage is controlled using the Select runs link on the 3D Montage tab. The images can be set to **Rotate** automatically or you can rotate them manually by clicking and dragging them with the mouse.

Editing of features in the View Results stage

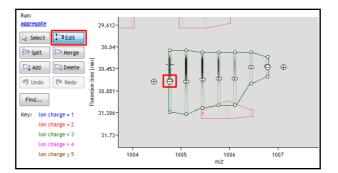
As an example of using the editing tools which are located on the left of the LC-MS Run view, we will remove and add back the 'monoisotopic peak' for the detected feature selected below. A feature can be selected from the 'Features' list or located using the various image views .



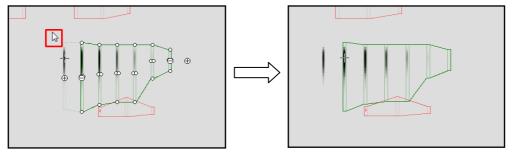
1. Locate the feature at approx 1005 m/z and 30.453 min using the Find tool.

Find specified location				X
Mass		1005.0000	<i>➡ m/z</i>	
Retention time on:	Alignment reference	30.453	🚔 minutes	
or	select a sample	30.453	minutes	
			Go	

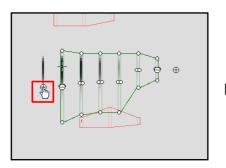
2. Select the Edit tool and click on the feature to reveal the 'edit handles'

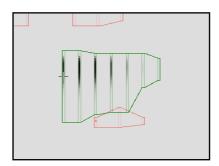


- 3. Click on the 'minus' handle over the monoisotopic peak to remove it.
- 4. Click outside the boundary of the feature to update the view.



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





- 6. Click on the 'plus' handle on the peak to add it.
- 7. Then click outside the feature to update the view.
- 8. Note: If you are not satisfied with the editing use the Undo button and retry.

Revi	ew Featu	res Gro	uping AC		
# 🔺	Anova (p)	Fold	Tag	▼ Notes	^
180	0.548	1.15	\checkmark \times	Add note	. —
181	0.00296	1.95	\checkmark \times	🔬 Add note	
182	0.00014	4.36	\checkmark \times	Add note	
183	0.938	1.03	✓ ×	Add note	
184	0.00227	399	< X	Add note	

9. Finally note: that a tag is automatically added to the edited feature in the table and the features 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 features for Progenesis Stats

There are a number of ways to 'refine' your 'Ranked List' of analysed features before examining them with the Statistical tools in Progenesis Stats. These make use of simple 'Selection' and 'Tagging' tools that can be applied to the various Groupings created in Stage 6 (page 21). An example is described below.

Revi	iew Featu	res Grou	ping AC	
	Anova (p)	Fold	Tag	▼ Notes ▲
6612	3.3E-13	Infinity	\checkmark ×	Add note.
7765	8.87E-13	Infinity	\checkmark \times	Add note.
3680	1.4E-12	Infinity	\checkmark \times	Add note.
2851	1.85E-12	Infinity	\checkmark \times	Add note.
1447	3.11E-12	Infinity	\checkmark ×	Add note.
4173	3.31E-12	Infinity	\checkmark ×	Add note.
2089	3.89E-12	Infinity	\checkmark \times	Add note.
263	4.49E-12	Infinity	\checkmark ×	Add note.
3217	6.76E-12	Infinity	\checkmark \times	Add note.
7088	6.9E-12	Infinity	\checkmark \times	Add note.
1280	8.09E-12	Infinity	\checkmark ×	Add note.
6693	1.48E-11	Infinity	\checkmark ×	Add note.
5412	1.64E-11	Infinity	\checkmark ×	🔬 Add note. 🚽
•	m			÷
🗸 Inc	lude 1 feature	e in results		8550 matched
X Do	n't include 1 fe	ature in re:	sults	0 matched

First expand the 'Features' table to show all the details by clicking on the 'Feature details' tab on the bottom left of View Results. Then order on Abundance and select the top 4000 features

Ei	15 Data Re	ference Run Selection	Alignmen	C Filtering	Group Setup 11e	w Results	Progenesis St.	ats Peptide Se	arch Peptide F	filter Protein 1	llew Rep	nonlin	ea
Rev	iew Featu	ires Group	ing AC		•								
*	Anova (p)	Fold	Tag	 Notes 	m/z	z	Mass	RT (mins)	RT window	Abundance	Intensity	MS/MS Protein	
	0.247	1.15		Add note	805.441	3	2413.301	54.743	4.9	1.14E+08	1.08E+08	100	
	0.87	1.01		Add note	1207.6552	2	2413.296	54.76	3.75	7.82E+07	5.04E+07	77	
1	1.7E-07	5-35E+04		Add note	1100,5863	з	3298.737	44.787	1.94	6.33E+07	7.87E+07	18	
	3.74E-05	2.85E+03		Add note	1176.2272	3	3525.66	47.91	3.37	6.13E+07	2.22E+07	31	
	6.71E-07	1.26E+03		Add note	656.8613	2	1311.708	43.853	2.95	5.92E+07	1.17E+08	29	
	4.82E-08	1.78E+03		Add note	988.9849	2	1975.955	50.538	2.64	5.4E+07	9.19E+07	30	
	0.0729	1.5		Add note	763.4082	3	2287.203	43.151	2.59	4.88E+07	7.86E+07	50	
	6.77E-06	4.74E+03		Add note	1061.0072	2	2120	53.397	4.27	4.528+07	2.52E+07	44	
	6.985-07	121		Add note	663,8693	2	1325.724	46.65	3.27	4.24E+07	1.69E+08	49	
	1.62E-07	1.17E+03		Add note	997.4478	2	1992.881	31.606	2.51	4.21E+07	3.81E+07	26	
)	7.09E-06	7.07E+03	V X	Add note	1032.4669	3	3094.379	32.837	1.9	3.95E+07	3.95E+07	18	
	0.317	1.18	V X	Add note	753.8284	2	1505.642	30.419	2.58	3.41E+07	8.23E+07	54	
	1.572-05	3.37E+04		Add note	774.6028	4	3094.382	32.793	1.86	3.23E+07	4.95+07	19	
< Do	clude 1 featur n't include 1 f Nete 1 selecte Splay 2D Mor	eature in res		0 marked 0 marked	Elli Spét	Edit Merge Delete Redo	31.306 32.135- (au) 0.45 33.015- 33.917-			j,	0.000 (mi) (mi) (mi) (mi) (mi) (mi) (mi) (mi)		
					Key: Ion charge Ion charge Ion charge Ion charge Ion charge	- 3	34.8-	1032 10	133 1034 m2	1035 1036		500 1000 1500 m2	20

With the 4000 features still highlighted right click on them and select 'New Tag'

		Reference Run Selection	Algement	Filtering	Group Setup	Vie	w Results	Progenesis Stats	Peptide Search	Peptide Filte	Protein	ópr	Report	non	linear
Revie	ew Featu	ires Grouping	ĸ		•										
	Anova (p)	Fold Ta	ng • No	tes	m/z	z	Mass	RT (mins)	RT window	Abundance	Intensity	MS/MS	Protein	Peptide Score	Peptide
2757	0.000221	45.5	X I	Add.note	613.3048	2	1224.595	24.857	0.678	4.552-04	5.05E+05	6			
5450	0.00807	2.92		Add note	#50.1033	3	2547.288	26.589	0.773	4.555-04	1.08E+05	0			
3122	0.0894	4.02		Allouten	\$81.3031	3	1740.887	15.278	1.89	4.558+04	1.58E+05	17			
5536	0.0199	5.99	18 90	Allenten	883,7081	4	3530.803	49.825	0.699	4.552-04	1.555-05	0			
4527	0.322	1,43	1× 11	Altratem	1133.072	2	2254.129	53.929	0.716	4.55E+04	1.55E+05	0			
3680	0.000992	59.4		Add.note	734.0079	3	2199.002	54.537	0,391	4.54E+04	2.17E+05	0			
1975	0.00211	97.7		Add note	668.64	3	2002.898	36.537	0.603	4.545+04	2.83E+05	5			
\$\$77	0.003	No tags to essi	pai 🖬	Ant note	789.71	3	2366.108	55.473	0.917	4.546-04	1.062-05	1			
9896	0.025	New tag_		Altoute	1360.8788	5	6799.358	54.025	0.679	4.540-04	7.875+04	0			
1609	0.002 9	Edit tags	1	Add mote	711.3412	2	1420.668	32.183	0.733	4.536-04	1.31E-05	2			
5428	0.00351	3.41	100	Add note	961.978	2	1921.941	45.333	0.869	4.532+04	1.47E+05	0			
5225	0.0244	3.68	100 100	Add note	645.2938	4	2577.146	56.174	1.04	4.535+04	9.985+04				
5151	0.0041	1.976-03	N 10	Add note	952.9798	2	1903.945	42.886	0,458	4.53E+04	1.296-05	3			
3385	0.732	1.32	1	Add note	694.5409	4	2774.134	51,798	0.84	4.53E+04	1.62E+05	0			
6367	0.0171	235	1X 🖬	Add note	1046.8008	1	3137.381	40.936	0.578	4.53E+04	1.31E+05	1			
1241	0.596	1.08	1X 🖬	Add note	458.5924	3	1372.755	29,492	0.779	4.532-04	6.552+05	8			
3139	0.0551	1.45	(i) (i)	Add note	758.9216	1	1515,829	39.819	0.601	4.530+04	2.07E+05	5			- 6
V Inch	ude 4000 fer	itures in results	1	0 marked	C-MS Run Expressio	an Pro	fle					_	72525		
× Don	t include 40	00 features in res	uits.	0 marked	Pun: Accreate		51.40	Tail		2		8 ⁽	0.009		
C: Dek	rte 4000 sek	ected features			Select Date		53.651 F		114			10	2.60- 0		
1D Disp	say 20 Mo	otage 30 Monta	pe Feature de	etals	*) Unda (* Re		55.407				# >	whice time [8	
					Find Key: Ion charge - Ion charge -	1	a 0.201					2,	4.783-		
					Ion charge + Ion charge + Ion charge a	4		805	804	807 808 12	201	-	580	1000 1500 miz	2000
				-											nplete

Give the Tag a name. i.e. '4000 most abundant'.

Create new tag		X
4000 most abundant		
	ОК	Cancel
Des desse Frankrig		

#	Anova (p)	Fold	Tag	 Notes
2757	0.000221	95.5	< X 🗖	Add note
5450	0.00807	2.92	🗸 🗙 📒	Add note
3122	0.0894	4.02	🗸 🗙 📒	Add note
5536	0.0199	5.99	🗸 🗙 📒	Add note
4527	0.322	1.43	- 🗸 🗙 📒	Add note
3680	0.000992	59.4	🗸 🗙 📒	Add note
3975	0.00311	92.7	- 🗸 🗙 📒	Add note
5577	0.00321	2.25	🗸 🗙 📒	Add note
9896	0.0253	163	🗸 🗙 📒	Add note
3609	0.00253	3.07	🗸 🗙 📒	Add note
5426	0.00351	3.41		Add note
5225	0.0244	3.68		Add note
5151	0.0041	1.97E+03		Add note
3385	0.732	1.32		Add note
6367	0.0171	235		Add note
1241	0.596	1.08		Add note
3139	0.0551	1.65	V X	Add note
•				

Review Features Grouping AC Anova (p) Fold Notes Tag 0.0495 2.05 Add note. 86 7726 0.0495 3 Add note. 0.0495 Add note 5403 12.5 3.58 0.0495 3196 Add note. 6045 0.0495 13.1 Add note. 8055 0.049 😑 4000 most abundant Add note. 4970 0.049 Add note. New tag... Add note. 11545 0.049 1 Edit tags 8297 0.049 Add note. 15009 0.0497 4.43 Add note. 11806 0.0497 2.5 Add note. 1402 0.0499 1.91 Add note. 6409 0.0499 1.61 Add note. 10280 0.05 31.3 Add note. 7332 0.0501 2.25 Add note. 10147 0.0501 4.37 Add note.. 15233 0.0501 4.24 Add note.. ✓ Include 8375 features in results 0 marke X Don't include 8375 features in results 0 marke

Edit Tags
Create a new tag
Remove tag from experiment
Active Tag

Active Tag

Significant p<0.05

OK Cancel

Now re-order the table based on Anova p value and highlight all

On clicking **OK** the Tag is added to the features highlighted in

the table (signified by a coloured square).

Right click and call the new tag Significant p<0.05

values less than 0.05.

Create new tag				X
Significa	nt p<0.05			
	C	ОК	Cance	el 🚽

To delete and/or create additional Tags click on **Edit tags** and Create/Remove Tags as required.

		Revi	ew Featu	res Group	AC AC	
		#	Anova (p)	Fold	Tag 🔻	Notes
		7201	1.97E-13	Infinity		Add note
		14179	4.31E-12	Infinity		Add note
		10365	6.84E-12	Infinity	X	Add note
Now coloct all the features currently display	ad in the table	443	9.57E-12	Infinity	 × 	Add note
Now select all the features currently display		14762	2.15E-11	Infinity	 × 	Add note
click on a feature and press Ctrl_A. Then r		15129	2.37E-11	Infinity	 X 	Add note
the features are ticked by pressing Include features in results	14393	6240	3.32E-11	Infinity	 × 	Add note
leatures in results		12594	3.62E-11	Infinity	 ✓ × ■ 	Add note
		5479	3.78E-11	Infinity	 ✓ × ■ 	Add note
		9945	3.91E-11	Infinity	✓ × ■	Add note
		2310	6.84E-11	Infinity	 × 	Add note
		5926	1.12E-10	Infinity	 × 	Add note
		6790	1.21E-10	Infinity	✓ × ■	Add note
		1262	1.22E-10	Infinity	 ✓ × ■ 	Add note
		3327	1.46E-10	Infinity	 ✓ × ■ 	Add note
		6562	1.55E-10	Infinity	✓ × ■	Add note
		2767	1.75E-10	Infinity	 ✓ × ■ 	Add note
		13100	1.99E-10	Infinity	X	Add note
		•				
		√ Inc	lude 14393 fe	14393 marked		
		<u> </u>				
		X Dor	n't include 143	393 features	in results	0 marked
	# Anova (p) Fold	Tag	▼ Note:		m/z	z Mass
To view the Tags and also control the	7201 1.97E-13 Infinity	 Image: A second s	📲 🍋 Show	all 🛛 🔐 Edit ta	igs	2112.143
number of features displayed in the	14179 4.31E-12 Infinity	< ≥		4000 most	abundant (4000	features) 2001.181
table, click on the drop down selection	10365 6.84E-12 Infinity	< ≥		Significant	p<0.05 (8375 fe	atures) 1880.312

on the right of the Tag column header.

#	Anova (p)	Fold	Tag	 Notes 	m/z	z	Mass
7201	1.97E-13	Infinity	 ✓ × [🐁 Show all 🛛 🖀 Edit	tags		2112.143
14179	4.31E-12	Infinity	< ×	🖌 🖂 📒 4000 mo	st abundant (4000 f	eatures)	2001.181
10365	6.84E-12	Infinity	< ×	🔽 🔲 Significar	nt p<0.05 (8375 fea	tures)	1880.312
443	9.57E-12	Infinity	< ×	No tags a	assigned (4810 feat	ures)	1541.913
14762	2.15E-11	Infinity	< ×[Cancel	1436.824
15129	2.37E-11	Infinity	< ×		ОК	Cancel	2242.991

Now tick the tag for the 4000 most abundant features

To move to the next stage in the workflow, Progenesis Stats, click Section Complete.

Stage 8: Multivariate Statistics on Selected Features

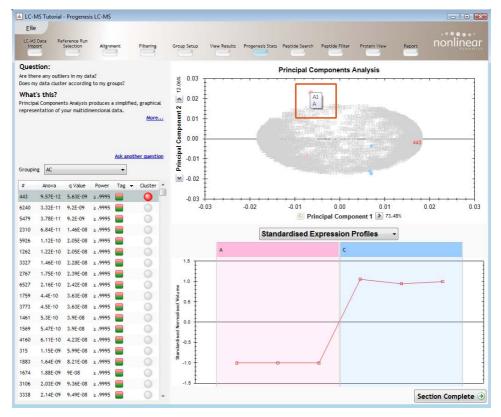
The tutorial displays the functionality of the Multivariate Statistics. This section is only available if Progenesis Stats is licensed.

Progenesis Stats opens calculating the Principal Components Analysis (PCA) for the active 'tag' in this case the 4000 most abundant features.



For this tutorial we will start by examining the behaviour of the **4000 most abundant** features from the previous stage, **View Results**.

The statistical analysis of the selected data is presented to you in the form of interactive graphical representations 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 **Progenesis Stats** the first statistically based question asked of the data takes the form of a Quality Control assessment:

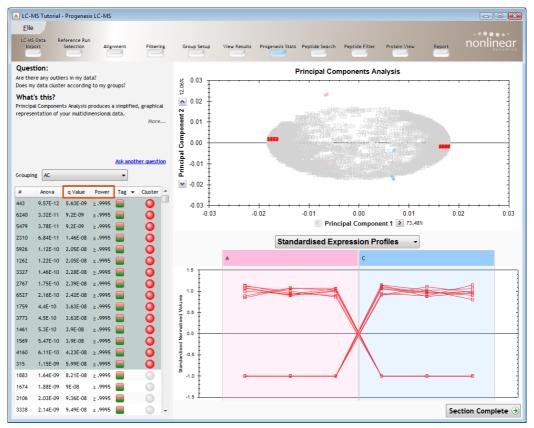
Are there any outliers in my data? And does my data cluster according to my groups?

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 groupings and conclusions can be drawn regarding possible outliers in your data.

Selecting spots in the table will highlight the spots 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 feature'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 feature, 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 4 (page 54)

Are there any outliers in my data? Does my data cluster according to my groups? Group my features according to how similar

their expression profiles are. How many replicates should I run? What is the power of my experiment?

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Correlation Analysis

Use the tags created in View Results to filter the features in the table. We are going to explore the Correlation Analysis for all the features that were tagged at the view results stage for having an Anova p<0.05.

				<u>Ask a</u>	nother question
Grouping	AC			•	ā
#	Anova	q Value	Power	Tag	✓ Cluster ▲
7201	1.97E-13	4.63E-10	≥.9995		Show all 🔮 Edit tags
14179	4.31E-12	5.07E-09	≥.9995		🕢 🖂 📒 4000 most abundant (4000 features)
10365	6.84E-12	5.36E-09	≥.9995		🗹 📃 🔳 Significant p<0.05 (8375 features)
443	9.57E-12	5.63E-09	≥.9995		No tags assigned (0 features)
14762	2.15E-11	9.2E-09	≥.9995		OK Cancel
15129	2.37E-11	9.2E-09	≥.9995		

On pressing OK the PCA will recalculate using these 8375 features, you can (to save time) stop this calculation by pressing **Cancel calculation** and then set up Correlation Analysis for the 8375 features.

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

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

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



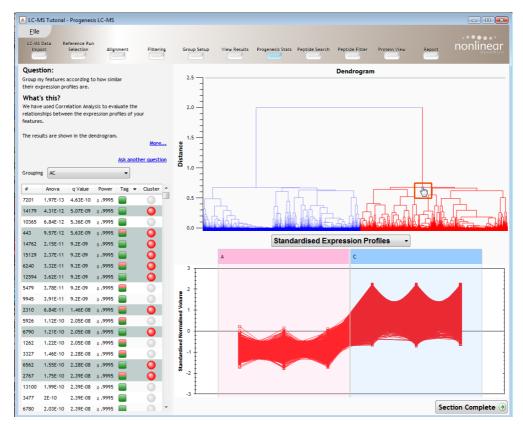
This time the statistically based question(s) being asked is:

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

The question is answered by:

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

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



To highlight all the features demonstrating **Increased expression in the C** group click on a 'node' for a branch of the Dendrogram (as shown above). As before create a Tag for these features.

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

Create new tag

Also create a tag for those features showing **Increased expression in A** by first clicking on the other 'main' node then right click on the highlighted features in the table and creating the New tag

Create new tag		.
Increased expression in A		
C	OK	Cancel

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

Stage 9: Peptide Search

Determining protein ID 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 dependent 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.

EN	100	ial - Progenesis LC	S-MS															
in the	port	Selection	Aligre	ment	Filtering	Group 5	lebup	View	Results	Progene	sis Stats Pept	ide Search F	eptide Filter	Protein View	Res	port	non	linear
Feat	ures					MS/MS S	pectr	ra								11		
	MS/MS	- Proteins	Score	Tags		Batch in	clusion	options f	for creati	ng MS/MS	query set							
2572	176	0	0	1	Show all	P Edit ta	105			h.	ber Exported	Feature intens	ity Precursor int	ensity (%)	Charge	Precursor m/z	Isotone	ld score
3714	67	0	0	V		4000 most					No	1.4++008	7 Se+007	55.8	2	656.8621	1	
1717	50	0	0			4000 most					No	9.3e+007	5.0e+007	53.5	2	656,8614	1	
10	49	0	0	1			_			ures)	No	1.4++008	3.3e+007	23.6	2	656.8621	1	
14	46	0	0	1		Significant					No	1.2e+008	2 5e+007	21.2	2	656.8630	1	
14	44	0	0			Increased				ures)	No	9.3e+007	1.7e+007	18.8	2	656.8613	1	
		0	0			No tags as	isigned	(4810 feat	tures)		No	1.4+008	1.6++007	11.2	2	656,8621	1	
17	42	0			-			OK	Car	ncel	No	1.2e+008	1.3e+007	10.9	2	656.8624	1	
25	42		0	*		1	12	9	A2	5103	No	1.4e+008	1.2e+007	8.6	2	656.8614	1	
1	42	0	0	*		1	13	9	A3	5210	No	9.3e+007	7.4e+006	8.0	2	656.8612		
660	41	0	0	~	.	N.	14	9	AZ	5312	No	1.4e+008	7.6e+006	5.5	2	656.8622		
9	38	0	0	*	_	1	15	9	A1	5175	No	12e+008	5.8e+006	49	2	656 8629	1	
5	37	0	0	1	S	1	16	9	A2	5354	No	1.4+008	4 3e+006	3.1	2	656.8621		
46	37	0	0	~		N.	17	9	AI	5217	No	1.2e+008	3.1e+006	2.6	2	656.8633		
48	37	0	0	1		1	18	9	A3	5292	No	9.3e+007	2.0e+005	2.2	2	656.8618		
79	37	0	0	× .		N N	19	9	A2	5392	No	1.4e+008	2.8e+006	20	2	656.8618		
1	37	0	0	× .			20	9	A1	5299	No	1.2e+008	1.9e+006	1.6	2	656.8617	÷.	
61	36	0	0	v .		N.	21	9	A2	5433	No	1.4e+008	2.2e+006	1.6	2	656.8612		
3	35	0	0	1		2	22	9	A3	5331	No	9.3#+007	1.2e+005	12	2	656.8613	1	
	34	0	0	1		1	23	9	A1	4966	No	1.2e+008	1.4e+006	12	-	656.8616	-	
24	34	0	0	1		a la	23	9	47	4200		1.4e+008	1.44+006	10	2	656 8613	1	
21	34	0	0	1	- ũ. I	1	. /4	3	A.	2011	No	146+1121	1.46+0.0	10	-	POP RELS	1	
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tow d		ms/ms ion search in current query se	_	ra		Run:A1	Scan r				30000	-						
Masco	xt					Juni) 044 787		(Q)	TT I	1	≥ 20000							
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	Import	t search results				Retention					10000	-			1 ii			
MSM	6 Preproce	essing				47.079	-						1. 1.					
-	1000	ment ion count 10		*			656	657	658 68 m2	59 660	- 0	0	50	0	and and a	1000		-
8	/ Deisoto	oping and charge de	econvolut	ion										m	κ.			
																Sec	tion Co	mplete

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

The query set can be searched using all the spectra, however the query 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.

Filter the table to show only the features tagged Significant p<0.05 as shown.

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

The total number of spectra included in the query set for this data set is 20873

Before exporting peak lists, the query set can be further refined.

Note: many features have a large number of spectra associated with them.

To control the number of spectra for each feature, expand the **Batch inclusion options** to reduce the size of the query set.

Progenesis	LC-MS	Tutorial
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Eile																	
		al - Progenesis L(2-1115														
LC-N	a AS Data Aport	Reference Run Selection	Alignr	nent	Filtering	Group S	etup	View Results	Progenesi:	s Stats Pept	tide Search	Peptide Filter	Protein Vie	w	Report	n	onlinear
Feat	ures					MS/MS S	pectra				_	_	_		_		
#	MS/MS	 Proteins 	Score	Tags	- N ^			tions for creati	ng MS/MS q	uery set							
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3714	67	0	0	✓ ×				greater an		10				1000 1101	-		
1717	50	0	0	 X 			Feature I	D less than	•			Precur	sor intensity	less than	•		
10	49	0	0	VX			~										
34	46	0	0	\checkmark \times			Charg	less than	•			Precursor	ntensity (%)	less than	•		
54	44	0	0	 X 		s	can numb	er less than	•				Run name	contains	•		
27	42	0	0	\checkmark \times													
125	42	0	0	 ✓ × 			Exporte	equal to	•		•	Peptid	e sequence	contains	•		
91	42	0	0	< ×	<u></u>		Isotop					Dentei	n accession				
6660	41	0	0	 ✓ × 			isotop	less than	•			Frotei	n accession	contains	•		
29	38	0	0	< X			ID scor	e less than	•			Protein	description	contains	•		
18	37	0	0														
546	37	0	0										Include i	in export	Exclude from	export	Clear all filters
148 179	37 37	0	0														
41	37	0	0			Export			Scan numb	- · · ·					rge Precursor		pe Id score
161	36	0	0				23 9 24 9		4966 5511	No No	1.2e+008 1.4e+008	1.4e+006 1.4e+006	1.2	2	656.8616 656.8613	1	
13	35	0	0				24 9		5042	No	9.3e+007	8.8e+005	1.0	2	656.8614	1	
78	34	0	0				26 9		5379	No	1.2e+008	1.0e+006	0.9	2	656.8610	i	
124	34	0	0				27 9		5374	No	9.3e+007	7.7e+005	0.8	2	656.8615	1	-
371	34	0	0	VX	-	4											•
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		ms/ms ion search				Run:A1	Scan nun	nber:4966									
		n current query se		5		42.643	_			30000	-						
Masco	ot		•											1			
	Export co	urrent query set				1 44.787	- 4		7	All 20000	-						
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	5 Preproce	-				47.079				o		Iliadad	. Late			J.	
V L	imit fragm.	ent ion count 10	000	*			656 6	57 658 65	9 660	U	0		500		1000		
5	/ Deisoto	ping and charge d	econvolut	ion				m/z						m/z			
																	Complete 🦻

For example: We will make use of the 'Rank' value to reduce the number of Spectra being used for each feature in the query set to a maximum of 10.

The 'Rank' of each MS/MS spectra is determined by comparing it's % value against all other spectra matched to the same feature.

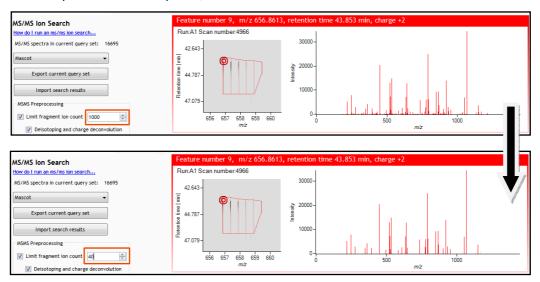
Export	Ran	c #	Run	Scan number	Exported	Feature intensity	Precursor intensity	(%)	Charge	Precursor m/z	Isotope Id so	core
	23 (0	4.1	4000	M-	1.0000	1.4000	10	1	050 0010		
	24	The rank	c of each	n MS/MS spect	rum found	by comparing it	s '%' values against	all other:	spectra n	natched to the	same feature.	
	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	
(•

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

Set the Rank filter to 'greater than' 10 and click **Exclude from export**. This will reduce the query set to 16695.

Limiting the 'fragment ion count' (FIC) for the spectra being used 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.

For this tutorial we will limit the fragment count to 1000.

Performing an MS/MS Ion Search

Having filtered the query set to 16695 spectra 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 5 (a and b) (pages 56 and 57) for details of the 'Search Engine' parameters

Note: the blue link tells you the appropriate formats for exporting ID results

Note: an example Search Results file, from a MS/MS Ion search, is available in the folder you restored the Archive to (Protein search results_v2.5.xml). Select the 'Mascot' method and import this file to see results like those below.



On importing the Search results the Features table updates to reflect the identified proteins and the relevant score for each searched feature.

LC-	MS Tutori	al - Progenesis LC-	-MS																		•
Eil	e																				
	AS Data	Reference Run Selection	Aligne	ment	F	iltering	Group 5	etup	Vier	w Results	Progenesi	s Stats Pep	tide Search	Peptide Filter	Prote	in View	R	eport		onlir	near
Feat	ures		_				MS/MS S	pectra													
	MS/MS	 Proteins 	Score	Tags	-	Ν.	 Batch in 	clusion o	ption	s for creating	ng MS/MS q	uery set									
2572	176	2 gi 135391	141	¥×				R	ank	greater the	an -	10		Fe	sature inter	nsity (less than	•			
3714	67	1 gi1145953	43	🖌 🗸		6															
1717	50	1 gi 145953	96.2	\checkmark ×		5		Feature	D	less than	•			Pre	cursor inter	nsity (less than	•			
10	49	1 gi 145953	90.6	✓ ×				Cha	rce	less than	•			Precurs	or intensity	(2) 6	less than	-			
34	46	2 gi 135391		✓ ×		54				ress man	•					1.00	ess man	•			
54	44	1 gil 135391		 ✓ × 	-	10.	s	can num	ber	less than	•				Runin	ame [contains	•			
27	42	2 gi 190015		∢ ×		-															
125	42	1 gi 135391		X		ba.		Expor	ted	equal to	•		•	Pe	ctide seque	ence [contains	•			
91	42	5 gil114961	_	× ×	-			Isot		less than	•			Pro	tein acces	sion (contains	•			
6660	41	1 gi 135391 3 gi 135391		1	-					icaa man	•					1	concarna	•			
29	38 37	3 gi 135391	_	1	=			ID so	ore	less than	•			Prot	tein descrip	tion [contains	•			
546	37		0	1	-										_						
148	37	6 gi1114565		28		5									Inc	slude in	export	Exclude from	export	Clear a	ll filters
179	37	1 gil126697		1	-		E Court	Death		0.0	C	. Current	E						andra Librari		
41	37	1 gi 118443		1	5		Export	Rank 19	10	Run C2	5950	No No	Feature inter 1.9e+008	3.0e+00		(%)	2	e Precursor 663.8693	m/z isot	ope io	score
161	36	2 gi 126699		1	5			26	10	C2	5992	No	1.9e+008	1.9e+00		10.2	2	663.8695	- 1		
13	35	1 gil145953	89	VX	ī.			31	10	C2	6034	No	1.9e+008	1.1e+00		6.0	2	663.8692	1		
78	34	1 gi 135391	71.6	V X		-		35	10	C2	6075	No	1.9e+008	6.1e+00	6	3.2	2	663.8692	1		
124	34	0	0	V X				36	10	C2	6116	No	1.9e+008	4.2e+00	6	2.2	2	663.8690	1		-
371	34	0	0	V×		- 14	4]					
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Masc	x		-				11 9 44.787	1	φī	TTT		≥ 2000)_								
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In order to review, and refine the quality of the **Peptide Search** results click on the next stage in the workflow, **Peptide Filter**.

Stage 10: Peptide Filter

In this tutorial example the organism under study is Clostridium difficile

In this tutorial as an example Acceptance Criteria on which to base the sequential filtering of the Peptide results, the following thresholds can be applied:

- 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 Doesn't contain 'Clostridium difficile'

	Set the S	Score to les	s than 40.	, then	Delete matching	search result
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LC-MS Tutori	al - Progen	esis LC-N	15														
Eile																	
LC-MS Data Import	Reference Selection		Alignment	,	Filtering	Gro	up Setup	View Re	rsults P	rogenesi	s Stats	Peptide Search	Peptide Filter	Protein View	Report		nonlinea
Features				Pe	ptide S	earch	Results										
# Total Hit	ts m/z	RT(mins	Charge -		atch delet	ion option	ns										
9 10	656.86	43.85	2 #			Score	less than	-	40				Sequence	contains •			
10 9	663.87	45.66	2				less than	•	40					contains •			
11 8	595.32	36.58	2			Hits	less than						Accession	contains •			
13 5 14 30	573.80 573.32	24.10	2											(
15 30	573.32	40.32	2			Mass	less than	-					Description	contains •			
18 30	498.26	25.07	2														
20 10	988.98	50.54	2			Charge	less than	-					Modification	contains +			
22 6 23 19	614.35 601.83	19.27 40.12	2			M/Z	-										
27 17	601.83	41.29	2			MIL	less than	•									
28 12	1100.59		3		Retenti	on Time	less than										
29 23	484.26	24.05	2				tess unan	•							_		
11 10	600.98	39.13	3		Sequence	- Length	less than	-					Delete	matching search results	Dele	te non-matching search resi	uits Clear all filter
13 2 14 20	726.68 941.79	32.54 58.18	3										·		- 1		
38 11	997.45	31.61	2			Score	Hits	m/z	RT(mins)	Charge	Mass	Sequence	Accessio	n Modificatk	ons		Description
19 11	980,48	41.97	2		9	84.38	10	656.86	43.85	2	1311.7	VFFEGTLAS	TIF 🕥 gi 13539	182		5-layer protein [Clostridik	
1 10	814.93	24,18	2	V	10	90.58	9	663.87	46.66	2	1325.7	B IFFEGTLAST	NK 🕥 gi 14595	3274		hypothetical protein Cdiff	Q_04003257 [Clostridi
44 10	900.97	39.43	2		11	83.22	8	595.32	36.58	2	1188.6	LGDSDIIDITY	K 🕥 gi 13539	182		S-layer protein [Clostridit	am difficile]
54 10 52 10	1061.01 623.83	53.40 37.42	2		13	89.03	5	573.80	24.10	2	1145.5	GOSDTINL	AK 🕥 gi 14595	3274		hypothetical protein Cdiff	Q_04003257 [Clostridi
52 10 55 10	1176.23		5		14	77.04	10	573.32	41.51	2	1144.6	GILDGSITER	K 🕥 gi 13539	182		5-layer protein [Clostridik	am difficile]
56 10	760.90	39.03	2		14	29.74	10	573.32	41.51	2	1144.6	B SUGGLTVTL	.EK 💊 gi 16381	6188		hypothetical protein COP	EUT_02372 [Coprococo
70 10	557.64	42.07	3		14	77.04	10	573.32	41.51	2	1144.6	GLLDGSITEI	K 👔 gi 14595	3274		hypothetical protein Cdiff	Q_04003257 [Clostridi
77 1	1052.05		2		15	31.08	10	573.32	40.32	2	1144.6	8 🕥 EVGGLTVTL	.EK 💊 gi 16381	6188		hypothetical protein COP	EUT_02372 [Coprococo
78 9 84 30	882.42 749.73	47.89 52.30	4		15	73.33	10	573.32	40.32	2	1144.6	s 💿 gildgsiter	K 🛛 🎯 gi 13539	182		5-layer protein [Clostridik	um difficile]
17 10	1170.55		2	1	15	73.33	10	573.32	40.32	2	1144.6	s 🚳 GLLDGSITEI	K 🛛 🎯 gi 14595	3274		hypothetical protein Cdiff	Q_04003257 [Clostridi
18 20	976.48	52.06	3		18	24.61	10	498.26	25.07	2	994.51	S TOLLKPTK	📦 gi 16774	7123 [7] Phospho (ST)		hypothetical protein ANA	CAC_01836 [Anaerosti
91 50	668.38	20.03	2		18	61.72	10	498.26	25.07	2	994.51	TOUNTLYR	🌚 gi 14595	3274		hypothetical protein Cdiff	Q_04003257 [Clostridi
93 10	702.36	42.97	3	1		20.38	10	498.26	25.07	2	994.51	SLNN/KK	🕥 gi 12669			exonuclease subunit C [Cl	ostridium difficile 630
96 16 101 50	832.18 445.93	54.46 19.99	4			103.11	10	988.98	50.54	2	1975.9	-				hypothetical protein Cdiff	Q_04003257 [Clostridik
102 20	611.99	25.51	3	2	22	87.81	6	614.35	19.27	2	1226.6					S-layer protein [Clostridio	um difficile]
103 3	1017.49		2	1		23.84	10	601.83	40.12	2	1201.6	5 🕥 LNIDNVCVKI				sensor histidine kinase (B	acillus cereus)
105 -40	528.82	39.69	2		23	21.31	9	601.83	40.12	2	1201.6			(1)	ethyl (C)		
113 22	564.36	36.32	2			26.60	10	601.83	41.29	2	1201.6	-				sensor histidine kinase (B	
122 9 125 10	825.69 707.67	44.74 53.40	4		27	25.22	7	601.83	41.29	2	1201.6	5 🕥 NUNLEČAKIR	C 💊 gi 182.41	8612 [6] Carbamidom	ethyl (C)	transcriptional regulator,	AraC family [Clostridk
128 20	613.31	42.64	2														•
133 10	835.95	42.07	2 *	129	9 search r	esults. 59	8 matching	batch dele	rte option:	5.						C	tion Complete
(•	_						_						Sec	complete (

Note: the search results matching the filter criteria turn pink and the total is displayed at the bottom of the table (598 matching out of 1299)

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

Delete 59	98 search results?	23
?	Are you sure you want to permanently delete 598 peptide search results?	
	Yes No	

Now **Clear all filters** and then apply the next filter (Hits: less than 2) followed by the remaining two filters (page 39)

Διο	-MS Tutoria	al - Progen	esis LC-P	иs													
E	le																
	MS Data	Reference															nonlinear
	nport	Selectio	n	Alignment		Filterin	g Gro	up Setup	View Re	Isuits	Progenesi	s Stats	Peptide Search R	eptide Filter	Protein View R	eport	normineur
																_	
Feat							Search										
	Total Hit		RT(min		1	Batch de	letion optio	ns									
9	10	656.86	43.85	2 1			Score	less than	-					Sequence g	contains +		
10	0	663.87	46.66	2													
11 13	0	595.32 573.80	36.58 24.10	2			Hits	less than						Accession a	contains •		
14	10	\$73.32	41.51	2													
15	10	573.32	40.32	2			Mass	less than	•					Description e	doesn't contain 🔹	Clostridium difficile	
18	0	498.26	25.07	2													
20	0	988.98	50.54	2			Charge	less than						Modification	contains •		
22	6	614.35	19.27	2													
23	0	601.83 601.83	40.12 41.29	2			M/Z	less than	-								
28	0	1100.59	41.27	3													
29	9	484.26	24.05	2		Nece	ntion Time	less than	•								
31	0	600.98	39.13	3		Campa	nce Length		_	_		_		Delete ma	atching search results	Delete non-matching search	h results Clear all filters
33	0	726.68	32.54	3		and an	the senger	less than	•					Develop Inte	atomic search reserve	server marining server	Citie an Inters
34	10	941.79	58.18	3	L Č				1								Description
38 39	10 10	997.45 980.48	31.61 41.97	2			Score		m/z) Charge	Mass	Sequence VFFEGTLASTI	Accession	Modification		and a second sec
41	0	814.93	24.18	2		9	84.38	10	656.86	43.85	2	1311.71				S-layer protein [Clos	
44	ő	900.97	39.43	2		2 11	83.22	8	595.32	36.58	2	1188.62	LGDSDIIDITK	gi 1353918		5-layer protein [Clos	
54	10	1061.01		2	6		77.04	10	573.32	41.51	2	1144.63	GILDGSITEIK	🔮 gi 13539183		S-layer protein [Clos	
62	0	623.83	37.42	2		15	73.33	10	573.32	40.32	2	1144.63	-	gi 13539183		S-layer protein [Clos	
65	10	1176.23	47.91	3	6		87.81	6	614.35	19.27	2	1226.69		-		S-layer protein [Clos	
66	10	760.90	39.03	2		29	\$9.36	9	484.26	24.05	2	966.50	TOUNTLYK	gi 13539183		S-layer protein (Clos	
70	10	557.64	42.07	3		7 34	98.13	10	941.79	58.18	3	2822.35	-	-		S-layer protein [Clos	tridium difficile]
77	0	1052.05 882.42	46.85 47.89	4		/ 38	102.5	10	997.45	31.61	2	1992.88	AGETTYSTGL1			S-layer protein [Clos	tridium difficile]
84	20	749,73	52.30	3	6		107.2	10	980.48	41.97	2	1958.94	VLNGDEADTN	 gi 13539183 	2	S-layer protein [Clos	tridium difficite]
87	0	1170.55	\$9.07	2	6	V 54	126.3	10	1061.01	53.40	2	2120.00	LAMSAIFDTAY	🛯 🌚 gi 1353918;	2	S-layer protein [Clos	tridium difficile]
88	10	976.48	52.06	3	1 6	V 65	115.9	10	1176.23	47.91	3	3525.66	DLTGASADAIU	e 👔 👔 13539183	2	S-layer protein [Clos	tridium difficile]
91	40	668.38	20.03	2	6	✓ 65	113.9	10	760.90	39.03	2	1519.79	ALAESGADFSI	🕤 🎯 gi 1267001:	29	putative translation	inhibitor endoribonuclease
93	0	702.36	42.97	3	1 6	/ 70	63.88	10	557.64	42.07	3	1669.89	IADELLQLKDE	🛛 🕥 gi 5668937		flagellin (Clostridiun	n difficile)
96	0 40	832.18	54.46	4	6	78	71.61	9	882.42	47.89	4	3525.66	DLTGASADAIU	🕯 🕥 gi 13539183	2	S-layer protein [Clos	tridium difficite]
101	10	445.93 611.99	19.99	3	6	84	75.97	10	749.73	52.30	3	2246.17	AFVVGGTGLA	l 🕥 gi 1353918;	2 [13] Oxidation (M)	S-layer protein (Clos	tridium difficile]
103	0	1017.49		2	1	2 84	75.97	10	749.73	52.30	3	2246.17	AFVVGGTGLA	(🌒 gi 7063280	6 [13] Oxidation (M)	S-layer protein preci	ursor (Clostridium difficile)
105	10	528.82	39.69	2	6	/ 88	72.46	10	976.48	52.06	3	2926.43	TINNGYSNAIE	🛯 🌚 gi 13539183	2	S-layer protein [Clos	tridium difficile]
113	10	564.36	36.32	2	1	91	82.36	10	658.38	20.03	2	1334.75	KVYLAGGVNSI	🕥 gi 7063281	3	S-layer protein preci	ursor (Clostridium difficile)
122	0	825.69	44,74	4		91	82.36	10	668.38	20.03	2	1334.75	KV/LAGGVNS	🕥 gi 1149615	0	SIpA [Clostridium dit	
125	10	707.67	\$3.40	3	16	<u>(</u>								-			
128 133	10	613.31 835.95	42.64	2 ~		C cearch	results, 0 r	hatching bat	ch delete	options	1						
133	10	835.75	-x.07	4 *	-	a search	results VI	accricity par	un geleber	vgrand Da	1						Section Complete

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

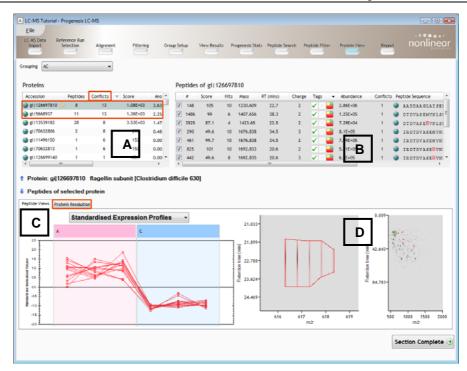
Stage 11: Protein View

The Protein View combines the quantitative LC-MS data with the qualitative MS/MS results at the protein level, highlighting proteins of interest between experimental groups. This stage allows you examine the behaviour of the assigned peptides and resolve any conflicts for the various peptide assignments at the protein level.

The Protein view 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.

When you open the Protein View 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 **'flagellin subunit'** the current protein by clicking on it in Window A (a circular orange symbol indicates current protein). **Flagellin subunit** has 8 peptides assigned (window B) which have a total of 13 conflicts. To view the conflicting assignments click on the **Protein Resolution** tab (window C) and then step through each assignment on window B.

	erence Run election	Alignment	Filterin	Group	Setup	View Resu	Its Prog	enesis Stats	Peptide Sear	rch Pepti	de Filter	Protein View	Repor	nonlin
suping AC		•												
roteins					Рер	tides of g	i 12669	97810						
Accession	Peptides	Conflicts v	Score	Anova (p)*		e Scor	e Hit	ts Mass	RT (mins)	Charge	Tags	 Abundance 	Confi	licts Peptide Sequence
🕽 gi 126697810 🛛 😐	8	13	1.08E-03	3.63E-05		461 99	k.7 1	0 1676.83	8 34.5	2	 ✓ × 	7.99E+05	1	IRDIDVASEMVNLSK
gi 5668937	11	13	1.36E+03	2.25E-05	2000	442 41	8 6.	1692.83	5 20.6	3	✓ ×	6.2E+05	1	IRDTDVASERVNLSK
gi170632806	2	8	295	0.484		825 1	01 1	0 1692.83	3 20.6	2	✓ ×	5.21E+05	1	🔮 IRDTDVASERVNLSK
gi 13539182	28	8	3.53E+03	1.47E-06		542 1	D4 1	0 1700.863	3 36	2	 ✓ × 	5.36E+05	1	🔮 VNINVSALIANNOMGR
gi 70632813	1	6	152	0.00384	~	1227 43	.3 7	1700.863	3 36	3	✓ ×	1.25E+05	1	🔮 VNINVSALIANNQMGR
gi 11496150	1	6	152	0.00384		1486 9	9 6	1407.65	6 38.3	2	✓ ×	1.25E+05	1	🕥 DIDVASENVNLSK
g1126698718	4	1	254	0.00099		3525 83	51 4	1423.65	5 22.5	2	- 🖌 🖂	7.29E+04	1	🕥 DIDVASE <mark>R</mark> VNLSK
Protein: gi 126 Protein: gi 126 Protein: gi 566 ptide Views	68937 fl: rin Resolutio	agellin (Clo	stridium diff											
Protein: gi 126 Protein: gi 126 Protein: gi 566 ptide Views Prote Conflicting pro	m 6697810 68937 fla rin Resolutio itelns for	flagellin su agellin (Clo n feature 35	abunit [Clos stridium diff 25	۰ tridium diffi ficile]	icile 630 Peptide	es of gi i								
erit26699140 Protein: gi[126 Protein: gi[566 ptide Views Prote Conflicting pro Accession	m 5697810 58937 fla ein Resolutio telins for Peptides	flagellin su agellin [Clo n feature 35: Conficts Pro	ubunit (Clos Istridium diff 25 Stein Score	Ficile]	Peptide	es of gi 5	Hits	Mass I	RT (mins)	Charge		Abundance	Conflicts	Peptilo Sequence
eii126699140 Protein: gi[126 Protein: gi[560 ptide Views Prote Conflicting pro' Accession @ gi[5669937	m 6697810 68937 fla ein Resolutio telins for Peptides 11	flagellin si agellin [Clo n feature 35: Conficts Pro 13 1.2	abunit [Clos stridium diff 25 stein Score 60-03	⊧ tridium diffi ficile] Peptis	Peptide	es of gi 5 Score	Hits 4	Mass 4	RT (mins) 22.5	Charge 2	<	7.29E+04	1	DTDVASERVNLSK
eii126699140 Protein: gi[126 Protein: gi[560 ptide Views Prote Conflicting pro' Accession @ gi[5669937	m 5697810 58937 fla ein Resolutio telins for Peptides	flagellin si agellin [Clo n feature 35: Conficts Pro 13 1.2	ubunit (Clos Istridium diff 25 Stein Score	Ficile]	Peptide	es of gi 5 Score 5 87.1 105	Hits 4 10	Mass 4 1423.65 1230.609	RT (mins) 22.5 22.7	Charge 2 2	/ X 🖬	7.29E+04 2.86E+06	Conflicts 1	 DIDVASERVNLSK AADDAAGLAISEK
eii126699140 Protein: gi[126 Protein: gi[560 ptide Views Prote Conflicting pro' Accession @ gi[5669937	m 6697810 68937 fla ein Resolutio telins for Peptides 11	flagellin si agellin [Clo n feature 35: Conficts Pro 13 1.2	abunit [Clos stridium diff 25 stein Score 60-03	⊧ tridium diffi ficile] Peptis	Peptide	es of gi 5 Score 5 87.1 105 125	Hits 4 10 10	Mass 4 1423.65 1230.609 2317.115	RT (mins) 22.5 22.7 38.7	Charge 2 2 2		7.29E+04 2.86E+06 5.55E+06	1	 DIDVASERVNISK AADDAAGLAISEK LESTONNINNTIENVIAAES
eii126699140 Protein: gi[126 Protein: gi[560 ptide Views Prote Conflicting pro' Accession @ gi[5669937	m 6697810 68937 fla ein Resolutio telins for Peptides 11	flagellin si agellin [Clo n feature 35: Conficts Pro 13 1.2	abunit [Clos stridium diff 25 stein Score 60-03	⊧ tridium diffi ficile] Peptis	Peptide	es of gi 5 Score 5 87.1 5 105 7 125 6 0.3	Hits 4 10 10 9	Mass 4 1423.65 1230.609 2317.115 2317.115	RT (mins) 22.5 22.7 38.7 38.7	Charge 2 2 2 3		7.29E+04 2.86E+06 5.55E+06 3.09E+06	1	 DTDVASERVNLSK AADDAAGLAISEK LESTQNNLNNTIENVTAAES LESTQNNLNNTIENVTAAES
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eii126699140 Protein: gi[126 Protein: gi[560 ptide Views Prote Conflicting pro' Accession @ gi[5669937	m 6697810 68937 fla ein Resolutio telins for Peptides 11	flagellin si agellin [Clo n feature 35: Conficts Pro 13 1.2	abunit [Clos stridium diff 25 stein Score 60-03	⊧ tridium diffi ficile] Peptis	Peptide	es of gi 5 score 5 87.1 105 125 60.3 107 5 1.2	Hits 4 10 10 9 10 4	Mass 4 1423.65 1230.609 2317.115 2317.115 1716.857 1716.858	RT (mins) 22.5 22.7 38.7 38.7 30.4 30.4	Charge 2 - 2 - 3 - 3 - 3 -		7.29E+04 2.86E+06 5.55E+06 3.09E+06 1.8E+05 3.69E+05	1	DTDVASERVNLSK AADDAAGLAISEK LESTONNLINTIENVTAAES LESTONNLINTIENVTAAES VITTVSALIANNORGR VITTVSALIANNORGR
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eii126699140 Protein: gi[126 Protein: gi[560 ptide Views Prote Conflicting pro' Accession @ gi[5669937	m 6697810 68937 fla ein Resolutio telins for Peptides 11	flagellin si agellin [Clo n feature 35: Conficts Pro 13 1.2	abunit [Clos stridium diff 25 stein Score 60-03	⊧ tridium diffi ficile] Peptis	Peptide	es of gils Score 5 87.1 105 125 60.3 107 5 1.2 49.6 7 42.3	Hits 4 10 10 9 10 4 10 7	Mass 4 1423.65 1230.609 2317.115 2317.115 1716.857 1716.858	RT (mins) 22.5 22.7 38.7 38.7 30.4 30.4	Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		7.29E+04 2.86E+06 5.55E+06 3.09E+06 1.8E+05 3.69E+05	1	DTDVASERVNISK ADDAACLAISK LESTONHLINTIENVTAAES LESTONHLINTIENVTAAES VHINVSALIANNGRe VHINVSALIANNGRe VHINVSALIANNGRE VHINVSALIANNGRE
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p (112669140) Protein: gi[32 Protein: gi[56 gatas Vexs] Prote Conflicting pro- Accession → gi1568937 → gi152097810	m 6697810 68937 fla ein Resolutio telins for Peptides 11	flagellin si agellin [Clo n feature 35: Conficts Pro 13 1.2	abunit [Clos stridium diff 25 stein Score 60-03	⊧ tridium diffi ficile] Peptis	Peptide	es of gi 5 5 core 5 87.1 125 125 125 125 125 125 125 125 125 12	Hits 4 10 10 9 10 4 10 7 6 8	Mass 4 1423.65 1230.609 2317.115 2317.115 1716.857 1716.858 1676.838 1700.863 1407.656 1692.835	RT (mins) 22.5 22.7 38.7 30.4 30.4 30.4 34.5 36 38.3 20.6	Charge 2 2 2 3 3 3 3 3 3 2 3 3 2 2 3 3 2 2		7.29E+04 2.86E+06 5.55E+06 3.09E+06 1.8E+06 3.49E+05 8.1E+05 1.25E+05 1.25E+05 6.2E+05	1	DTOVASERVNISK AADDAAGLAISK LESTONNINNTIENVTAAEG LESTONNINNTIENVTAAEG VHTVVSALIANNORGA VHTVVSALIANNORGA INTOVASENVNISK VHTVVSALIANNORGA DTOVASERVNISK INTOVASERVNISK

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In this case the conflicting peptide assignments are with the main **flagellin** protein which contains 3 additional peptides. To resolve this conflict un-assign all the peptides assigned to the **flagellin subunit** protein, by selecting and then unticking all the peptides in window B.

Import	ference Run Selection	Alignment	Filterin	Group	Setup	View R	esults Pri	ogenesis Stats	Peptide Search	Peptis	le Filter	Protein View	Report	nonlir	
uping AC		•													
oteins					Рер	tides o	f gi 1260	597810							
ccession	Peptides	Conflicts ~	Score	Anova (p)/ *		# 5	core H	tits Mass	RT (mins)	Charge	Tags	 Abundance 	Confi	icts Peptide Sequence	
gi1126697810	• •	0	0			-461	99.7	10 1676.83	8 34.5	2	X	7.99E+05	0	IRDTDVASEMVNLSK	
gi15668937	11	0	1.36E+03	2.256-05	10	442	49.6	8 1692.83	5 20.6	а	X	6.2E+05	0	IRDTDVASERVNLSK	
g1170632806	2	8	295	0.484		825	101	10 1692.83	3 20.6	2	 X 	5.21E+05	0	IRDTDVASERVNLSK	
gi 13539182	28	8	3.53E+03	1.47E-06	10	542	104	10 1700.86	3 36	2	 X 	5.368+0	B	VNTNVSALIANNQMGR	
gi170632813	1	6	152	0.00384		1227	42.3	7 1700.86	3 36	3	< ×	1.25E+05	0	VNTNVSALIANNOMGR	
gi 11496150	1'A	6	152	0.00384		1486	99	6 1407.65	6 38.3	2	 X 	1.258+05	0	DT DVASEMVNLSK	
		1	254	0.00099	101	3525	87.1	4 1423.65	5 22.5	2	✓ ×	7.295+04	0	🕥 DT DVASERVNLSK	
gi1126698718	4		8.01	0.00077											
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Note: as you un-assign the peptides the number of conflicts update 'on the fly' in all the windows A similar argument can be applied to the next set of conflicting assignments

	ference Run Selection	Alignment	Filterin	e Grou	p Setup	Vie	ew Results	s Proj	penesis Stats	Peptide Searc	h Pepti	ide Filter	,	Protein View	Repor	t		nlinea
rouping AC		•																
Proteins					Pe	ptide	es of gi	7063	2806									
Accession	Peptides	Conflicts	Score	Anova (p)*			Score	н	ts Mass	RT (mins)	Charge	e Taga		 Abundance 	Conf	licts	Peptide Sequence	
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g gi 13539182	28	8	3.53E+03	1.47E-06	V	1718	5 66.9	9	8 2246.17	72 52.3	4	1	-	7.21E+04	1		AFVVGGTGLADA	REINDAR
🚽 gi 170632806 🛛 🤇	<mark>o 2</mark>	8	295	0.484	1	84	76		0 2246.13	74 52.3	3	1	-	4.28E+06	1		AFVVGGTGLADA	RSIAPVAS
gi 70632813	1	6	152	0.00384														
🕥 gi i 11496150	1	6	152	0.00384														
gi 126699140	1	1	45.7	0.000605														
🔮 gi 126698718	4	1	254	0.00099	• •													
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In this case the conflicting peptides are unassigned from the 'precursor' protein.

In many cases the rationale for resolving a peptide assignment conflict is based on the number of peptides assigned to each protein, often the conflict(s) being resolved are in favour of the protein with the greater number of assigned peptides.

LC-MS Tutorial -	 Progenesis Li 	C-MS												
<u>F</u> ile														
LC-MS Data R Import	leference Run Selection	Alignment	Filterin	Grou	o Setup	View R	esults P	rogenesis Stat	s Peptide Se	earch Pep	tide Filter	Protein View	Report	nonlinoc
rouping AC		•												
Proteins					Рер	tides of	gi 1266	98718						
Accession	Peptides	Conflicts v	Score	Anova (; 🔺		# So	ore H	its Mass	RT (mins)	Charge	Tags	+ Abundance	Conflict	s Peptide Sequence
🗿 gi 11496150	0	0	0		V	3156	60.5	2 1580.79	2 30.4	2	 ✓ × 	6.51E+04	0	NTDIKEEYLSEIK
谢 gi 126699140	1	1	45.7	0.000605	V	449	49.9	8 1153.63	5 37.1	2	√ ×	4.33E+05	0	LVPEIDVDVR
🌛 gi 126698718	<u> </u>	1	254	0.00099	V	578	76.5	9 1344.77	6 46.5	2	\checkmark \times	5.29E+05	0	FIVDGGTVVLAVR
gi 126700090	1	0	80	0.0344		891	67.5 1	10 1175.60	9 36.3	2	\checkmark \times	📒 1.71E+05	1	🕥 ALLDAFHYAR
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Accession	Peptides	Conflicts Prof		Pepti	#	Score	Hits	Mass	RT (mins)	-	Tags 🔹			Peptide Sequence
Accession	Peptides	Conflicts Prot 1 254	tein Score	Pepti		Score	Hits		RT (mins) 36.3	-	Tags 🔹	Abundance	Conflicts P	Peptide Sequence
Conflicting p Accession gil126698718 gil126699140	Peptides	Conflicts Prof	tein Score	Pepti	#	Score	Hits	Mass		-	-			
Accession	Peptides	Conflicts Prot 1 254	tein Score	Pepti	#	Score	Hits	Mass		-	-			

In the above example the conflict would be resolved in favour of the protein with 4 peptides.

Note: the number of conflicts you have to resolve will depend on the scope and stringency of the filters you apply at the **Peptide Search** stage

For this tutorial use of the 'suggested' **Peptide Filter** criteria, (page 39), results in a low number of conflicts requiring 'subjective' resolution. A less stringent application of filters at the Peptide Filter stage will increase the time spent resolving conflicts.

Finally order the Protein table (A) using descending score, and then scroll to the right to locate the 'tags' column.

You can now select proteins on the basis of the tagged features.

For example you can filter the list to show only these proteins that contain features with $\ensuremath{\text{Increased expression in A}}$

Progenesis LC-MS Tutorial

Protein	5				Peptides	s of gi∣13	53918	32						
re 🔻	Anova (p)*	Fold	Tags	✓ Abundance Mass ▲	#	Score	Hits	Mass	RT (mins)	Charge	Tags 🔻	Abundance	Conflicts	Peptide Sequence
53E+03	1.47E-06	5		🗲 Show all		84.4	10	1311.708	43.9	2	✓ ×	5.92E+07	0	VFFEGTLASTII
37E+03	3.85E-05	2.45		V V = 4000 most abundant (92 prote	eins)	78.4	7	1311.707	42.6	2	< × 🞴	1.7E+05	0	VFFEGTLASTII
36E+03	2.25E-05	3.69		Increased expression in A (80	<u> </u>	80.8	4	1311.708	50.2	2	< X 🞴	7.15E+04	0	VFFEGTLASTII
19E+03	0.114	1.2		V V Increased expression in C (38		78.6	6	1311.708	52.5	2	< X 📮	5.34E+04	0	VFFEGTLASTII
686	0.00177	2.24		V V Significant p<0.05 (112 protei	· · · I	43.4	8	1311.708	53.3	2	< X 🞴	1.38E+05	0	🔮 VFFEGTLASTI
683	0.000375	2.43		No tags assigned (5 proteins)		83.2	8	1188.624	36.6	2	✓ ×	2.9E+07	0	LGDSDIIDITK
507	0.00675	1.89				53.9	2	1188.621	41	2	< X 🞴	8.15E+04	0	LGDSDIIDITK
437	2.91E-05	5.68		ОК	Cancel	77	10	1144.634	41.5	2	< × 📒	2.27E+07	0	🌒 GILDGSITEIK
•				4	•									-

This will filter the Protein list so that it now only displays the 80 proteins containing peptides that show **Increased expression in A**

Export Protein List
Choose properties to be included in exported file
✓ Spectral counts✓ Tags
OK Cancel

You can export this filtered Protein list (csv format) by selecting this option from the **File** menu. You can control the data output required, using the dialog provided.

Now return to the **Peptide Search** stage by clicking on the icon in the **Workflow** at the top of the screen.

LC-MS Data	Reference Run Selection	Alignment	Filtering	Group Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report	
------------	----------------------------	-----------	-----------	-------------	--------------	------------------	----------------	----------------	--------------	--------	--

Note: in the Features list, if you have resolved all the conflicts, there will only be one protein assigned to each feature.

Feat	ures				MS/MS Spectra
#	MS/MS	Proteins 👻	Score	Tags	Batch inclusion options for creating MS/MS
9672	6	1 <mark>(</mark> i 126697	74.9	🖌 🔨	Show all 🚰 Edit tags
105		1†i 126697		 ✓ × 	🔽 🖂 📒 4000 most abundant (4000 features)
3109	7	1 <mark>1</mark> 1 101802			🕢 🖂 📒 Assigned features (392 features)
		1 ₁ i 101802		 ✓ × 	🔽 🖂 Increased expression in A (4317 features)
5965	4	1 i 101802	141	 ✓ × 	🔽 🖂 🔳 Significant p<0.05 (8375 features)
1114	13	0	0	✓ ×	V I Increased expression in C (4058 features)
5907	13	0	0	< X	No tags assigned (4810 features)
23	13	0	0	✓ ×	
14922	0	0	0		OK Cancel

Create a new tag for the selected features and call it **Assigned Features**

Create new tag		×
Assigned Features		
	OK Cance	*

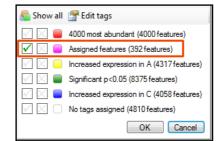
Now move to the Report section by clicking on **Report** icon on the workflow at the top of the screen.

Stage 12: 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 features**.

As an example we will create a report for **only** the features with identified proteins and showing an 'Infinite fold' difference between the groups AC.

- 1. First reduce the features to report on by selecting the 'Assigned Features' tag. In this example it reduces the number of features in the table to 392.
- 2. Expand the various Report Design options (by default they are all selected)
- 3. Un-tick as shown below



4. Click Create Report

<u>F</u> ile	Tutorial - Prog						
LC-MS Data Import	Reference Selection		ment	Filtering	Group Setup	View	Results Progenesis Stats Peptide Search Peptide Filter Protein View Report
eature	25			_			Report Design
Grouping	AC			-			Title LC-MS Tutorial
#	Anova (p)	Fold	Tag 👻	Report	Notes	*	
3993	0.004	3.2		1	2	E	Select the sections you wish to include in your report:
3970	0.002	5.0		1	2		🔍 🔲 Overview run
4086	0.005	6.5			D		🕑 🔲 Data processing methods
4022	0.003	39.4		V	a.		📀 🔲 Experiment design
3946	3.363e-004	38.5		1	a.		Note in report
3839	2.967e-004	2.9		1	19		Include tables showing protein abundances and peptides identified for each protein
3794	0.014	1.6		1	D		V Protein table
2510	0.002	1.8		1	D		Peptide tables
3875	0.002	11.3		-	D		🕞 🥅 Feature table
4573	0.004	2.9		1	- D		✓ ▼ Feature details
4538	0.015	2.2		1	D		
4736	0.044	2.4		1	10		
4599	6.063e-004	8.6		1	D		Create Report
4516	0.047	2.2	-	1	a.		
4152	0.010	3.9		√	9		Export Inclusion List
4096	0.016	2.0		1	12		
4434	7.508e-004	5.0		1	19.		
4265	0.002	7.1		1			
2840	0.023	1.8			D		
2827	2.545e-004	8.8		-			
2976	0.001	3.6		1	a		
2877	8 5760.004	12.2				Ψ.	

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

LC-MS Tutorial Experiment: LC-MS Tutorial Reperiment: LC	Experiment: LC-MS Tutorial Report created: 21/07/2009 11:47:11 Poteins Accession Perifes Nova (p) Fold Tag Description Nova (p) Fold Tag Description Nova (p) Nova (p) Fold Tag Description Nova (p) Nova (p) <th></th>																		
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gl1126699063 6 368.44 2.16e-0 AAADEIGLP/EQT.GGYNAX 10690 41.75 5 204.0799 3 0 1111.22 1.21e-004 ni1126498647 A 3.93 3 3 3 3 0 101 1111.22 1.21e-004 GIBYONYAKE 775 88.49 10 1127.7599 2 0 7.22e-004 2.06e+005 GIBYONYAKE/KY 1127 49.5 4 1237.7599 2 0 1.27e+004 2.06e+005 GLAZGVGDEGGFAPHLGSMR 5630 88.0 2 1946.8905 2 0 [4] Carbamidomethyl (C) 9050.97 3.84e+004 LGLWGDELFYINTER 1009 9.243 10 118.8842 2 0 [11] Oxidation (M) 5626.65 2.75e+004 SOETEDSTLADLAVANHAQQIK 2228 106.386 2 8 2 0 [11] Oxidation (M) 5626.65 2.75e+004	gl 126699063 6 368.44 2.16e-0 AAADEKICPUTOT.GGYNAAK 10690 41.75 5 2040.0789 3 0 11141.22 1.21e-004 ni11726498643 4 373 30 3 9 0 7.22e-004 2.06e-005 GIEROYANSILVK 1327 49.25 4 1312.7343 2 0 1.27e-005 4.47e-004 GLEGOYANSILVK 1327 49.25 4 1312.7343 2 0 1.27e-005 4.47e-004 GLEGOYANSILVK 1327 49.25 4 1312.7343 2 0 1.27e-005 4.47e-004 GLEGOYANSILVK 1327 49.25 4 1312.7343 2 0 1.27e-005 4.47e-004 GLAGOYANGGARHUGSH 5530 8.03 2 194.57895 2 0 [111] 0xidation (M) 3626.54 2.75e-004 LGAVADUPTOTYNTER 1009 92.43 10 178.8842 2 0 9.53e-004 2.77e-005 SGETEDSTIADLAVANHAGQIK 2	gi 126697969 gi 126698640 gi 126697690	5	507.41 481.67 437.19	6.75e-0 1.48e-0 2.91e-0	rgeviald vyvADDAI gi 120 enolase [4 8 peptide	Clostrie	534 255 790	83.64 75.03	8 1107. 10 1234.	.5815	5 2 5 2	0			8.10	e+005	3.64e+005	bundances
nil126498643 4 339 3 3 10.07 GIENGVANSLUK 1227 49.25 4 1312.759 2 0 7.22e-004 2.06e-005 GIENGVANSLUK 1227 49.25 4 1312.7543 2 0 1.27e-005 4.47e-004 GLACGVC0EGGFAPHLGSNR 530 88.03 2 1946.8905 2 0 [4] Carbamidomethyl (C) 9050.77 3.84e-004 LGANALGVSMAAVAR 5916 52.36 2 1457.8024 2 0 [11] Oxidation (M) 3626.54 2.75e-004 LQLVGODLVTNTER 1009 92.43 10 1788.882 2 0 [11] Oxidation (M) 3626.54 2.77e-005 SGETEDSTUDLVAVNAQQIK 2226 104.385 2 0 6.23e-004 1.55e-005	All 1724AB8.43 A 339 39 3.9.16.1 GENEYAYSELVK 1327 49.25 4 1327.759 2 0 7.22e-004 2.06e-005 GENEYAYSELVK 1327 49.25 4 1327.759 2 0 1.27e-005 4.47e-004 GLACGYGEGGFAPHLGSNR 5630 88.03 2 1946.805 2 0 [4] Carbanidomethyl (C) 905.97 3.8e-004 LGANALGYBAVAR 5916 52.36 2 1457.8024 2 0 [1] Oxidation (M) 3626.54 2.78e-004 LQLYGODLYTNTER 1009 92.43 10 178.8842 2 0 9.53e-004 2.77e-005 SGETEDSTIADLAVANAGQIK 2226 104.36 6 2188.0855 2 0 6.23e-004 1.55e-005 SVIELVYAR 896 5.755 10 1048.5911 2 0 3.78e-004 1.32e-005	gi 126697969 gi 126698640 gi 126697690 gi 54781345	5 5 4 5	507.41 481.67 437.19 405.08	6.75e-0 1.48e-0 2.91e-0 1.84e-0	rgeviald vyvADDAI gi 120 enolase [4 8 peptide	Clostrie	534 255 790	83.64 75.03	8 1107. 10 1234.	.5815	5 2 5 2	0			8.10	e+005 Avera	3.64e+005	bundances
n11726498.43 J 239 9 9 19.01 GIENGVANSILVK 1327 49.25 4 1312.7343 2 0 1.27e=005 4.47e=004 GLENGVANSILVK 1327 49.25 4 1312.7343 2 0 1.27e=005 4.47e=004 GLENGVANSILVK 5503 85.03 2 194.8905 2 0 [4] Carbamidomethyl (C) 995.09 3.84e=004 LGANALGVSÄMAVAR 5916 52.36 2 1457.8024 2 0 [11] Oxidation (M) 3626.54 2.75e=004 LQLYGODLVYINTER 1009 92.42 10 178.8842 2 0 9.5a=004 2.78e=004 SGETEDSTUDLVAVMAGQIK 2220 106.36 6 2188.0855 2 0 0 6.23e=004 1.55e=005	mil 126608643 d 320 3 9 flan GIENGVANSILVK 1327 49.25 4 1312.7343 2 0 1.27e=005 4.47e=004 GIENGVANSILVK 1327 49.25 4 1312.7343 2 0 1.27e=005 4.47e=004 GLALGVODEGGFAPHLGSNR 5530 88.03 2 146.8905 2 0 [4] Carbamidomethyl (C) 9050.97 3.84e=004 LGANLIGVOM 92643 10 178.8842 2 0 [11] Oxidation (M) 9.526.54 2.77e=005 SGETEDSTUDLAVAMAQQIK 2226 106.36 6 2188.0855 2 0 6.32e=004 1.55e=005 SVIELVAR 99.6 57.95 10 1048.5911 2 0 0 3.78e=004 1.12e=005	gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752	5 4 5 5 5 5	507.41 481.67 437.19 405.08 370.14	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0	rigeviale yvvaddal gij120 enolase [8 peptide Sequence	Clostrie	534 255 dium diff	83.64 75.03 icile 630	8 1107. 10 1234. Score 106.02	.5819 .6445 Hits	5 2 5 2 2 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	0 0 Charge	Conflic	ts Modificatio	8.10	e+005 Avera	3.64e+005 ge Normalised A C 8171.92	1.40e+005
GLAĽGVGDEGGFAPHLGSAR 5530 88.0 2 1946.8905 2 0 [4] Carbamidomethyl (C) 9050.97 3.84e=004 LGANALIGVŠIKAVAR 5916 52.36 2 1946.8905 2 0 [4] Carbamidomethyl (C) 9050.97 3.84e=004 LGANALIGVŠIKAVAR 5916 52.36 2 1578.024 2 0 [11] Oxidation (M) 5626.54 2.75e=004 LQLVGDDLFVINTER 1009 92.421 10 1718.8842 2 0 9.53a=004 2.77e=005 SOETEDSTLALAVAVINAGQIK 2228 106.38 6 2188.0855 2 0 6.33e=004 1.55e=005	GLAČOVODEGGFAPHLGSNR 55.0 8.0 2 1946.8905 2 0 [4] Carbamidomethyl (C) 905.97 3.84e+004 LGANAUGOSMAVAR 5916 52.36 2 1946.8905 2 0 [11] Oxidation (M) 3626.54 2.75e+004 LQLVGDOLEVTNITER 1009 92.43 10 178.8842 2 0 9.53e+004 2.77e+005 SGETEDSTIADLAVANAQUK 2226 106.36 6 2188.0855 2 0 6.23e+004 1.55e+005 SVIELVYAR 896 57.95 10 1048.5911 2 0 3.78e+004 1.13e+005	gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752	5 4 5 5 5 5	507.41 481.67 437.19 405.08 370.14	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0	TGEVIALE TGEVIALE VYVADDAI gi 120 enolase [: 8 peptide Sequence AAADEIGI AAADEIGI	LLEK 57007 Clostrie 5 PLFQYL PLFQYL	534 255 dium diff	83.64 75.03 icile 630	8 1107. 10 1234. Score 106.02 41.75	.5815 .6445 Hits 10 5	5 2 5 2 2 4 4 4 5 2 4 4 5 2 4 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4	0 0 Charge 2 3	Conflic	ts Modificatio	8.10	Avera A	3.64e+005 ge Normalised A C 8171.92 1141.22	1.40e+005 1.21e+004
LGANAILGVŠIMAVAR 5916 52.36 2 1457.8024 2 0 [11] Oxidation (M) 3626.54 2.75e+004 LQLVGDDLFVTNTER 1009 92.43 10 1718.8842 2 0 [11] Oxidation (M) 3626.54 2.77e+004 SGETEDSTIADLAVANNAQUK 2226 106.36 6 2188.085 2 0 6.23e+004 1.55e+005	LGANAILGVŠMAVAR 5916 52.36 2 1457.8024 2 0 [11] Oxidation (M) 3626.54 2.75e-004 LQLVGDOLVTNTER 1009 92.43 10 1718.8842 2 0 95.3e-004 2.77e-005 SGETEDSTIADLAVANAGUK 2226 106.36 6 2188.0855 2 0 6.23e-004 1.55e-005 SVIELVYAR 896 57.95 10 1048.5911 2 0 3.78e-004 1.13e-005	gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VYVADDAI gi 120 enolase [8 peptide Sequence AAADEIGI AAADEIGI EALELIVE	LLEK 57007 Clostrie 5 PLFQYL PLFQYL AITK	534 255 dium diff	83.64 75.03 icile 630 Feature 3910 10690 795	8 1107. 10 1234. Score 106.02 41.75 88.49	.5815 .6445 Hits 10 5 10	5 2 5 2 4 2 6 2 7	0 0 Charge 2 3 3 2	Conflic	 Modification 0 0 	8.10	Avera A	3.64e+005 ge Normalised A C 8171.92 1141.22 7.22e+004	1.40e+005 1.21e+004 2.06e+005
SGETEDSTIADLAVAVNAGQIK 2226 106.36 6 2188.0855 2 0 6.23e-004 1.55e-005	SGETEDSTUADLAVANNAGQIK 2226 106.36 6 2188.0855 2 0 6.23e-004 1.55e-005 SVIELYYAR 896 57.95 10 1048.5911 2 0 3.78e-004 1.13e-005	gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE YYYADDAI gi 12.0 enolase [8 peptide Sequence AAADEIGI AAADEIGI EALELIVE GIENGVAN	LLEK 57007 Clostrie S PLFQYL PLFQYL AITK KSILVK	534 255 dium diff	83.64 75.03 icile 630 Feature 3910 10690 795 1327	8 1107. 10 1234. Score 106.02 41.75 88.49 49.25	5815 6445 Hits 10 5 10 4	5 2 5 2 2 4 4 4 5 2 4 2 4 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4 5 4	0 0 Charge 2 3 3 2 2 2	Conflic	ts Modificatio	ns 8.10	Avera A	3.64e+005	1.40e+005 1.21e+004 2.06e+005 4.47e+004
	SVIELVYAR 096 57.95 10 1040.5911 2 0 3.78e-004 1.13e+005	gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VYVADDAI gi 120 enolase [r 8 peptide Sequence AAADEIGI AAADEIGI EALELINE GIENGVAI GLAČGVO	LLEK 7007 Clostrie PLFQYL PLFQYL AITK KSILVK DEGGFA	534 255 dium diff	83.64 75.03 icile 630 Feature 3910 10690 795 1327 5630	8 1107. 10 1234. Score 106.02 41.75 88.49 49.25 88.03	5815 6445 Hits 10 5 10 4 2	5 2 5 2 2 4 4 5 2 2 046.0782 2 2046.0782 2 2046.0789 1 327.7599 1 312.7343 1 946.8905	0 0 Charge 2 3 2 2 2 2 2 2	Conflic	ts Modificatio	ns	Avera A	3.64+005	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004
SVIELVYAR 896 57.95 10 10.48.5911 2 0 2.78e-004 1.13e-005		gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VYVADDAI gi 120 enolase [8 peptide Sequence AAADEIGI AAADEIGI GIENGVA GIENGVA GIAČGVO LGANALO	LLEK Clostrie S PLFQYL PLFQYL AITK SILVK DEGGF/ SVSMAV	534 255 dium diff GGVNAK GGVNAK APNLGSNR AR	83.64 75.03 icile 630 5910 10690 795 1327 5630 5916	8 1107. 10 1234. Score 106.02 41.75 88.49 49.25 88.03 52.36	5815 6445 Hits 100 5 100 4 2 2	5 2 5 2 2 4 4 2046.0782 2046.0782 2046.0789 1312.7599 1312.7599 1312.7599 1312.7599 1312.7599 1312.7599	0 0 Charge 2 3 3 2 2 2 2 2 2 2	Conflic	ts Modificatio 0 0 0 0 0 (4) Carbam 0 [4] Carbam	ns	Avera A	3.64e+005	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004 2.75e+004
		gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VVVADDAI gill20 enolase [8 peptide Sequence AAADEIGI AAADEIGI EALELIVE GIENGVAI GLACGVO LGANALC LQLVGDD SGETEDS	PLFQYL PLFQYL AITK SUVK DEGGFA SVSMAV LFVTNTI TIADLAV	534 255 dium diff GGVNAK GGVNAK APNL.GSNR AR ER	83.64 75.03 ctile 630 Feature 3910 10690 7955 11227 5530 5916 1009	8 1107. 10 1234. 10 1234. 106.02 41.75 88.49 49.25 48.03 52.36 92.43 106.36	5815 6445 100 5 100 4 2 2 100 6	5 2 5 2 2 4 5 2 2 4 5 2 2 4 5 2 2 4 5 2 2 4 5 2 4 4 5 2 4 4 5 2 4 5 2 4 5 2 4 2 4 5 2 4 4 5 2 4 4 5 4 4 5 4 4 5 4 4 5 4 4 5	0 0 Charge 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Conflic	ts Modificatio 0 0 0 0 0 0 0 0 0 0 0 0 [4] Carbam 0 0 [11] (0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ns	Avera A	3.64e-005	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004 2.75e+004 2.77e+005
gi <u> 126698631</u>		gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VYVADDAI gi 120 enolase [8 peptide Sequence AAADEIGI AAADEIGI EALELIVE GIENGVAI GLAĞIYOC LGANALO LGANALO LQLYGOD SGETEDS' SVIELIVVA	LLEK 57007 Clostric 5 PLFQVL ATTK SILVK DEGGFA SVSTAVA LFVTNTT TIADLAV R	534 255 dium diff GGVNAK GGVNAK APNLGSNR AR ER /AVNAGQIK	83.64 75.03 icile 630 Feature 3910 10690 795 1327 5630 5916 1009 2226	8 1107. 10 1234. 10 1234. 106.02 41.75 88.49 49.25 48.03 52.36 92.43 106.36	5815 6445 100 5 100 4 2 2 100 6	5 2 5 2 2 4 5 2 2 4 5 2 2 4 5 2 2 4 5 2 2 4 5 2 4 4 5 2 4 4 5 2 4 5 2 4 5 2 4 2 4 5 2 4 4 5 2 4 4 5 4 4 5 4 4 5 4 4 5 4 4 5	0 0 Charge 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Conflic	ts Modificatio 0 0 0 0 0 0 0 0 0 0 0 0 [4] Carbam 0 0 [11] (0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ns	Avera A	3.64e+005	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004 2.75e+004 2.77e+005 1.55e+005
		gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VYVADDAI gi 120 enolase [8 peptide Sequence GIENGVAI GLACGVO LGANALC LQLVGDD SGETEDS SVIELVYAI gi 120	LLEK 57007 Clostri- 55 PLFQYL PLFQYL 451LYK KSILVK KSILVK R 8 66986	534 255 790 dium diff 	83.64 75.03 Feature 3910 10690 795 1327 5630 5916 1009 2226 896	8 1107 10 1234 Score 106-02 41.75 88.49 49-25 88.03 52.36 92.43 106.36 57.95	Hits 10 5 10 4 2 2 10 6 10	J 2 5 2 5 2 Mass 2046.0782 2046.0782 2046.0789 1327.7393 1946.8905 1457.8024 1718.884.8 2188.0855 1048.5911	0 0 Charge 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Conflic	ts Modificatio 0 0 0 0 0 0 0 0 0 0 0 0 [4] Carbam 0 0 [11] (0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ns	Avera A	3.64e+005	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004 2.75e+004 2.77e+005 1.55e+005
gi 126698631 cell surface protein [Clostridium difficile 630] 5 peptides		gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VIVADDAI gil120 enolase [8 peptide Sequence AAADEIGI AAADEIGI AAADEIGI CALGIVGOD LGANALC LQLVGOD SGETEDS SVIELVVA gil120 cell surfa	LLEK S7007 Clostri- S5 PLFQYL AITK KSILVK KSILVK KSILVK R R C66986 Cce pro'	534 255 790 dium diff 	83.64 75.03 Feature 3910 10690 795 1327 5630 5916 1009 2226 896	8 1107 10 1234 Score 106-02 41.75 88.49 49-25 88.03 52.36 92.43 106.36 57.95	Hits 10 5 10 4 2 2 10 6 10	J 2 5 2 5 2 Mass 2046.0782 2046.0782 2046.0789 1327.7393 1946.8905 1457.8024 1718.884.8 2188.0855 1048.5911	0 0 Charge 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Conflic	ts Modificatio 0 0 0 0 0 0 0 0 0 0 0 0 [4] Carbam 0 0 [11] (0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ns	Avera A	3.64e+005	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004 2.75e+004 2.77e+005 1.55e+005
cell surface protein [Clostridium difficile 630]	5 peptides Sequence Feature Score Hits Mass Charge Conflicts Modifications Average Normalised Abundances	gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VYVADDAI Sel 120 8 peptide 8 peptide Sequence AAADEIGI AAADEIGI CALELIVE GIENGVA GLAČGVO LGANALC LQLVGOD SOETEDS' SVIELVYA gi 120 cell surfa 5 peptide	LLEK 57007 Clostri- 55 LPLFQYLL AITK 45ILVK 10EGGF4 5VSMAV2 LEVTNTT TIADLAV R 66986 ce pro' 15	534 255 290 dium diff 	83.64 75.03 Feature 3910 10690 795 1327 5633 5916 1009 2226 896	8 1107 10 1234 Score 106.02 41.75 88.03 52.36 92.43 106.36 57.95 difficile	Hits 100 5 100 4 2 2 100 6 300	5 2 5 2 5 2 Mass 2046.0782 2046.0782 1027.7599 1312.7743 1946.8905 1457.8024 1718.8842 1048.5911	0 0 0 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2	Conflic	ts Modificatio 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	idomethyl (C) Dxidation (M)	Avera A a	3.64e+005 ge Normalised A ge N	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004 2.75e+004 2.77e+005 1.55e+005
cell surface protein [Clostridium difficile 630] 5 peptides Sequence Feature Score Hits Mass Charge Conflicts Modifications Average Normalised Abundances	Sequence Feature Score Hits Mass Charge Conflicts Modifications Average Normalised Abundances	gi 126697969 gi 126698640 gi 126697690 gi 54781345 gi 126697752 gi 126699063	5 5 4 5 5 6	507.41 481.67 437.19 405.08 370.14 368.44	6.75e-0 1.48e-0 2.91e-0 1.84e-0 2.61e-0 2.16e-0	TGEVIALE VIVADDAI gi 120 enolase [8 peptide 8 peptide AAADEIGI AAADEIGI EALELINE GENGVAI GLAGAVALC LGAVALC LGAVALC LGAVALC LGAVALC SGETEDS' SVIELVVAI gi 120 cell surfa S peptide	LLEK 57007 Clostri 5 PLFQYL PLFQYL AITK SELVK LEVTNT TIADLAV R 66986 ce pro' 15	534 255 255 255 255 257 257 257 257 257 257	83.64 75.03 Feature 3910 10690 2226 096 tridium Feature	8 1107. 10 1234 Score 106.02 41.75 88.49 49.25 88.49 106.62 41.75 88.49 49.25 88.49 49.25 88.49 89.49 89.49 89.49 89.49 89.49 89.49 89.49 89.49 106.62 89.49 89.49 89.49 89.49 89.49 89.49 89.49 89.49 80	5815 6445 10 5 10 4 2 2 10 6 10 6 30 6 10	2 2 5 2 5 2 Mass 2046.0782 2046.0782 2046.0789 13127.739 1946.8905 1457.8024 1718.8842 2188.0855 1048.5911	0 0 Charge 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Conflict	ts Modificatio 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	idomethyl (C) Dxidation (M)	Avera A A I I I I I I I I I I I I I I I I I	3.64=*005	1.40e+005 1.21e+004 2.06e+005 4.47e+004 3.84e+004 2.75e+004 2.77e+005 1.55e+005

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.

Creating an Inclusion list

As an example of creating an **Inclusion list** you are going to create an inclusion list for all the features that show a Significant difference between groups A and C (Anova p<0.05) and have no MS/MS spectra.

	LC-MS Data Import	Reference Run Selection	Alignment	Filtering	Group Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report
--	----------------------	----------------------------	-----------	-----------	-------------	--------------	------------------	----------------	----------------	--------------	--------

First return to **View Results** using the Workflow icons. Then select the **Feature details** tab to expand the table.

1D Display 2D Montage 3D Montage Feature details

Filter the table so that it is only showing features with a **Significant p<0.05** then order the table on ascending MS/MS. Highlight all features with **No** MS/MS spectra and create a new tag for them called **Inclusion_1**.

#	Anova (p)	Fold	Tag	▼ Notes	m/z	z	Mass	RT (mins) RT window	Abundance	Intensity	MS/MS	Protein	Pej
13662	0.00611	20.1		🐁 Show all 🛛 😭 Edit tag	IS		.9	16 28.624	0.127	2.78E+03	4.97E+04	0		
4268	0.00614	1.16E+03			ibundant (4000 fea	atures)	1.2	08 40.168	0.328	4.32E+04	1.56E+05	0		
7460	0.0109	773	V X		atures (392 feature			31 29.133	0.47	6.09E+04	7.66E+04	0		
552	0.0262	2.04	V X		xpression in A (43)		ines) 1.3	28 52.003	0.545	2.6E+04	9.32E+04	0		
3983	0.0229	72.5	V X		<0.05 (8375 featu			06 46.145	0.219	4.84E+03	5.34E+04	0		
13166	0.0262	61	V X		xpression in C (40			15 35.238	0.269	4.13E+03	2.59E+04	0		
3488	0.0062	8.6	V X		igned (4810 featur			67 47.079	0.414	5.98E+03	3.3E+04	0		
1027	0.00607	2.14	V X			_		52 21.947	0.634	7.01E+04	1.93E+05	0		
5288	0.00608	40.9	V X		OK	Cano	cel 1.1	17 46.465	0.745	2.93E+04	8.31E+04	0		
5117	0.0262	470	V X	Add note	1183.7853	4	4731.1	12 57.899	0.548	8.74E+04	1.22E+05	0		
3062	0.00605	3.71	V X	Add note	1151.0519	2	2300.0	89 52.343	0.95	1.33E+05	1.9E+05	0		
10438	0.0279	85.4		Add note	668.3301	3	2001.9	68 59.372	0.407	4.57E+03	6.62E+04	1		_
5440	0.0182	51.3	VX	Add note	632.791	2	1263.5	67 41.208	0.572	1.87E+04	2.01E+05	1		
4454	2.23E-06	Infinity	VX	Add note	1138.5733	2	2275.1	32 44.292	0.277	2.06E+04	2.98E+05	1		
5360	0.015	35		Add note	595.651	3	1783.9	31 45.016	0.597	2.2E+04	1.15E+05	1		
•														+

Now use the new tag to filter the table to display only those features that show a Significant Change and DO NOT have any MS/MS spectra.



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

Ei	ile													-
	Save												nonli	
5	Close		Alignment	Filtering	Group Setup V	'iew Res	ults Progenesi	s Stats Peptic	de Search Pep	tide Filter Pr	otein View	Report	nonii	dynamic
	Export Featur	e Data												
	Export Inclusi	ion List	AC		•									
:	Exit		ag 🔻	Notes	m/z	z	Mass	RT (mins)	RT window	Abundance	Intensity	MS/MS	Protein	Pe
388	2.52E-05	6.22 C	reate an inclusio	n list containing all f	eatures satisfying th	ne curre	ent tag filter	31.649	0.872	1.75E+05	6.91E+05	0		
89	0.000221	381		Add note	694.2952	3	2079.864	20.17	1.56	2.94E+05	8.47E+05	0		
	0.000264	453	1 × ы	Add note	696.6377	3	2086.891	52.295	1.33	2.06E+05	1.4E+06	0		
00				Add note	1118.5556	3	3352.645	44.736	1.55	1.02E+06	1.54E+06	0		
	1.26E-05	604							1121220	8.4E+05	1.13E+06	0		
18	1.26E-05 1.98E-05	604 587		Add note	1007.9291	2	2013.844	44.167	1.92	0.4C+UD	1.132+00	U		
00 18 24 35					1007.9291 996.1532	2 3	2013.844 2985.438	44.167 54.614	0.207	2.73E+05	1.19E+06	0		

48

Finally export the inclusion list in the appropriate MS machine format to use in the acquisition of additional MS/MS spectra from new LC-MS run(s).

Export Inclusion Lis	-MS_2.1 Test Set > + 4 Search P
File name: Save as type:	Inclusion
Browse Folders	Save Cancel

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

Congratulations!

This document has taken you through a complete analysis using Progenesis LC-MS, from Alignment through Analysis to generating lists of interesting features 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 LC-MS using your own data and explore the LC-MS Data Import module as well as the rest of the workflow please go to the next section.

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 (Thermo and Waters) or, alternatively convert them to mzXML format first.

To create a new experiment with your files select **New** 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.

New Experiment	- E
Create a new label-free experiment called	
LC-MS Tutorial	
Data type	
Profile data Centroided data	
Resolution (full width at half maximum) 20000 $\stackrel{\wedge}{[r]}$	
Machine type	
Default FTMS	
C Low resolution Ion Trap	
Experiment folder	
Save experiment in the same folder as the run data	
Choose an experiment folder	
Browse	Create Don't Create

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

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

Then locate your data files using the Add data files... link.

LC-MS Tutorial - Progenesis LC	C-MS						
Eile LC-MS Data Reference Run Import Selection All	ground Fit	ering Group Setup Vie	w Results Progenesis Stats P	eptide Search Peptide Pilte	Protein View	nonlin	ear
Import Data m2XML files • 60 m2XML files Thermo .RAW files Waters .RAW folder NetCDF files	id data files Include?	Data processing me Feature detection n Peak processing n					
		Select files	🗸 👻 📑 New Folder	• 47 Search	<u>ب</u> ر	ρ	
		Favorite Links Documents Recent Places Computer Pictures Recently Changed Recently Changed Searches	Name AL.mcoml A2.mcoml A3.mcoml C1.mcoml C2.mcoml C3.mcoml	Date modified 03/06/2008 08:10 03/06/2008 08:10 03/06/2008 08:10 03/06/2008 08:10 03/06/2008 08:10 03/06/2008 08:10	Type MZXML File MZXML File MZXML File MZXML File MZXML File		
✓ Include run in analysis	1	Public Folders	< ["C3.mpmi" "A1.mpmi" "A2	‴ mzeni" ". ♥ [mzXML file Open	s (".mzml) v Cancel	,	
$\boldsymbol{\chi}$ Don't include run in analysis							
Exclude areas from selected ru	un					Section Comple	te (

50

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 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.

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.

LC-MS Tutorial - Progenesis l	LC-MS								- • •
Eile									
LC-MS Data Reference Run Import Selection A	Alignment	Filtering Group Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report	nonlinear
Import Data		Data processing	methods:						
mzXML files 👻 🔺	Add data files	Feature detect							
	Include?	Peak process	ng method:	Profile dat	a				
No problems found		No prob	lems	found					Â
A1	\checkmark X	A1							
A2 A3	Dending								E
AS C1	Pending								
C2	Pending								
C3	Pending								
									-
		No problems fo	und						
		The data file was		th no proble	ns.				
		The data appears				ed by this sol	ftware		
		The data appears		on cer format	to be unarys	cu by and so	- Condi Ci		
✓ Include run in analysis									
X Don't include run in analysis									
Exclude areas from selected	run							Sec	tion Complete 🏵

Note: as each data file is loaded the progress is reported in the **Import Data** list. The dialog below the image reports on the QC of the imported Data files. In this case 'No problems found' with the this data file

Now move to the next stage in the workflow (page 6 in this tutorial) by clicking **Section Complete.**

Add data files.

-

Import Data

Thermo .RAW files

Appendix 2: Stage 1 Data QC review and addition of exclusion areas

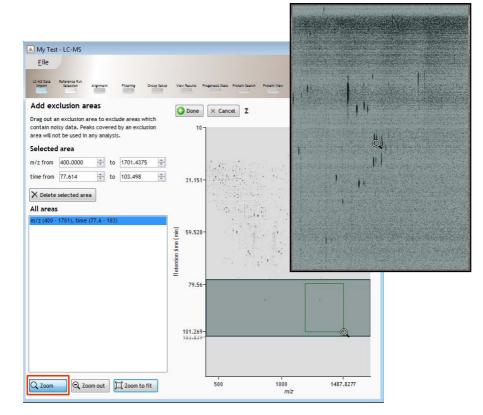
During the process of Data QC you may identify areas of the raw data for a particular run that appear 'noisy' yet still have identifyable 'isotopic patterns'.

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 features in the replicate group.

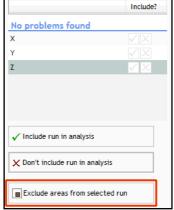
To do this select Exclude areas from selected run on the bottom left of the Software.

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

Note: if you now zoom into the masked area using the **Zoom** tool you will see the isotopic features in the noise.



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



Appendix 3: Licensing Runs (Stage 3)

When setting up a **New experiment** if you are evaluating LC-MS with unlicensed Runs then the licensing page will open after **Reference Run Selection**

LC-MS Data Import	Reference Run Selection		Alignment
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If you already have a programmed dongle attached to your machine then the following License Images page will not appear.

To use this page to License your Runs you must first either obtain an 'Evaluation' License Code from a Nonlinear Sales Person or have purchased a license code directly from Nonlinear.

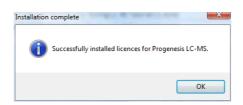
Each code will allow you to license a set number of Runs.

The Runs you wish to License will be listed as shown below.

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

CLC-MS_Tutorial - Progenesis LC-MS Elle LC-MS Data Reference Run Import Selection Licensing Alignme	nt Filtering Group Setup View Results Progenesis Stats Peptide Search Peptide Filter Protein View		nlinear
Dongle License Runs			
This installation is currently restricted to analyse licensed runs only.	Run name	Licence state	License this run
To license your runs, you need an	C:\Users\Andy.Borthwick\Desktop\Tutorial\A1.mznld	Unlicensed	V
evaluation licence code which can be	C:\Users\Andy.Borthwick\Desktop\Tutorial\A2.mznld	Unlicensed	V
obtained from a sales representative.	C:\Users\Andy.Bothwick\Desktop\Tutorial\A3.mznld	Unlicensed	V
Once licensed, your runs can be	C:\Users\Andy.Borthwick\Desktop\Tutorial\C1.mznld	Unlicensed	
analysed on any installation of the	C:\Users\Andy.Borthwick\Desktop\Tutorial\C2.mznld	Unlicensed	
software. The licence is automatically included when archiving an experiment.	C:\Users\Andy.Borthwick\Desktop\Tutorial\C3.mznld	Unlicensed	
If your runs have been licensed on another computer, <u>click here</u> to make the licences available on this computer. If you have one, you can <u>open a licence</u> <u>file</u> to install. If you have just installed a dongle, <u>click</u> <u>here</u> .			
	Run licence code: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx		nce Code
		Section (Complete 🤆

A message confirming successful installation of your image licenses will appear.



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

Appendix 4 Power Analysis (Progenesis Stats)

To explore the third Statistical analysis of the data click on the blue link Ask another question at the top of the table. The selection of 3 tools will appear in the form of questions.

v	Are there any outliers in my data? Does my data cluster according to my groups?
ሐ	Group my features according to how similar their expression profiles are.
Ľ	How many replicates should I run? What is the power of my experiment?

Select the third option to explore the number of replicates required and obtain a measure of the 'power' of the current experiment.

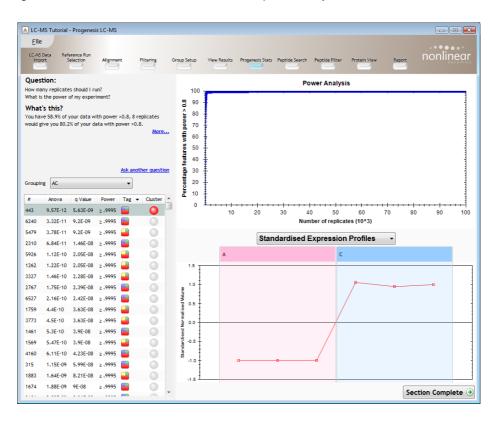
This time the statistically based question(s) being asked of the application is:

'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 features with a power >0.8'

Using all 4000 most abundant features view the power analysis.



This is displayed graphically showing that 58.9% of the 4000 features have a power of 80% or more and therefore 9 replicates would be required to give you 80% 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 5 (a): Search engine parameters (Stage 9) Mascot

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

MASCOT	MS/MS Ions Search		
Your name	Andy	Email	andy.borthwick@nonlinear.com
Search title	Tutorial v2		
Database	NCBInr -		
Taxonomy	Firmicutes (gram-positive bacteria)	•	
Enzyme	Trypsin 👻	Allow up to	1 • missed cleavages
Fixed modifications	Acetyl (Protein N-term) Amidated (C-term)	Variable modifications	
Quantitation	None 👻		
Peptide tol. ±	9 ppm ▼ # ¹³ C 0 ▼	MS/MS tol. ±	0.6 Da 🔻
Peptide charge	1+ •	Monoisotopic	Average
Data file	C:\Users\Andy.Borthwick\Deskt 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 03/09) was used with the Taxonomy restriction set to Fermicutes Variable modifications: Carbamylation(C), OxidationM, Phospho (ST) and Phospho (Y) Peptide Tol: 9ppm Instrument: ESI-Trap

Appendix 5 (b): Search engine parameters (Stage 9) Phenyx

The parameters applied to the Phenyx search that yielded the search results used in this example tutorial are shown below:

IDs	60629	9
Title		
File(s)	C:\Users\Andy.Borthwick\Desktop\LCMS Tu	torial\Abundant C.mgf (mgf 108913 Kb)
Databank(s)	NCBInr (20080114)	
AC		
Taxonomy	Firmicutes	
Scoring Model	ESI-LTQ-Orbitrap (CID_LTQ_scan_LTQ)	
Parent Charge	1,2,3,4 (trust=medium)	
Round #	1	
Modifications	Oxidation_M[variable, <=4] PHOS[variable, <=4] Cys_CM[variable, <=4]	
Enzyme	Trypsin_(KR_noP) miss. cleav. 1 cleav. mode. normal	
Parent tol.	0.01Da	
Pept thresholds	length>=6 score>=6.0 p-value<=1.0E-6	
AC Score	6.0	
Conflict resolution	yes	
Turbo scoring	tolerance=0.5Da coverage >=0.2 series=b;b++;y;y++	

Database : NCBInr (circa 03/09) was used with the Taxonomy restriction set to Fermicutes Variable modifications: Carbamylation(C), OxidationM, Phospho Peptide Tol: 0.01Da

Instrument: ESI-Trap