

Progenesis LC-MS User Guide

Analysis workflow guidelines

for version 3.0



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Introduction

This user guide takes you through a complete analysis of 6 LC-MS runs with 2 groups (3 replicate runs per group) using the unique Progenesis 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 please refer to Appendix 1 (page 50) then start at page 6.

How to use this document

You can print this user guide to help you work hands-on with the software. The complete user guide takes about 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 Data Import 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 user guide document. Alternatively if you would like to arrange a demonstration in your own laboratory contact <u>support@nonlinear.com</u> and we will help you.

LC-MS Data used in this user guide

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

Workflow approach to LC-MS run analysis

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

	Reference Run			Experiment						
Data Import	Selection	Alignment	Filtering	Design Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report
										-

Stage	Description	Page
Data Import	LC-MS Data Import: Selection and review of data files for analysis.	6
Reference Run Selection	Reference Run Selection: Select run to align to.	7
Licensing	Licensing : allows licensing of individual data files when there is no dongle attached (Appendix 3)	7
Alignment	Alignment: automatic and manual run alignment	8
Filtering	Filtering : defining filters for features based on Retention Time, m/z , Charge and Number of Isotopes.	14
	Review Normalisation: explains LC-MS normalisation	17
Experiment Design Setup	Experiment Design Setup: defining one or more group set ups for analysed aligned runs	20
View Results	View Results : review and validate results, edit feature detection, tag groups of features and select features for further analysis	23
Progenesis Stats	Progenesis Stats : performing multivariate statistical analysis on tagged and selected groups	31
Peptide Search	Peptide Search : managing export of MS/MS spectra to, and import of peptide ids from Peptide Search engines	36
Peptide Filter	Peptide Filter: manage peptide ids and filters	39
Protein View	Protein View : validation and resolution of peptide id conflicts for data entered from Database Search engines	41
Report	Report: generate a report for peptides and/or 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.

A Progenesis LC-MS						
Eile						
Experiments						nonlinear
Perform analysis C	ombine analy	ysed fractions				
Recent experiments						Online content
)	Getting started • Progenesis LC-MS Tutorial • Video: quantify. then identify:
	Open Experim	ent				
	Look in:	LC-MS_Tutorial_3.0	•	G 🤣 📂 🛄 -		
	œ.	Name	Date modified	Туре	Size	unities to Version State Shall be and the set of th
	Recent Places	LC-MS Tutorial_3.0.Pro	20/10/2010 15:35	Progenesis LC-MS	278,76	A Data and the second s
	Desktop					
There	Andy Borthwick					• Frequently asked questions
						Who else is using Progenesis LC-MS?
	Computer					Published papers Conference posters
	1					<u>Customer quotes</u>
	Network	•	III		•	La contra c
		File name:			Open	Latest blog posts
		Files of type: Progenesis L	.C-MS Experiments and	Archives (* 🔻	Cancel	Getting increased protein coverage with Progenesis LC-MS
l						Establish and maintain 2D gel quality within your lab
						Wine and proteomics at Proteomlux
Other experiments						
New 🔗 Browse						
View online tutorial						3.0.3960.33326 check for updates

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).



Import	LC-MS Tutorial from archive	
Rep	lace an existing experiment	
Experin	nent to replace:	
Orea Name:	LC-MS Tutorial	

Then press Import.

Restoring tutorial	

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 Data Import Selection Alignment	Filtering Design Setup View Results Progenesis Stats Peptide Search Peptide Filter Protein View Repo	<pre>monlinear</pre>
Import Data mzXML files Add Files Add Files	Data processing methods: Feature detection method: Default Peak processing method: Profile data	
Include? No problems found A1 A2 A3 Pending C1 Pending C2 Pending C3 Pending	At	=
 ✓ Include run in analysis X Don't include run in analysis 	A2 Pending A3 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.	
Exclude areas from selected run		Section 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 Run 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 button (see Appendix 1, page 50).

Tip: the **'Exclude areas from selected run'** facility allows you to examine and exclude areas (usually early and/or late in the LC dimension (Retention Time) that appear excessively noisy due to capture of data during column regeneration (see Appendix 2, page 52). This is 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.



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 if you are using 'Unlicensed' data files to evaluate the software and have no dongle attached..



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 Search	Peptide Filter	Protein V
s 🕶 🎾 AL	utomatic Alignmen	t Vi
	Transition	n 😐

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.

Automatic Al	gnment	×
Select the ru Add Run	ns for automatic alignment vector generation Notes	Vectors
🔳 A1	this run does not need to be aligned as it is the alignment reference	Ref
🔽 A2	automatic alignment will be performed for this run	0
🔽 A3	automatic alignment will be performed for this run	0
🔽 C1	automatic alignment will be performed for this run	0
🔽 C2	automatic alignment will be performed for this run	0
🔽 C3	automatic alignment will be performed for this run	0
	ОК	Cancel

The following pages in this user guide explain in more detail the views and functions of the Alignment stage in the Progenesis LC-MS Alignment, focusing on the Program layout

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.

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.

For more details on manual assistance of Alignment refer to Appendix 4 page 54

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 view 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. This is the reference run you chose at the previous stage, in this case **A1**.

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 run is displayed in green and the chosen reference run 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 **Whole 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



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 view.

If the alignment has worked well then in Windows A and C the grid lines should show minimal distortion, Window B (Transition) 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 run alignment (for more information refer to Appendix 4 (page 54).





Rotate Chromatograms

When **show aligned** is pressed you will see the corresponding effect on the alignment of the 'Total lon Chromatograms'



Stage 5A: 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.



Detection Parameters

The Detection Parameters dialog, showing all the runs in the current experiment and a tick against each run, opens. This is the default setting, where the feature detection algorithm uses information from all of the runs to contribute to the pattern of feature outlines.

Detection Parameters		Detection Parameters	
Runs for detection Detection limits		Runs for detection Detection limits	
Choose runs for detection		Sensitivity	
You can tick or un-tick each run to control which will be used by the feature detection algorithm. Although any run which is left un-ticked will not affect the detected feature outlines, it will still have outlines added to it and will be available in the experiment design setup. Learn more about why you might not want to select all runs.	 ☑ A1 ☑ A2 ☑ A3 ☑ C1 ☑ C2 ☑ C3 	You can adjust the sensitivity of the detection algorithm using these different methods. Each sensitivity method examines the intensities of groups of MS peaks to judge if they are likely to form part of a peptide ion or whether they represent noise and so should be ignored. Peaks which are rejected as noise will not be used to build peptide ion outlines.	Absolute ion intensity Absolute ion intensity % Base Peak The automatic sensitivity method uses a noise estimation algorithm to determine the noise levels in the data. The higher the sensitivity value, the more features will be detected. fewer default more 3
		Minimum retention time wine	dow
		The retention time window is the period over which a peptide has eluted. If you set a retention time window limit, any peptide which has eluted over a shorter period will be rejected.	Apply a retention time window limit RT window limit:
	Detect Cancel		Detect Cancel

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

Note: features outlines will be added to 'un-ticked' runs; however, these runs will not contribute to the detection pattern.

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

The sensitivity of the detection can be controlled by adjusting the sensitivity method and settings under the **Detection Limits** tab. More details on the management of sensitivity are available in the How to do on **Adjusting the Sensitivity of Feature detection.**

For the runs in this user guide we will use the default settings for the Automatic method.

Press **Detect** to start the detection process.

During the few minutes that the automatic analysis requires, a progress bar will appear telling you that it is Analysing.

Analyzing		

On completion of analysis the Filtering stage will open displaying the number of features detected in this example, 14095.

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



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

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

 Inside area 					N	 Inside area 				
<i>m/z</i> from	440.024	to	2001.438		\square	<i>m/z</i> from	440	to	2000	
Time from	12.086	to	76.662	minutes	٢	Time from	10	to	75	minutes

To remove the (in this case 29) features outside of the selected area, press the **Delete 29 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.

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

Filter Features										
You can filter features that you do not wish to include in your analysis by using the criteria below.										
Select all features matching the following filters:										
✓ Inside area										
Nith charge										
Charge 1 (699 features)										
Charge 2 (5559 features)										
Charge 3 (5438 features)										
Charge 4 (1746 features)										
Charge 5 (489 features)										
Charge 6 (43 features)										
Charge 7 (25 features)										
Charge 8 (14 features)										
Charge 9 (6 features)										
Charge 10 (10 features)										

For this user guide, we will filter the area as shown above and also delete a further 766 features with a charge state of 1 or 8 and above by ticking the various options.

Hence all features with a charge state of 1 or 8 and above will appear blue (see above).

To remove these features press Delete 766 Non 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. Before moving on from filtering you should review the normalisation of the experiment.

Stage 5B: Reviewing Normalisation

Normalisation review is accessed from the button at the bottom right corner of the filtering page

Normalisation Note that all remaining features will be used in the normalisation calculation. If deleting a substantial number of features, you should review the normalisation afterwards.	
Review normalisation >>	440

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

Recalculating normal	sation

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

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

Alternatively, if you do not believe normalisation is necessary, you can opt to use un-normalised feature abundances for the rest of the analysis.

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



Calculation of Normalisation Factor:

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

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



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

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



Finally, if you do not wish to work with normalised data then you can **use the raw abundances** by switching off the normalisation.

🔼 LC-MS Tut	orial - Progene	sis LC-MS						N			
<u>F</u> ile Data Import	Reference Run Selection	Alignment	Filtering	Experiment Design Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report	nonlinear
Review no Normalisatio to allow com By assuming unaffected b the factor by normalise ba	ormalisation in is required in parisons acros that a signific y experimenta which the sar ack to its refere	n proteomics as different se ant number I conditions, nple as a wh ence.*	experiments ample runs. of proteins ar we can use iole varies to	Normali Norm	sation Graph nalise to all f	is Normalisa	tion Method	a set of spike	features ()	Don't use a	any normalisation

Note: once you have identified the spike features you can then apply the Normalise to a set of spike features by using this option to locate and select the features

For the experiment you should leave the Normalise to all features option selected.

Now return to filtering by clicking on the button

on the bottom left of the screen

Graph size:	
< Continue filtering features	0-1

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

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

Stage 6: Experiment Design Setup for Analysed Runs

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

There are two basic types of experimental designs:

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

LC-MS	Tutorial - Progenesis LO	C-MS												
<u>F</u> ile	Reference Run			Experiment									••••	
Data Imp	oort Selection	Alignment	Filtering	Design Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report		no	nline	ear
New														
Whic	h experiment de	sign type do y	you want	t to use for thi	s experimer	nt?								
	Between-sub	ject Design			Cı	reate	0-0 0-0 With 0-0	in-subject [Design					
Do only sub	samples from a given y one condition? The ject design.	n subject appea en use the betw	r in reen-	Control	D C1 R	emove	Have you tak under differe within-subjec	en samples fro ent conditions? ct design.	m a given subject Then use the		0 hours	2 hours	4 hours	
To set up this design, you simply group the runs according to the condition (factor level) of the samples. The ANOVA calculation assumes that			he runs of the es that		C2 R C3 R	emove emove	Note: you mu subject for ev subject design	ist have a sampl very condition to n.	le from every o use a within-	Patient 1	C1	A1	AA1	
give the	conditions are indep as a statistical test of conditions are all eq	whether the mouther	eans of	Treatment	D	elete	For example, design for a ti	you would choo ime series expe	choose this type of experiment where every	Dationt 2	C2	A2	AA2	
					A1 <u>R</u> A2 <u>R</u>	emove emove	subject has be To set up this	ubject has been sampled at each time point. To set up this design, you tell the software not only which condition (factor level) each run belongs to but also which subject it came from.	Facienc 2	\$\$6.	Q62.	92.		
				Add an diling	A3 R	emove	only which co belongs to but		. Patient 3	C3	A3	AA3		
			Add condition		The measurements		The software measures ANC	software will then perform a repeated sures ANOVA.	rm a repeated					
							A standard AN the data viola independence	NOVA is not app ates the ANOVA . With a repeat	ropriate because assumption of ted measures ANO	Patient 4	C4	A4	AA4	
							differences (v powerful test	rerences can be source of betwe vhich helps to c).	e eliminated or een condition create a more					
							The within-su an extension include compa repeated mea	bject design ca of the paired-sa arison between asures.	n be thought of as amples t-test to more than two					

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

Additional information on how to apply the Within-subject Design is in Appendix 5 page 59

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

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

Progenesis LC-MS	X									
Create a new experiment design										
Name: AC										
Start with an empty layou	ıt									
○ Copy layout from:	T									
	Create design Cancel									

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

🔺 LC-MS Tutorial - Progenesis LC-MS				
<u>File</u>	Experiment			
Data Import Selection Alignment Filtering	Design Setup View Results	Progenesis Stats Peptide Search	Peptide Filter Protein View	Report nonlinear
	•			DTNAMICS
AC I × New				
Conditions	Runs Add Selected Runs to	Condition 👻 🔎		
Setup the conditions that you want to compare below (e.g., control, drug A, etc), and then assign each of your	Add to new condi	tion C2		C3
samples to the correct condition.	A	Sec.		Sec.
A <u>Delete</u>				
A1 <u>Remove</u>				
A2 <u>Remove</u>				
A3 <u>Remove</u>				
Add condition				
				Section Complete 🧿

To create a new condition

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

Between-subject Design again.

for each condition, A and C, click on the New tab and click on

To create another Design, for example comparing only two replicates All_Conditions New Which experiment design type do 00 Between-subject Design 00 00

Give the new design a name, then tick the **Copy layout from** option and select the **AC** design.

🛆 Progenesis LC-MS 🛛 💌										
Create a new experiment design										
Name:	AC_2									
© Start	Start with an empty layout									
💿 Сору	/ layout from: AC 🔹									
	Create design Cancel									

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

Use the Delete link on the Conditions panel to remove replicates and/or conditions that are not required in this particular design.

🔼 LC-MS Tuto	orial - Progenesis LC-	-MS									
Eile											
Data Import	Reference Run Selection	Alignment	Filtering	Experiment Design Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report	nonlinear
				•							D Y N A M I C S
AC	AC_2 I ×	🗆 New									
Conditions				Runs Add S	elected Runs to	Condition 💌	P				
Setup the co	nditions that you	want to comp	are below		4.2						
(e.g., contro samples to t	l, drug A, etc), an he correct conditi	id then assign on.	each of your		A3						
				1	2349						
A			<u>Delete</u>								
			A1 <u>Remove</u>								
			A2 <u>Remove</u>								
C			<u>Delete</u>								
			C1 <u>Remove</u>								
			C2 <u>Remove</u>								
			C3 <u>Remove</u>								
Add conditi	<u>on</u>										
				<u> </u>							Section Complete
]							Section Complete 🥑

On deleting each replicate the runs will reappear in the Runs window.

Note: both designs are available as separate tabs.

To move to the next stage in the workflow click 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 and the expander bar to the right of the table. 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

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'

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

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

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

Progenesis LC-MS User Guide

LC-MS Run Expression Profile

The 1D Display

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).

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.



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.





Run: Aggregate Solut Solut Aggregate A1 A2 A3 C1 C1 C2 Salut C1 C2 Salut C3 Salut C3 C1 C2 C3





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 runs.

🔼 LC-MS Tutoria	al - Progenesis I	.C-MS										- • •
Eile												
Data import	Reference Run	Alignment	illering D	Experiment	ulla Dessenseis Chai	r Dephide Convels	Dephide Filter	Deskain Views	Depert			
Data Import	Selection	Augment	-ittering - L	lesign setup	auts Progenesis star		Peptide Fitter	Protein view	(eport			nonlinear
-			-	•								
Review Feat	tures Experi	ment design: AC		•								
No filter	applied	Create	A (c				
	(a) Eold	Top - Nator			111111	E GHEARE		. !!				
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3782 1.04E-12	2 Infinity	Add r		11						Late		
142 1.6E-12	Infinity	✓ X Addr			51111				 and and 		- 486 - CH	
1899 3.35E-12	2 Infinity	Add r	1.1 -		11							
10683 5.43E-12	2 Infinity	✓ X Add r	1.5									
7466 7.45E-12	2 Infinity	✓ X Add r			1.1		1.1		10.111		11111	
4126 8.28E-12	2 Infinity	🖌 🖂 🛛 Add r										
3575 1.63E-11	1 Infinity	🗸 📐 Add r				11						
12922 1.92E-11	1 Infinity	Add r		· · · · · · · ·			. E	3				
3642 2.03E-11	1 Infinity	🖌 🗧 🔍 Add r		HIRTON	111111				ingen i Simm			
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1629 3.01E-11	1 Infinity	🖌 🖂 🔜 Add r										
4466 3.28E-11	1 Infinity	🖌 🖂 🔤 Add r							aper 1.22 - 200			
8764 5.24E-11	1 Infinity	🖌 🖂 🔤 Add r			: I !!!!				11 July 111			
9705 5.92E-11	1 Infinity	🖌 🖂 🛛 Add r	1.1									
6615 7.68E-11	1 Infinity	V X Add r										
1385 1.16E-10	0 Infinity	V 🛛 Add r										
2556 1.26E-10	D Infinity	Add r							1111 1 1 1			
6839 1.34E-10	D Infinity											
308 1.38E-10	Jinninty		*									
		,										
✓ Include 1 featu	ure in results	13300 n	arked LC-MS F	Run Expression Profile								
X Don't include 1	1 feature in resu	ts O n	arked Aggre	egate	o l					0.009		
Delete 1 select	ted feature		↓ Se	elect 🔁 Edit							a <mark>ng Kana</mark> ngana	
					24.409	-					-	
				ipir			1 1			튙 42.643-		
1D Display 2D M	Nontage 3D Mo	ntage		Add 🔼 Delete 📲	24.903-	4	1 1			e l	a generation	
Show all outlin	nes		19 U	Indo 🕅 Redo 🦉			1 1			tion		
Multiple colum	nns per condition	ו	Fine	d	25.322-					50 50 50 50 50 50 50 50 50 50 50 50 50 5		
							-4			04./03		
Contrast:		_	Key:	ion charge = 1	25.747-							
				Ion charge = 3								
			-	Ion charge = 4	109	9 1	100	1101	1102	500	1000	1500 2000
Montage size:			_	Ion charge ≥ 5			m/2				m/z	
0 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 <u>Select runs</u> link on the 3D Montage tab. The views 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 views.



1. Locate the feature at approx 1004.77 m/z and 30.453 min using the Find tool.

Find specified location					×
Mass		1004.7700	*	m/z	
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.
- 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 20). An example is described below.

First expand the 'Features' table to show all the details by clicking on the 'Expander bar' to the right of the Review features table.

Review Features Experiment design: AC										
No filter applied Create										
#	Anova (p)	Fold	Tag	▼ Notes ▲						
4562	2.6E-09	Infinity	\checkmark \times	Add r						
6653	2.69E-09	Infinity	\checkmark \times	🔍 Add r						
11401	2.98E-09	3.09E+05	\checkmark \times	🔬 Add r						
5352	3.07E-09	Infinity	\checkmark \times	🔬 Add r						
3382	3.22E-09	Infinity	\checkmark \times	🔬 Add r						
1614	3.4E-09	Infinity	\checkmark \times	🔍 Add r						
2443	3.44E-09	Infinity	\checkmark ×	Add r						
6494	3.58E-09	Infinity	\checkmark ×	🔬 Add r						
5777	3.69E-09	Infinity	\checkmark \times	🔬 Add r	P					
470	3.72E-09	Infinity	\checkmark \times	🔬 Add r						
3940	3.85E-09	Infinity	\checkmark \times	🔍 Add r						
1064	3.87E-09	Infinity	\checkmark \times	🔍 Add r						
5786	3.96E-09	3.25E+06	\checkmark \times	🔬 Add r						
6919	4.09E-09	Infinity	\checkmark \times	🔬 Add r						
11049	4.34E-09	Infinity	\checkmark \times	🔍 Add r						
432	4.43E-09	Infinity	\checkmark \times	🔍 Add r						
9495	4 57F-09	Infinity	./ 🛛	T Add -						
	0			,						
🗸 Incli	ude 1 feature	in results		13300 mark	œd					
X Don	't include 1 fe	ature in resu	ilts	0 mark	ed					
Dele	ete 1 selecteo	l feature								

Then order on Abundance and select between 3000 to 4000 of the highest abundance features, the exact number is not important.

	CL-MS Tutorial - Prozenetis LC-MS																		
Fil	e	riogenesis	LC MD																
-	R	leference Rur	1	Citeria	Experiment	Mary Davida	Deservation Ch		Dantida Casuak	Destide Filter	Deshain Mi								• • •
Data		Selection	Augun	nent Pittering	besign setup	view Results	Progenesis su	ausi	eptide search	Peptide Fitter	Protein Vi	ew Repo	яс					nonii	near
	-																		
Revi	ew Featu	res Exper	iment desigr	AC AC	•														
Y	No filter ap	plied		Create															
#	Anova (p)	Fold	Tag	 Notes 	Highest	Lowest Mean	m/z	z	Mass	RT (mins)	RT window	Abundance	Intensity	Max CV (%)	MS/MS P	rotein	Peptic	e Score Pept	de 🔺
1	0.509	1.04	\checkmark \times	Add note	A	с	805.441	3	2413.301	54.743	7.43	1.24E+08	1.08E+08	8.8	126				
17	0.228	1.1	\checkmark \times	Add note	с	Α	1207.6552	2	2413.296	54.76	4.73	9.42E+07	5.04E+07	9.56	82				
29	1.95E-06	5.73E+04	\checkmark \times	Add note	с	A	1100.5863	3	3298.737	44.787	3.05	8.73E+07	7.87E+07	40.8	17				
64	2.04E-06	1.56E+03	\checkmark \times	Add note	A	с	1176.2271	3	3525.66	47.91	8.65	6.84E+07	2.22E+07	23.7	48				
9	1.32E-06	928	\checkmark \times	Add note	A	c	656.8613	2	1311.708	43.853	4.22	6.11E+07	1.17E+08	25.4	37				
20	1.75E-07	822		Add note	c	A	988.9849	2	1975.955	50.538	4.67	5.33E+07	9.19E+07	13.4	41				
10	5.96E-08	14/		Add note	c	A	663.8693	2	1325.724	46.66	5.08	5.19E+07	1.69E+08	6.49	60				
23 E2	9.25.04	1.12E+04		Add note	د ۱	A	900.9713	2	2120	39.125	4.62	4.73E+07	8.16E+U/	35.3	15				
20	4 955 04	4.1/E+03		Add note	A	C	007 4477	2	1002 881	21 404	4.03	4.255+07	2.520+07	24.7	44				
93	0.0574	2.93		Add note	с С	۵	976.813	3	2927 417	54 367	12.31	3.84F+07	1.58E+07	56.6	188				
48	6.27E-06	3.77E+03		Add note	c	A	1032,4669	3	3094,379	32,837	3.72	3.67E+07	3.95E+07	32.1	31				
12	0.719	1.06		Add note	c	A	763,4082	3	2287,203	43,151	3.51	3.59E+07	7.86E+07	30.5	53				
77	5.12E-06	612		Add note	A	c	882,4228	4	3525,662	47.888	5.26	3.4E+07	1.8E+07	27.1	34				
8	0.75	1.05		Add note	A	c	753,8284	2	1505.642	30,419	3.05	3.37E+07	8.23E+07	17.6	70				
59	3.88E-07	396		Add note	A	с	980.9712	2	1959.928	44.126	2.5	3.14E+07	2.27E+07	14.8	30				
44	6 67E-06	9 89F+03		Add note	r	۵	774 6027	4	3094 387	37 793	4 61	3F+07	4 9F+07	36.3	32	_			-
I							III	-											•
🗸 Inc	lude 1 feature	e in results		13300 marked	LC-MS Run Expressi	on Profile													
V Do	alt include 1 fr	anturo in roc	dt.c	0 mented	Run:								/	0.00	-				
2.00	remender re	eature in res	acs	0 marked	Aggregate														
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					Ion charge =	4	1024.5	-	1025	102	5	1026	1024		500	1000		1500	2000
					Ion charge a	5	1024.0		1025	102: m/z		1020	1020.5		500	1000	m/z	1000	2000
																		Section Con	nplete 🤿

With the 2000-4000 features still highlighted right click on them and select 'New Tag'.



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

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

new tag	9													
Most abundant														
OK Cancel														
Revie	Review Features Experiment design:													
No filter applied Create														
#	# Anova (p) Fold Tag 👻 Notes													
4559	0.0106	5.65		Add note										
1101	0.0149	2.49	< × 🗧	Add note										
3453	1.28E-06	9.71	 × 	Add note										
3342	0.00261	3.41	 × 	Add note										
3527	3.68E-05	186	 × 	Add note										
1597	0.184	1.16	 X 	Add note										
4583	0.0539	139	 X 	Add note										
4377	0.0126	27.7	🖌 🛛 📒	Add note										
1779	1.61E-05	7.96	\checkmark \times	Add note										
5047	0.0357	205	\checkmark \times	Add note										
2479	9.39E-05	157	\checkmark \times	Add note										
3518	0.0112	1.07E+03	\checkmark \times	Add note										
3315	6.23E-05	195	\checkmark \times	Add note										
1525 ∢	0.152	1.44	∢ ×	Add note										
🗸 Incli	ude 2170 feat	ures in result	ts	13300 marked										
× Don	't include 217	0 features in	results	0 marked										
Dele	ete 2170 selec	ted features	;											

Creat

Revi	Review Features Experiment design: AC											
	Vo filter applied											
#	Anova (p)	Fold	Та	g	▼ No	otes	1					
4559	0.0106	5.65	-	×	a	Add note	C					
1101	0.0149	2.49	✓	×	a	Add note	c					
3453	1 28F-06	9 71	-4	X		Add note	A					
3342	Edited			X	a	Add note	C					
3527	Most a	bundant		X	a	Add note	c					
1597	New ta	ig		X	a	Add note	C					
4583	Quick	Tags	•	4	Anova	p-value ≤ 0.05						
4377	🔗 Edit tad	qs		4	Anova	p-value ≤ 0.1						
1779	1.61E-05	7.96	V	4	Anova	p-value ≤ 0.2						
5047	0.0357	205	~	1	Max fo	old change ≥ 1.5						
2479	9.39E-05	157	~	1	Max fo	old change ≥ 2						
3518	0.0112	1.07E+03	~	1	Max fo	old change ≥ 2.5						
3315	6.23E-05	195	~	1	Max fo	old change ≥ 3						
1525	0.152	1.44	1	X	a	Add note	A					

Now right click on any feature in the table and select **Quick Tags** this will offer you a number of standard tags. Select **Anova p-value=<0.05** and either accept the offered name clicking OK or overtype it as required, then click OK.

<u> </u>			
Create	e new tag		×
	Anova p-value ≤ 0.05		
		ОК	Cancel
Y	No filter applied		Create

Click Create to open the Filter dialog and drag the new tag on to the Show features with all of these tags.

Filter the features	
Create a filter Show or hide features based on a selecti to create the filter. For more guidance, p	on of their tags. Move tags to the appropriate boxes lease see the <u>online reference</u> .
Available tags:	Show features that have all of these tags:
Most abundant (2170 features) Anova p-value \$ 0.05 (7847 features)	Show features that have at least one of these tags:
Clear the filter	OK Cancel

Now order the current features in the table by the Highest mean so that all the features showing the Highest mean for condition C are at the top of the list.

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

#	Anova (p)	Fold	Tag 🔻	Notes	Highest	Lowest /		#	Anova (p)	Fold	Tag 🔻
10116	0.05	3.96		Add note	с	A		10116	0.05	3.96	🖌 🗶 📃
12607	8.39E-07	Infinity		Add note	с	А		12607	8.39E-07	Infinity	VX 🔤
3727	0.022	1.42		Add note	с	А		3727	0.022	1.42	<
8745	0.0221	117		Add note	с	А		8745	0.0221	117	✓ × ■
9955	0.0221	60.4		Add note	с	А		9955	0.0221	60.4	
10295	0.0222	1.9		Add note	c	A		10295	0.0222		
6660	0.0219	5 16		Add note	6	Δ		6669	0.0219	Edited	
0007	0.0217	5.10		<u>Add notenn</u>	č	<u></u>	v	040	0.005.07	Most a	bundant
960	8.38E-07	269	 <td>Add note</td><td>С</td><td>A</td><td></td><td>900</td><td>0.30E-07</td><td>Anova</td><td>p-value ≤ 0.05</td>	Add note	С	A		900	0.30E-07	Anova	p-value ≤ 0.05
1171	8.1E-07	350	< × 💻	Add note	с	A		1171	8.1E-07	_	
6903	0.0222	208		Add note	с	А		6903	0.0222	New ta	g
11149	8.31E-07	Infinity		Add note	с	А		11149	8.31E-07	Quick 1	lags
5863	0.022	1.55		Add note	с	А		5863	0.022	🔗 Edit tao	15
10862	0.0219	1.69	 ✓ ✓ 	Add note	С	A		10862	0.0219	1.69	🖌 🖂 🔤
643	8.19E-07	215		Add note	с	A		643	8.19E-07	215	

Create a tag for them called **Significantly up in C**, tagging 3817 features

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

Tag filter applied features may be hidden	Edit
---	------

Filter the features										
Create a filter Show or hide features based on a selection of their tags. Move tags to the appropriate boxes to create the filter. For more guidance, please see the <u>online reference</u> .										
Available tags:	Show features that have all of these tags:									
 Anova p-value ≤ 0.05 (7847 features) Significantly up in C (3817 features) 	Most abundant (2170 features) Show features that have at least one of these tags: Hide features that have any of these tags:									
Clear the filter	OK Cancel									

Notes -

Add note.. Add note...

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Make sure that only the tag for the Most abundant features is shown and press OK.

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

Stage 8: Multivariate Statistics on Selected Features

The user guide now describes 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 Most abundant features.



As an example we will start by examining the behaviour of the **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 representation of answers to questions asked of the analysed data.



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

Principal Component Analysis (PCA)

In **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 experimental conditions?

It answers this question by:

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

PCA can be used to determine whether there are any outliers in the data and also look at how well the samples group. The groupings that can be observed on the 2D PCA plot can be compared to your experimental conditions and conclusions can be drawn regarding possible outliers in your data.

Selecting features in the table will highlight the features 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 6 (page 61)

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-value=<0.05.

On pressing OK the PCA will recalculate using these 7847 features, you can (to save time) stop this calculation by pressing **Cancel calculation** and then set up Correlation Analysis for the 7847 features.

Filter the features Create a filter Show or hide features based on a selection of their tags. Move tags to the appropriate boxes to create the filter. For more guidance, please see the <u>online reference</u> .								
Available tags:	Show features that have all of these tags:							
 Most abundant (2170 features) Significantly up in C (3817 features) 	Anova p-value ≤ 0.05 (7847 features) Show features that have at least one of these tags: Hide features that have any of these tags:							
Clear the filter	OK Cancel							

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

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



Select the second option to explore '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.



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

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 right click on the highlighted features in the table and create a Tag for these features (Up regulated in C).

Create new tag			×
Up regulated in C			
	ОК	Cance	1

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 (Up regulated in A).

Create new tag		X
Up regulated in A		
	OK Cance	1

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

Filter the features	×
Create a filter Show or hide features based on a selection of create the filter. For more guidance, please set	their tags. Move tags to the appropriate boxes to a the <u>online reference</u> .
Available tags:	Show features that have all of these tags:
Most abundant (2170 features) Up regulated in A (4030 features)	Anova p-value ≤ 0.05 (7847 features)
 Significantly up in C (3817 features) Up regulated in C (3817 features) 	Show features that have at least one of these tags:
	Hide features that have any of these tags:
Clear the filter	OK Cancel

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

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

Stage 9: Peptide Search

Progenesis LC-MS does not perform peptide identifications itself. Instead it supports identifications by allowing you to export a set of MS/MS peak lists in formats which can be used to perform peptide searches by various search engines. The resulting identifications can then be imported back into Progenesis LC-MS, using a number of different file types, and matched to your detected features.

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

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Ei	le																					
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	Tag filter	applied						34	9	A1	4/91	No		1.2e+008	2.7e+005	0.2	2	656.8609	1			
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124	42	0	0	 ✓ × 	Add													656.8617	1			
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20	41	0	0	✓ ×	Add									Je reatures triat	nave any or these	tays:		656.8621	1			
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For this example we are using LC-MS runs containing MS/MS data where the data was acquired in a data dependent mode.

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

The first step is to decide which MS/MS scans you wish to export to be identified. By default this is all the available spectra for the Features displayed in the Features list (in this case all the features that have a p value of less than and equal to 0.05. This number is visible on the Export button.

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

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 this set is **20773** as shown on the Export button.

Performing the search Select the search program you're using:
Mascot 👻
Help
Export 20773 ms/ms spectra
Import search results
Import Sour en results
MSMS Preprocessing
MSMS Preprocessing
MSMS Preprocessing Limit fragment ion count 1000

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

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

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

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	Tag filter	applied							Feat	ure ID					Precure	or intensit	_						
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64	48	0	0	× ×		Add			ID	score	less than		•		Protein	description	n cont	tains	•				
62	40	0	0	× 10		Add												_					
53	44	0	0			Add										Include	e in exp	ort	Exclude from	export	Clear all filters		
28	42	0	0			Add	10					~											
124	42	0	0	VX		Add		Expor	rt Han	k i	Kun	Scan nu	Imber Exported	1 2ex 009	2 Zev 005	tensity	(%)	Charge	e Precursor	m/z Isotop	e Id score	Peptide	
6363	41	0	0	VX	1	Add			33	9	Δ1	4/31	No	1.2e+008	2.7e+005	0.	2 4	2	656 8611	1			
20	41	0	0	VX	1	🔬 Add			37	9	A1	4874	No	1.2e+008	1.2e+005	0.	1	2	656.8621	1			
411	39	0	0	V×		🔬 Add			24	9	A1	4966	No	1.2e+008	1.4e+006	1.	2	2	656.8616	1			
165	38	0	0	√ ×		🔬 Add			2	9	A1	5006	No	1.2e+008	1.0e+008	86	5.8	2	656.8612	1			
76	38	0	0	 X 		🔬 Add		V	4	9	A1	5048	No	1.2e+008	6.8e+007	57	7.8	2	656.8624	1			
405	38	0	0	 ✓ × 		🔬 Add			8	9	A1	5094	No	1.2e+008	2.5e+007	21	1.2	2	656.8630	1			
30	38	0	0	 ✓ × 		Add			11	9	A1	5133	No	1.2e+008	1.3e+007	10	0.9	2	656.8624	1			-
9	37	0	0	✓ ×		Add		-		4	A1	51/5	No	11	5 Sealth	^	4	<i></i>	656 X679				+
147 Porf	37 ormina t	0 the search	0	.		Add		Featur	e nun	nber 9), m/z	656.86	13, retentio	n time 43.85	3 min, cha	rge +2							^
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For example: We will make use of the 'Rank' value to reduce the number of Spectra being used for each feature 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.

Ex	port F	lank	#	Run	Scan number	Exported	Feature intensity	Precursor intensity	(%)	Charge	Precursor m/z	Isotope Id so	ore 🔺
	2	3 🖵	· ·		4000	N-	1.0000	1.4000	10	2	CEC 0010	1	
	2	4 Ľ	he rank o	of each	1 MS/MS spect	trum found	d by comparing it	s '%' values against	all other	spectra n	natched to the	same feature.	
	2	5	9	A3	5042	No	9.3e+007	8.8e+005	1.0	2	656.8614	1	
	2	6	9	A1	5379	No	1.2e+008	1.0e+006	0.9	2	656.8610	1	
	2	7	9	A3	5374	No	9.3e+007	7.7e+005	0.8	2	656.8615	1	-
۰													- F

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 reduces the number to spectra to export to **16362**.

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

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



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

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

Performing an MS/MS Ion Search

Having chosen 16362 spectra to export, as described above:

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

Please refer to Appendix 7 (a and b) (pages 62 and 63) for details of the 'Search Engine' parameters

Note: the blue link tells you the appropriate formats for exporting search 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.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.

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link pro	tein IDs to o	detected peaks.							F	Rank	oreater t	han	▼ 10		Featur	e intens	ity les	s than	•				
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#	MS/MS 🔻	Proteins	Score	Tags	•	Notes	*		Ch	arge	less than		•		Precursor int	ensity (%) les	s than	•				
10		1 gi 145953	90.5	V X		Add			Scan nur	nhar				-		Run nar							
35	58	2 gi 145953	95.2	 × 		🔬 Add			Juanna		less than		•			nun nar	cor	itains	•				
115	53	0	0	 × 		Add			Expo	orted	equal to		•	•	Peptide	sequen	ce cor	ntains	•				
123	52	0	0	✓ ×		🔬 Add								_									
87	49	2 gi 145953	72.5	√ ×	1	🔬 Add			lso	tope	less than		•		Protein	accessi	on cor	ntains	•				
64	48	1 gi 255101	117	 ✓ × 		🔬 Add			ID s	core	(In on the second		-		Protein	lescrinti	00	talaa.					
66	46	0	0	 ✓ × 		🔬 Add			10 3	COIC	less than		•		Troteint	leachpu	cor	itains	•				
62	45	0	0	 ✓ × 		Add										Inclu	de in exr	nort	Exclude from exr	ort	Clear all filters		
53	44	1 gi 255101	126	 ✓ × 		Add										mona	ao in esp		Exclude norm exp				
28	42	2 gi 190015	26.6	 ✓ × 		Add		Export	Rank	#	Run	Scan nu	imber Exported	Feature intensity	Precursor inte	ensity	(%)	Charg	e Precursor m/z	Isotop	e Id score	Peptide	
124	42	1 g1/255101	82.3	✓ ×		Add			34	9	A1	4791	No	1.2e+008	2.7e+005		0.2	2	656.8609	1			
6363	41	1 g1/255101	6.48			Add			33	9	A1	4830	No	1.2e+008	4.8e+005).4	2	656.8611	1			
20	41	1 gi 145955	102			Add			37	9	A1	4874	No	1.2e+008	1.2e+005		0.1	2	656.8621	1			
165	38	1 01/257884	10.7			Add			24	9	A1	4966	No	1.2e+008	1.4e+006		1.2	2	656.8616	1	70.0		
76	38	0	0			Add			4	9	Δ1	5048	No	1.2e+008	6.8e+007		57.8	2	656 8624	1	82.4	VFFEGTLAST	
405	38	- 1 gi 126697	109	v x		Add			8	9	A1	5094	No	1.2e+008	2.5e+007		21.2	2	656.8630	1	79.0	VFFEGTLAST	
30	38	3 gi 255101	59.4	VX		Add			11	9	A1	5133	No	1.2e+008	1.3e+007		10.9	2	656.8624	1		•	
9	37	1 gi 255101	82.5	X		Add			15	٩	۸1	5175	No	1 20+002	5 804006		19	2	656 8629	1			Ψ.
147	37	8 gil114565	101	JX		Add	-	4															•
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	Deisotopi	ing and charge de	convolut	ion							m/z								m/z				~
																					Se	ction Complet	e 🤿

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 example the organism under study is *Clostridium difficile*

As an **example** 'Acceptance Criteria' on which to base the sequential filtering of the Peptide results, the following thresholds will 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'

On the Batch detection options panel, set the Score to less than 40, then **Delete matching search results**.

📥 LC-	MS Tutorial	- Progene	esis LC-M	IS														- 0 🔀
Eil	e																	
		Reference	Run				Exp	eriment										
Data	Import	Selectio	n	Augnment		Filtering	Des	ign Setup	View Re	sults P	rogenesi	s Stats	eptide Sear	rch Peptide Hito	er P	Protein view	Report	nonlinear
								•						-			-	DYNAMICS
Feat	ures				Pe	eptide S	earch	Results										
#	Total Hits	m/z	RT(mins	Charge 🔺		Batch delet	ion optio	ns										
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10	9	663.87	46.66	2			3.0010	tess than	•	40				C.I.	10 LIC	ess than	•	
11	8	595.32	36.58	2			Hite	(Inco Albert						Seque	ance 🗔		-	
13	5	573.80	24.10	2				tess than	•					Seque		ontains	•	
14	30	573.32	41.51	2			Macc	(Incombined	_					Acces	sion 🗔		_	
15	30	573.32	40.32	2			11025	less than	•					Acces.		ontains	•	
18	30	498.25	25.07	2		Mass erro	or (oom)	lerr than	_					Descript	tion 🗔	ontains	-	
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23	10	900.97	39.13	2			M/Z	less than	_					Modifica	tion 🗔	ontains	_	
24	16	601.83	40.12	2				tess triain	•						6	ontains	•	
28	17	601.83	41.29	2		Retenti	ion Time	loss than	_									
29	12	1100.59	44.79	3				tess trian	•									
30	21	484.26	24.05	2		Sequence	e Length	less than						De	lete ma	tching search	results Delete non-mat	ching search results Clear all filters
32	10	600.98	39.13	3				tess than								•		
35	20	941.79	58.18	3														
39	10	997.45	31.61	2		#	Score	HITS	m/z	RT (mins)	Charge	Mass	Mass erro	Sequence	A	CCESSION	Modifications	
53	10	1061.01	53.40	2		<u> </u>	02.50	10	000.00	43.05	-	1311.71	0.39		9 El	11255101905		cett surrace protein (s-tayer precursor
61	19	623.83	37.42	2		/ 10	90.54	9	663.87	46.66	2	1325.72	0.69	W IFFEGTLASTIK	S 61	1 145953274		hypothetical protein CdifQ_04003257 [
64	10	1176.23	47.91	3		Z 11	83.08	8	595.32	36.58	2	1188.62	0.12		S 61	1 255101963		cell surface protein (S-layer precursor
65	10	760.90	39.03	2		/ 13	89.03	5	573.80	24.10	2	1145.59	0.67	LGDSDTINLAK	🎯 gi	1 145953274		hypothetical protein CdifQ_04003257 [
69	10	557.64	42.07	3		Z 14	73.23	10	573.32	41.51	2	1144.63	0.11	GILDGSITEIK	🌍 gi	i 255101963		cell surface protein (S-layer precursor
77	9	882.42	47.89	4		/ 14	29.70	10	573.32	41.51	2	1144.63	0.20	EVGGLTVTLEK	🛛 🌒 gi	i 163816188		hypothetical protein COPEUT_02372 [C
86	10	1170.55	59.07	2		/ 14	73.23	10	573.32	41.51	2	1144.63	0.11	GLLDGSITEIK	🌍 gi	i 145953274		hypothetical protein CdifQ_04003257 [
87	20	976.48	52.06	3		Z 15	29.56	10	573.32	40.32	2	1144.63	0.26	EVGGLTVTLEK	🛛 🌒 gi	i 163816188		hypothetical protein COPEUT_02372 [C
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95	20	611 00	25 51	2		/ 15	73.29	10	573.32	40.32	2	1144.63	0.35	GLLDGSITEIK	🌍 gi	i 145953274		hypothetical protein CdifQ_04003257 [
104	40	528.82	39.69	2		/ 18	24.61	10	498.26	25.07	2	994.51	2.48	TDLLKPTK	🕥 gi	i 167747123	[7] Phospho (ST)	hypothetical protein ANACAC_01836 [/
112	20	564.36	36.32	2		/ 18	45.37	10	498.26	25.07	2	994.51	0.77	SELNTIYR	🕥 gi	i 266623151		putative di-trans, poly-cis-decaprenvlc
118	1	1013.46	43.42	3		/ 18	61.72	10	498.26	25.07	2	994.51	0.77	TDLNTLYR	🕥 gi	i 145953274		hypothetical protein CdifO 04003257 [
121	9	825.69	44.74	4		2 20	102.49	10	988.98	50.54	2	1975.96	1.19	FGLVDGTTYST	- a gi	1145953274		hypothetical protein CdifO, 04003257 [
124	10	707.67	53.40	3		2 22	87.81		614 35	19.27	-	1226 69	0.24		a oi	1255101963		cell surface protein (S-laver precursor
127	20	613.31	42.64	2		2 22	110.45	10	000.07	20.12	2	1700.02	0.87			1145953274		hunothetical protein (3/layer precursor
132	10	835.95	42.07	2		2.3	24.20	, 10	/00.9/	40.40	4	4204 (5	5.00		a a a a a a a a a a a a a a a a a a a	1100300214	K1 Contraction attack (C)	hypothecical protein curro_04003257
135	8	929.48	39.95	4		24	21.28	•	001.83	40.12	4	1201.65	5.09		S SI	11102410012	toj carbamidometnyl (C)	transcriptional regulator, Arac family
145	80	616.31	27.70	2	4	24	23.82	10	601.83	40.12	2	1201.65	5.00	CNIDNVCVKGK	21 CT	11190015/03		sensor histidine kinase (Bacillus cereus
148	20	917.48	25.46	2 -	13	53 search r	esults, 60	3 matching	batch dele	ete option	s.							
٠				•	-													Section Complete

Note: the search results matching the filter criteria turn pink and the total is displayed at the bottom of the table (603 matching out of 1353)

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

Delete 60	03 search results?	83
?	Are you sure you want to permanently delete 603 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)

🛆 LC-I	VIS Tutorial	- Progen	esis LC-M	IS															- • •
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Data	Import	Reference	Run	Alignment	Fil	tering	Exper	riment Setup	View Per	culte D	rogeneci	c State - B	Contida Sas	rch Ren	tide Filter	Protein View	Penort		nlinger
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10	0	663.87	45.05	2		So	ore	less than	•						Charge	e less than 🔹			
11	8	595.32	36.58	2															
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32	10	600.98	39.13	3															
39	10	997.45	31.61	2		# 5	core	Hits	m/z	RT(mins)	Charge	Mass	Mass erro	Seque	ence	Accession	Modifications		<u>م</u>]
40	10	980.48	41.97	2		9 82	2.50	10	656.86	43.85	2	1311.71	0.39	VFFEG	TLASTIK (gi 255101963		cell surface protein	(S-layer precursor
53	10	1061.01	53.40	2		11 83	8.08	8	595.32	36.58	2	1188.62	0.12			gi 255101963		cell surface protein	S-laver precursor
61	0	623.83	37.42	2		14 73	3.23	10	573.32	41.51	2	1144.63	0.11	GILDG	SITEIK	gi 255101963		cell surface protein	S-laver precursor
64	10	1176.23	47.91	3		15 73	3.29	10	573.32	40.32	2	1144.63	0.35	GILDG	SITEIK	gi 255101963		cell surface protein	S-laver precursor
65	10	760.90	39.03	2		22 87	7.81	6	614.35	19.27	2	1226.69	0.24	TPSAS	VQPVITE	gi 255101963		cell surface protein	S-laver precursor
77	9	882.42	47.89	4		30 59	.36	9	484,26	24.05	2	966.50	0.75		LYK (gi 255101963		cell surface protein	S-laver precursor
86	0	1170.55	59.07	2		35 95	5.15	10	941.79	58,18	3	2822.35	1.83		VKDFLD	gi 255101963		cell surface protein	(S-laver precursor
87	10	976.48	52.06	3		39 10	2.53	10	997.45	31.61	2	1992.88	0.48	AGETT	EVISTIGET	ei 255101963		cell surface protein	(S-laver precursor
92	0	702.36	42.97	3		40 11	4.37	10	980.48	41.97	2	1958,94	0.38			ei 255101963		cell surface protein	(S-laver precursor
95	0	832.18	54.46	4		53 12	6.15	10	1061.01	53,40	2	2120.00	0.68	CAMSA		a ei 255101963		cell surface protein	S-laver precursor
101	10	611.99	25.51	3		64 11	6.91	10	1176.23	47.91	3	3525.66	1.16		ASADAIIA	a gi 255101963		cell surface protein	(S-laver precursor
112	10	564.36	36.32	2		65 11	3.91	10	760.90	39.03	2	1519.79	0.10	AILAF	SGADESN	a gi 126700129		putative translation	inhibitor endoribe
118	0	1013.46	43.42	3		69 63	88	10	557.64	42 07	-	1669.89	0.03			a gi 5668937		flagellin [Clostridium	difficile
121	0	825.69	44.74	4		77 60	0 14		882.42	47.89	4	3525.66	1.84		ASADAIIA	a gi 255101963		cell surface protein	Slaver precursor
124	10	707.67	53.40	3		87 7	47	10	976 49	52.06	2	2026 /2	0.52	TYNN	GYSNAIF 4	ai 255101963		cell surface protein	S-laver precursor
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1/15	0	929.48 610.00	30 GE	2 2 7	477 5	search results	. 0 ma	tching bate	h delete o	options.								Sectio	n Complete 🕀
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Having applied all 4 filters the Peptide Search Results should be reduced to 478.

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 identified 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.

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

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



Depending on the ordering, make '**cell surface protein precursor etc**' the current protein by clicking on it in Window A (a circular orange symbol indicates current protein).

This protein has 23 peptides assigned (window B) which have a total of 13 conflicts. To view the conflicting assignments click on the **Protein Resolution** tab (window C).

🔼 LC-MS Tutorial	- Progenesis LC-	-MS															- 0	x
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– R	eference Run	Alignment	Filterin	E)	perimen	t	View Decu	lte De	ogenerir Stat	n Deptide Cearch	Deptide Filter	- Brot	ain Vie		Peport			
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🎯 gi 260682215	 23 (11) 	13	2.03E+03	4.08E-(210) 102	10	1502.79	0.223	28.7	2	4		8.36E+05	1	3	(≡
🎯 gi 209571234	24 (12)	13	2.4E+03	2.51E-(V	230	44.5	10	1732.89	0.454	28.9	3	- 1	X	6.89E+05	1	۵.	1
🎯 gi 126700407	9 (2)	9	1.04E+03	0.0007	V	472	77.5	10	1732.89	0.555	28.9	2	1	X	5.28E+05	1	۵.	1
🎯 gi 255656776	9 (2)	9	925	0.0001	V	914	61.4	8	1115.57	0.153	27.5	2	1		1.42E+05	1	S	1
🎯 gi 126699128	4 (2)	3	386	0.0036	V	285	55	10	1051.55	0.206	23.3	2	<	×	4.99E+05	1	9	1
🎯 gi 126699078	3 (1)	3	315	0.0432	V	401	127	10	1692.93	0.108	45.3	2	 ✓ 		8.5E+05	1	S	1
gi 126698718	5 (4)	1	301	0.0004	· 🔽	473	124	10	2096.02	0.506	42	2	~		1.49E+06	1	<u></u>	1 *
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I Totelli. gijz		cell surfac	e protein (putative	hema	gglu	itinin/adl	hesin)	precurso	r [Clostridium	difficile CD	196]						
Protein: gi 2	209571234 0	cell surfac	ce protein (rotein V [C	putative lostridiu	hema m diffi	gglu cile]	itinin/adl	hesin)	precurso	r [Clostridium	difficile CD	196]						
Protein: gi[2 Peptide Views Pr	209571234 c	cell surfac	ce protein (rotein V [C	putative Iostridiu	hema m diffi	gglu cile]	ıtinin/adł	hesin)	precurso	r [Clostridium	difficile CD	196]						
Protein: gip Peptide Views Pr Conflicting p	209571234 c rotein Resolution	cell wall p	ce protein (rotein V [C	putative lostridiu	hema m diffi Pepti	gglu cile] des (of gi 209	hesin) 957123	precurso	r [Clostridium	difficile CD	196]						
Protein: gip2 Protein: gip2 Peptide Views Pr Conflicting p Accession	209571234 c rotein Resolution proteins for f Peptides	cell wall p cell wall p ceature 21 Conflicts Pr	ce protein (rotein V [C 0 rotein Score	putative Iostridiu Pe	hema m diffi Pepti #	gglu cile] des (of gi 209 Score	h esin) 957123 Hits	precurso 34 Mass	r [Clostridium	difficile CD	196] Charge	Tags	•	Abundance	Conflict	s Peptid	•
Protein: gip2 Protein: gip2 Peptide Views Pr Conflicting p Accession gip20957123-	209571234 c rotein Resolution proteins for f Peptides 4 24 (12)	ell wall p eature 21 Conflicts Pr 13 2.4	ce protein (rotein V [C 0 rotein Score 4E+03	Putative Iostridiu Pe V 10	hema m diffi Pepti	gglu cile] des (of gi 209 Score 77.5	957123 Hits	precurso 34 Mass 1732.899	r [Clostridium Mass error (p 0.555	RT (mins) 28.9	196] Charge 2	Tags ✓ ×	•	Abundance 5.28E+05	Conflict:	s Peptid	•
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In this case the conflicting peptide assignments are with **Cell wall protein V** (from a different strain) which contains 13 conflicts. To resolve this conflict un-assign all the conflicting peptides (showing 1) assigned to the **Cell wall protein V**, by selecting and then unticking all the peptides in window D.

Data Import Sele	lection /			Exp	erimen											
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gi 255656776	(2)	9	925	0.0001	V	828	93.4	6 1823.8	78 0.428	54.7	2	<	\times	5.64E+05	0	9
gi 126699128	A (2)	3	386	0.0036	V	1212	114	10 2079.9	0.767	56	2	✓	\times	4.62E+05	0	3
gi 126699078	3 (1)	3	315	0.0432	V	1589	48.3	8 1698.	1 0.0809	30.6	2	<	\times	2.26E+05	0	3
gi 126698718	5 (4)	1	301	0.0004 ~	V	1670	68.4	5 1293.6	0.743	29.2	2	- 1	\times	7.14E+04	0	3
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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

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.

<u>File</u>	e Pue			Eve	arimant												•	
ata Import Select	ion A	Alignment	Filterin	g Desi	gn Setup	Vie	w Results	Proger	esis Stats	Peptide Search	Peptide Filter	· Protein	View	Re	eport	non	lin	e
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gi 126698450 (+1)	12	0	1.21E+03	4.05E-0	V	2986	57.8	2	1580.792	0.764	30.4	2			6.65E+0	4	0	6
gi 5668937 (+1)	12	0	1.41E+03	2.03E-0	V	2567	49.4	3	1335.644	1.22	23.8	2			6.15E+0	4	0	6
gi 209571234	12	0	1.25E+03	2.51E-0	V	437	49.9	8	1153.635	0.268	37.1	2		(4.23E+0	5	0	6
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Protein: gi 12669 Protein: gi 12669 Protein: gi 12669 Nide Views Protein R	8718 nit 9140 fer lesolution	roreduct	ase-family	y protein	[Clostr e subu	idium nit alp	difficile ha [Clo	630] stridiu	m difficil	m e 630]								
Protein: gi 126694 Protein: gi 126694 Protein: gi 126694 Dide Views Protein R Conflicting protein Accession F	8718 nit 9140 fer tesolution ns for fea	troreduct rredoxin- ature 84	ase-family NADP(+)	y protein reductase	<pre><[Clostr Clostr e subu Peptide #</pre>	idium nit alp es of g Sco	difficile oha [Clo gi 12669 re Hi	630] stridiu 09140 ts Mat	m difficil	m e 630]	Π (mins)	Charge 1	Tags	✓ At	oundance	Confli	ets Pe	epti
Protein: gi 126699 Protein: gi 126699 Protein: gi 126699 titide Views Protein R Conflicting protein Accession F ati 126698718 •	8718 nit 9140 fer lesolution ns for fea peptides Co	ature 84	ase-family NADP(+) I	protein reductase	(Closti e subu Peptide #	idium nit alp es of g sco 4	difficile ha [Clo gi 12669 re Hi 5.7 !	630] stridiu 09140 ts Mat	m difficil	m e 630] ss error (p F 7.86	T (mins) 36.3	Charge 1	Tags	✓ At 1.8	oundance 3E+05	Confli	ts Pe	epti
Protein: gi 126699 Protein: gi 126699 ptide Views Protein R Conflicting protein Accession F g 126698718 • g 126698718 •	8718 nit 9140 fer tesolution ns for fea 2eptides Co 5 1	ature 84 0 301 0 40.	ase-family NADP(+)	0.021 y protein reductase P ♥ 67 ■ 45	<pre>(Clostr e subu Peptide # 849 261</pre>	idium nit alp es of g Sco 4 9 4	difficile ha [Clo gi 12669 re Hi 5.7 !	630] stridiu 09140 ts Mat 5 117 5 143	m difficil ss Ma 5,609 2.755	m e 630] ss error (p F 7.86 0.768	T (mins) 36.3 29.7	Charge 1 2 v 3 v	Tags	 At 1.8 3.5 	pundance 3E+05 57E+04	Confli 0 0	ts Pe	eptic I I

In the above example the conflict would be resolved in favour of the protein with 5 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

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



Having performed the conflict resolution with **Group similar proteins** and **Quantitate from non-conflicting features** now switch off the protein grouping.

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

roteins		No. Chara				Pep	tides (of gi 566	68937							T (11 11- d		
l'occinis	Y	NO TILLER	appued	Create											7	features may be h	idden	Edit
Accession	Peptides	Conflicts	Score	Anova (p)*	*		#	Score	Hits	Mass	Mass error (p.	. RT (mins)	Char	rge Ta	ags	 Abundance 	Cont	flicts P
gi 5668937	12 (3)	14	1.41E+03	4.15E-06	μ.		446	93.3	10	1676.838	1.2	34.5	2	 ✓ 	$ \times $		1	i 🎯
gi 126697810	9 (0)	14	1.13E+03			V	3335	84.7	4	1423.65	0.405	22.5	2		X	7.39E+04	1	3
gi 255654924	7 (0)	9	645			\checkmark	147	101	10	1230.609	0.44	22.7	2	 ✓ 	'X	3.07E+06	1	3
gi 126698450	12 (5)	9	1.21E+03	1.28E-06		\checkmark	166	125	10	2317.115	0.168	38.7	2	 ✓ 	X	5.55E+06	1	3
gi 10180205	1 (0)	1	63			V	179	60.9	9	2317.115	0.201	38.7	3	✓	X	3.09E+06	1	3
gi 255101963	29 (28)	1	3.37E+03	2.31E-05		V	238	107	10	1716.857	0.429	30.4	2	 ✓ 	X	1.73E+06	1	Ś
gi 70632806	1 (0)	1	66.9			V	564	51.2	4	1716.858	0.394	30.4	3	✓		3.57E+05	1	Ś
Protein: gi 56 Protein: gi 12	1 (0) (68937 fl: 6697810	agellin flagelli	⁶³ Clostridiun n subunit [(n difficile) Clostridiur	m dif	ficile	• 630]	/0 5	10	<u>1676 838</u> II	1 27	34 F	3			7 505105	1	•
Protein: gi 56 Protein: gi 56 Protein: gi 12 ptide Views Pro Conflicting pro	1 (0) 668937 fla 6697810 tein Resolution oteins for	agellin flagelli	63 Clostridium n subunit [4 2 446	… n difficile] Clostridiur	m dif Pe	ficile	• 630]	^{≠0 E}	7810	1676 939 II	1 37	34 5	3			7 ROFANK	4	•
Protein: gi 12 Protein: gi 12 Protein: gi 12 Protein: gi 12 Protein: gi 12 Protein: gi 12	1 (0) 10 10 10 10 10 10 10 10 10 10	agellin flagelli on feature Conflicts	63 Clostridium n subunit (4 e 446 Protein Score	n difficile] Clostridiur	m dif Pe	ficile	283 • 630] es of g	10 5 i 12669 e Hit:	7810 s Mass	1474 939 III S Mat	1 37 1 ss error (p	₹4 5 RT (mins)	Charge	Tags		Abundance	Conflicts	Pepti
Protein: gi 1255555774 Protein: gi 12 protein: gi 12 ptide Views Pro Conflicting pro Accession gi 15668937	1 (0) 668937 fl: 6697810 tein Resolution oteins for Peptides 12 (3)	agellin flagelli flagelli flagelli conflicts 14	63 Clostridium n subunit (4 2 446 Protein Score 1.41E+03	n difficile] Clostridiur Pep	m dif Pe	ficile	283 e 630] es of g Scor	10 F (i 12669 e Hit: 01 10	10 7810 s Mass 0 1230	s Ma:	1 27 1 ss error (p 1 0.44	34 5 RT (mins) 22.7	Charge 2	Tags		Abundance 3.07E+06	Conflicts	Pepti
Protein: gi 56 Protein: gi 56 Protein: gi 12 ptide Views Pro Conflicting pr Accession	1 (0) 66937 fl: 6697810 tein Resolution oteins for Peptides 12 (3) 9 (0)	agellin flagelli on feature Conflicts 14	63 Clostridiun n subunit [4 2 446 Protein Score 1.41E+03 1.13E+03	n difficile] Clostridiur Pep V 93.3 V 93.3	n dif Pe	ficile	283 630] es of g Scor 10 11	10 F i 12669 e Hit: 11 10 25 10	10 7810 5 Mass 0 1230 0 2317	s Ma: 0.609 7.115	1 27 55 error (p 1 0.44 0.168	34 E RT (mins) 22.7 38.7	Charge 2 2	Tags ✓×		Abundance 3.07E+06 5.55E+06	Conflicts 1 1	Pepti
Protein: gi 56 Protein: gi 12 Protein: gi 12 Protein: gi 12 Protein: gi 12 Protein: gi 12 Conflicting pr Accession gi 12668937 gi 126697810	1 (0) 66937 fl: 6697810 tein Resolution oteins for Peptides 9 (2) 9 (0)	agellin flagelli feature feature 14	63 Clostridium n subunit [4 2 446 Protein Score 1.41E+03 1.13E+03	n difficile] Clostridiun Pep Ø 93.3 Ø 93.3	m dif Pe	ficile	283 630] es of g Scor 7 10 60	x0 E i 12669 e Hit: 01 10 25 10 0,9 9	10 7810 s Mass 0 1230 0 2317 2317	s Mas 0.609 7.115 7.115	1 27 ss error (p 1 0.44 0.168 0.201	24 E RT (mins) 22.7 38.7 38.7	Charge 2 2 3	Tags ✓ × ✓ ×		Abundance 3.07E+06 5.55E+06 3.09E+06	Conflicts 1 1 1	Pepti A L L L
Protein: gi 56 Protein: gi 12 Protein: gi 12 Protein: gi 12 Protein: gi 12 Conflicting pr Accession gi 5668937 gi 126697810	1 (0) 168937 fl: 16697810 16697810 16697810 16697810 16697810 16697810 16697810 16697810 169	agellin flagelli on feature Conflicts 14 14	A3 Clostridium n subunit [4 2: 446 Protein Score 1.41E+03 1.13E+03	n difficile) Clostridiun Pep V 93.3 V 93.3	m dif Pe	ficile	283 630] es of g Scor 10 60 11 10 10 10 10 10 10 10 10 1	10 F 11 12669 e Hit: 10 10 25 10 9,9 9 07 10	7810 s Mass 0 1230 0 2317 2317 0 1716	1474 838 m s Mas 0.609 7.115 7.115 5.857	ss error (p 0.44 0.168 0.201 0.429	24 E RT (mins) 22.7 38.7 38.7 30.4	Charge 2 2 3 2	Tags ✓ × ✓ ×		Abundance 3.07E+06 5.55E+06 3.09E+06 1.73E+06	Conflicts 1 1 1 1	Pepti S A S L S V
Protein: gi[36 Protein: gi[12 Protein: gi[12 Protein: gi[12 Protein: gi[12 Protein: gi[12 Conflicting pr Accession gi[126697810	1 (0) 66937 fl: 6697810 tein Resolution oteins for Peptides 9 (2) 9 (0)	agellin flagelli on feature Conflicts 14 14	A3 Clostridium n subunit [0 2: 446 Protein Score 1.41E+03 1.13E+03	n difficile] Clostridiun	n dif	ficile	283 630] es of g Scor 10 10 10 10 10 10 10 10 10 10	40 F ii 12669 e Hits 01 10 25 10 0.9 9 907 10 .2 4	7810 5 Mass 0 1230 0 2317 2317 0 1716 1716	1474 838 m s Mas 0.609 7.115 5.857 5.858	1 33 ss error (p 1 0.44 0.168 0.201 0.429 0.394	24 E RT (mins) 22.7 38.7 38.7 30.4 30.4	Charge 2 2 3 2 3 2 3	Tags ✓ × ✓ × ✓ ×		Abundance 3.07E+06 5.55E+06 3.09E+06 1.73E+06 3.57E+05	1 Conflicts 1 1 1 1 1 1 1 1	Pepti Pepti A L L V V V V
Protein: gi[36 Protein: gi[12 ptide Views] Pro Conflicting pr Accession gi[126697810	1 (0) 66937 fl: 6697810 tein Resolution oteins for Peptides 12 (3) 9 (0)	agellin flagelli on feature Conflicts 14	A3 Clostridium n subunit (2: 446 Protein Score 1.41E+03 1.13E+03	m difficile] Clostridiun Pep ♥ 93.3 ♥ 93.3	Pe	ficile	283 e 630] es of g scor 10 0 10 10 10 10 10 10 10 10 10 10	iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii	7810 s Mass 0 1230 0 2317 2317 0 1716 1716 0 1676	1476 838 II s Mai 0.609 7.115 5.857 5.858 5.838	1 32 ss error (p 0.44 0.201 0.429 0.394 1.32	24 E RT (mins) 22.7 38.7 30.4 30.4 30.4 34.5	Charge 2 2 3 2 3 3 3	Tags ✓ × ✓ × ✓ × ✓ × ✓ ×		Abundance 3.07E+06 5.55E+06 3.09E+06 1.73E+06 3.57E+05 7.59E+05	Conflicts 1 1 1 1 1 1 1 1 1 1	Pepti Pepti A L L V V V V I I
e11255656774	1 (0) 68937 fl: 6697810 tein Resolutio oteins for Peptides 9 12 (3) 9 (0)	agellin flagellin feature Conflicts 14	43 Clostridium n subunit (2: 446 Protein Score 1.41E+03 1.13E+03	n difficile] Clostridiur Ø 93.3 Ø 93.3	Pe	<pre></pre>	283 630] es of g Scor 7 10 60 61 61 64 61 64 60 61 60 60 60 60 60 60 60 60 60 60	19 5 i 12669 e Hits 10 10 25 10 0.9 9 07 10 .2 4 0.5 10 .5 10	7810 s Mass 0 1230 0 2317 2317 0 1716 1716 0 1676	1474 838 II s Mas 0.609 7.115 5.8557 5.858 5.838	1 22 ss error (p 0.44 0.168 0.201 0.429 0.394 1.32	24 E XT (mins) 22.7 38.7 30.4 30.4 34.5 34.5 34.5	Charge 2 2 3 2 3 3 3	Tags ✓ × ✓ × ✓ × ✓ × ✓ ×		Abundance 3.07E+06 5.55E+06 3.09E+06 1.73E+06 3.57E+05 7.59E+05	1 1 1 1 1 1 1 1 1 1 1 1	Pept

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

Proteins	V No	filter appl	ied	Create	Pep	otides	of gi 566	8937				7	Tag f featu	filter applied ures may be hidd	len Ec	dit
Accession	Peptides	Conflicts	Score	Anova i 4	•	#	Score	Hits Mass	Mass error (p	RT (mins)	Charge	Tags	•	Abundance	Conflic	ts F
🌍 gi 255101963 (+1)	29	0	3.37E+03	2.31E-0		69	63.9	10 1669.8	89 0.0302	42.1	3	\checkmark	< 📒	4.75E+06	0	G
🗿 gi 260682215	23	0	2.03E+03	6.28E-0	V	132	103	10 1669.8	38 0.646	42.1	2	\checkmark	< 📒	4.38E+06	0	6
🗿 gi 209571234	12	0	1.25E+03	2.51E-0	V	147	101	10 1230.6	09 0.44	22.7	2	\checkmark	< 📒	3.07E+06	0	6
🗿 gi 126698450 (+1)	12	0	1.21E+03	4.05E-0	V	166	125	10 2317.1	15 0.168	38.7	2	\checkmark	(5.55E+06	0	6
gi 5668937 (+1)	12	0	1.41E+03	2.03E-0	V	179	60.9	9 2317.1	15 0.201	38.7	3	\checkmark	< 📒	3.09E+06	0	6
gi 126697969 gi 56	68937 - fla	gellin [Clo	stridium diffi	cile]		238	107	10 1716.8	57 0.429	30.4	2	\checkmark	(1.73E+06	0	(
gill/	ISSU IVIII		double the locks	a selection and a selection of the selec												
Protein: gi 56689: No protein select	37 flage ted	ellin [Clo	ostridium d	ifficile]	IE 050]		P1 A			<u> </u>				A F7F AF	î	
Protein: gi[56689: No protein select ptide Views Protein Re Conflicting protein	37 flage	ellin [Clo	ostridium d	ifficile]	Peptid	es of o	conflicting	g protein	117 2.20 <i>1</i>						^	
Protein: gi[56689: No protein select eptide Views Protein Re Conflicting proteir Accession Pep	37 flage ted esolution tides Cont	ellin [Clc	ein Score	ifficile]	Peptid	es of (conflicting re Hits	g protein Mass	Mass error (p R	(mins) C	harge Ta	gs	▼ Ab	undance (Conflicts P	ept
Protein: gi 56689: No protein select ptide Views Protein R Conflicting proteir Accession Pep	37 flage red esolution 15 tides Cont	Ricts Prot	ein Score	ifficile]	Peptid	es of (conflicting re Hits	g protein Mass	Mass error (p., R1	r (mins) C	harge Ta	gs ·	✓ Ab	undance (Conflicts P	ept

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

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 C

LC-MS Tutorial	- Progen	nesis LC-N	4S														
<u>F</u> ile																	
Re Re	eference Selectio	Run /	lianment	Filtering	Experi	ment Setup	View Perults	Pro	generic State	Pentide S	aarch	Pentide Filte	r Protein View	. Report	n	nli	noar
					Congri						Jaron				μΥ		neai
Experiment design Proteins	n: AC			•	ag filter appl	ied e bidden	Edit		Peptides	of gi 12	66986	540		No filter	applied		reate
					locents may b	e maden	Edit							1			eate
Accession		Peptides	Conflicts	Score v	Anova (p)*	Fold	Tags	<u> </u>	#	Score	Hits	Mass	Mass error (p	RT (mins)	Charge	Tags	✓ Abu
🎯 gi 255101963	(+1)	29	0	3.37E+03	2.31E-05	4.87		Ε	573	60.8	6	1395.834	0.00058	50.4	2	\checkmark \times	2.9E
S gi 260682215		23	0	2.03E+03	6.28E-05	3.52	•		745	102	10	1541.867	0.147	63.1	2	\checkmark \times	2.44
S gi 126698450	(+1)	12	0	1.21E+03	4.05E-06	16.8			1077	112	10	1585.883	0.139	62.6	2	\checkmark \times	1.88
🎯 gi 126700407		9	0	1.04E+03	0.945	1.01			1928	62	9	1585.882	0.343	62.6	3	\checkmark ×	4.75
🔮 gi 126700790		8	0	687	3.18E-05	2.46			1265	70.6	10	1088.55	0.624	35.1	2	\checkmark \times	8.22
🎯 gi 126698640	0	7	0	597	0.000651	1.79			1363	77.8	9	1271.65	0.461	23	2	\checkmark \times	8.25
🎯 gi 126698631		5	0	574	0.000151	2.77	-		3310	66.8	5	1125.649	1.64	34.3	2	\checkmark \times	2.4E
🎯 gi 126699063		6	0	368	2.03E-06	18.5	-		6805	44.9	2	1211.582	0.504	22.8	2	\checkmark \times	- 1.14
🎯 gi 126698643		4	0	365	0.0132	2.26	-	-									
•							+		•								۰.
1 Protein: gi	26698	640 el	ectron tra	ansfer flav	oprotein a	Ipha-s	ubunit [Clo	strid	lium diffic	ile 630]							
Peptides of	selec	ted prot	tein														
Peptide Views P	rotein Re	solution															
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Protein options	1														Section	n Comp	olete 🌛

This will filter the Protein list so that it now only displays only the proteins containing peptides that show increased expression in ${\bf C}$

	Peptide count
	Confidence score
You can export this filtered Protein list (csy format) by selecting	📝 Anova (p)*
the estimation from the Fi le end of Management of the later of the	Max fold change
this option from the File menu. You can control the data output	Description
required, using the dialog provided.	Normalized abundance
	Raw abundance

Accession	
Peptide count	
Confidence score	
📝 Anova (p)*	
Max fold change	
Description	
Vormalized abundant	ce
Raw abundance	
Spectral counts	
Tags	

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 proteins that show increased expression for strain C.



- 1. First reduce the features to report on by selecting the '**Up regulated in C**' tag. In this example it reduces the number of features in the table to 3817.
- 2. Expand the various Report Design options (by default they are all selected)
- 3. Un-tick as shown below
- 4. Click Create Report

.C-MS Tu File	itorial - Prog	enesis LC-	-MS										
ata Import	Reference Selectio	Run Al	lignment	Filteri	ng l	Experiment Design Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report	nonline
atures							Report	Design					
xperimer	nt design:	AC				•	Title	LC-MS Tutorial					
feati	ures may be	e hidden				Edit	Select th	ne sections you	wish to include	in your report	:		
# /	Anova (p)	Fold	Tag 💌	Report	Notes			Overview run					
0442 5	.821e-005	Infinity		V	D			Data processir	ig methods				
0454 5.	.024e-007	Infinity		V	a		List th	e processing me	ethods used for	r peak and feat	ure detection		
250 1.	.132e-007	Infinity		V	a								
0405 1.	.124e-007	Infinity		V	a			Experiment de	sign				
278 5.	.170e-010	Infinity		V	D		Includ	Protein report	a protein abun	dances and ner	tides identified	for each pr	otein
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.0557 2.	.724e-008	Infinity		1	D			Peptide table	5				
.0571 2.	.879e-004	Infinity		1	a			Feature table					
.0484 1.	.358e-004	Infinity		V	a			Feature details	•				
.0511 5.	.337e-007	Infinity		V	a								
.051€ 8.	.951e-008	Infinity		1	12		Crea	te Report					
.0388 5.	.619e-006	Infinity		1	a								
.0294 4.	.868e-006	Infinity		V	a								
.0307 1.	.215e-006	Infinity		V	a								
.0313 1.	.371e-005	Infinity		1	12								
.0284 5.	.761e-008	Infinity		1	a								
.028€ 3.	.275e-004	Infinity		1	a								
.0291 3.	.795e-006	Infinity		1	12								
282 1	116e-006	Infinity			-	*							

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-M	S Tut	oria	al																
Experime	ent: LC-/	MS 11	Itorial																
Report ci	reated:	25/1	0/2010	14:	51:51														
Proteins	s																		
Protein bui	ilding opt	tions																	
Protein gro	ouping	Gro	up simila	ar p	roteins	L		61:											
Protein qu	Idnuiduu	on Usi	ng only te	eati	ures wit	n no pr	otein	conti	CES										
Accession	Pept	tides	Score	An	ova (p)	Fold	Tags	Descr	iption					Aver	age Norma	lised			
														Abu	ndances				
gi1255101	963	29	3369-31	2	310-005	4 87		coll si	irface prof	nin (S.Ia	ver pr	ecursor prot	oin)	A	368+008	8 96e+007			
51255101	<u></u>	27	5567.51	2	510 005	4.07	-	[Clost	ridium dif	ficile QC	D-63q	42]	ciny		500.000	0.700.007			
gi 2606823	215	23	2034.50	6.:	28e-005	3.52	•	cell su hema diffic	urface prot gglutinin/a ile CD196]	tein (put adhesin)	ative precu	rsor [Clostri	dium	2.1	38e+006	1.01e+007			
gi 1266984	450	12	1205.26		gi 1	2669	845	0											
gi 1267004	407	9	1039.27		ABC tr	anchor	tor r		ato-bindi	ng lino	prote	vin [Clostri	idium di	fficil	- 620l				
gi 126700	790	8	686.67		12 pep	tides	ter, 5	ubsu a	ate-bindi	ng upo	prote	in [Closur		mena	- 030]				
gi 126698	640	7	597.15																
gi 126698	631	5	574.32		Seque	nce			Feature	Score	Hits	Mass	Charge	Tags	Conflicts	Modificati	ons	Average Norm Abundances	alised
gi 126699	063	6	368.44															A	С
ai142((00)	(42)		2/E 00		ALGM	EKPSNEI	NISYVK		3803	53.86	3	1778.8867	2		0			920.05	4.57e+004
<u>gi 126676</u>	<u>643</u>	4	365.00		ALG	EKPSNEI	NISYVK		3085	40.19	5	1794.8816	3		0	[4] Oxi	dation (M)	1949.77	4.26e+004
gi 255101	959	4	359.78		DIPILI	TAVSDP	VAAGL	VK	1226	81.49	10	1891.1038	3		0			3946.33	1.56e+005
					DIPILI	TAVSDP	VAAGL	VK	324	102.06	10	1891.1034	2		0			2.66e+004	1.32e+006
					GALA	QGINY	zafn, Ek	41K	2029	74.72	8	1965.0197	2		0	Carban	[5] nidomethyl	1.31e+005 1.88e+004	7.98e+005 9.41e+004
					GITTS	NEVNQO	GISSLV	GK	413	78.36	6	1802.9354	2		0		(C)	3.96e+004	9.45e+005
					IATEN	KIPVIAA	ESGPV	EK	2207	60.13	3	1965.0773	2		0			1710.03	1.71e+005
					IATEN	KIPVIAA	ESGPV	EK	679	51.81	6	1965.0768	3		0			2.05e+004	3.79e+005
					IDVLY	/PTDNL	VASSM	PIVSK	3477	72.50	5	2260.2017	2		0			537.79	7.04e+004
					IGITQ	LVEHPA	LDATR		926	75.70	6	1732.9471	2		0			9505.86	3.28e+005
					IGITQ	LVEHPA	LDATR		298	62.06	10	1732.9469	3		0			4.76e+004	6.76e+005
					IPVIAA	ESGPVE	K		398	92.32	8	1308.7284	2		0			3.19e+004	5.36e+005
					KIGITO			R	4088	55.57 89.09	3	1861.0416	2		0			65 70	4 79e+005
					TLEKP	GTNVSC	GTSDF	/SVDK	752	82.58	6	2080.0317	2		0			2.39e+004	5.88e+005
					TLEKP	GTNVSC	GTSDF	/SVDK	406	51.30	9	2080.0302	3		0			5.13e+004	7.28e+005
					Tags M A S U	ost abu nova p- ignifica p regula	ndant value s ntly up ited in	≤ 0.05 in C C											

Creating an Inclusion list

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

As an example we 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.

First return to View Results using the Workflow icons.



Then click on the **Expander button to the right of the Review Features table** to expand the table.

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**.

A Filter the features	
Create a filter Show or hide features based on a selection of create the filter. For more guidance, please se	their tags. Move tags to the appropriate boxes to te the <u>online reference</u> .
Available tags:	Show features that have all of these tags:
Most abundant (2170 features) Up regulated in A (4030 features) Significantly up in C (3817 features) Up regulated in C (3817 features)	Anova p-value ≤ 0.05 (7847 features) Show features that have at least one of these tags: Hide features that have any of these tags:
Clear the filter	OK Cancel

Review	Features	Experim	ent d	lesign: AC		•											
Tag feat	filter applied ures may be	d hidden		Edit													
Anova (p)	Fold	Tag	•	Notes	Highest Mean	Lowest Mean	m/z	z	Mass	RT (mins)	RT window	Abundance	Intensity	Max CV (%)	MS/MS	Prot ^	П
0.000284	Infinity	\checkmark \times		Add note	A	С	1426.6914	3	4277.052	34.931	0.125	5.15E+03	3.7E+04	112	0		
0.000404	123	 ✓ × 		Add note	С	Α	882.4745	2	1762.934	59.891	0.423	8.46E+03	5E+04	56.4	0		П
1.19E-07	Infinity	✓ ×		Add note	A	с	535.6629	5	2673.278	27.064	0.391	7.25E+03	7.39E+04	18.8	0		
0.000537	20.5	\checkmark X		Add note	с	Α	1137.227	3	3408.659	44.646	1.46	2.37E+05	5.2E+05	39.3	0		
3.42E-08	Infinity	 ✓ × 		Add note	Α	с	916.9336	4	3663.705	29.396	0.219	1.9E+04	7.94E+04	15.3	0		
3.69E-09	Infinity	\checkmark X		Add note	с	Α	1011.7922	3	3032.355	47.031	1.09	3E+04	1.34E+05	9.72	0		
0.000402	12	 ✓ × 		Add note	Α	с	891.4206	2	1780.827	45.934	0.443	2.03E+04	6.67E+04	42	0		4
0.0259	145			Add note	Α	С	630.703	3	1889.087	57.35	0.563	5.66E+03	2.97E+04	86.6	1		11
0.000432	23.6	< ×		Add note	Α	С	671.3484	3	2011.023	53.274	0.647	1.5E+04	6E+04	44.4	1		П
0.00179	5.13	<×		Add note	С	A	1024.5299	2	2047.045	50.882	0.539	4.13E+04	1.41E+05	30.2	1		П
0.000103	23.5	<×		Add note	С	A	927.1203	3	2778.339	49.085	1.03	6.33E+04	2.49E+05	32.1	1		П
0.00852	6.98	\checkmark \times		Add note	С	A	1198.2335	3	3591.679	44.053	0.706	1.53E+05	2.81E+05	71.4	1		
0.00715	957	< × ×		Add note	Α	с	742.7004	3	2225.079	24.176	0.551	4.47E+04	1.41E+05	173	1	-	
•																F.	

Create new tag	×
Inclusion_1	
	OK Cancel

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.

Filter the features Create a filter Show or hide features based on a selection of boxes to create the filter. For more guidance	of their tags. Move tags to the appropriate e, please see the <u>online reference</u> .
Available tags: Most abundant (2170 features) Up regulated in A (4030 features) Significantly up in C (3817 features) Up regulated in C (3817 features) Anova p-value ≤ 0.05 (7847 features)	Show features that have all of these tags: Inclusion_1 (4540 features) Show features that have at least one of these tags: Hide features that have any of these tags:
Clear the filter	OK Cancel

Then select Export Inclusion List... from the file menu and select the appropriate format.

۵ ل	C-MS 1	Futorial - Pro	ogenesis L	C-MS					
	Eile Save Close					Fil	tering	Experiment Design Setup	View Results
	Expor Impo Expor	t Feature Da rt Feature Ni t Thermo Fii	ta umbers as nnigan inc	Tag Iusion li	ist	t]	·	
	Expor	t Inclusion L	ist		•	[Beta] Agil	lent Preferred MSN	/IS Table
×	Exit	Infinity			Add note]]	Beta] Agil Beta] Brul	lent Targeted MSN ker Maxis inclusion	/IS Table n list
0.04	437 5E-06	3.71 6.59E+04	 ✓ × ✓ × ✓ × 		Add note	A N T	ABI inclusi MassLynx Thermo Fi	ion list mass inclusion lis nnigan inclusion l	t ist
0.03	339 0115	2.92 22.7	✓× ✓×		Add note	N	More Inclu	usion List Formats	

Finally save the file to an appropriate location

🔼 Export Inclusio	on List				×
Save in:	Inclusion Lists		-	G 🏚 📂 🛄 🗸	
Ca.	Name		Date modified	Туре	Size
Recent Places			This folder is empty		
Desktop					
Andy Borthwick					
Computer					
	•				•
Network	File name:	Inclusion_1			Save
	Save as type:	Thermo Finni	igan inclusion list files (*.b	d) 🔻	Cancel
					Help

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

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

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

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

Congratulations!

This document has taken you through a complete analysis using Progenesis 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 SameSpots using your own runs and explore the Progenesis LC-MS workflow please go to Appendix 3: Licensing Runs (page 53).

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, for other Vendors, 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.

Create New Experiment	
Create a new label-free experiment named:	
LC-MS Tutorial	
Data type Profile data Centroided data Resolution (full width at half maximum) 5000	
Machine type High resolution mass spectrometer Experiment folder Save experiment in the same folder as the run data	High resolution mass spectrometer e.g. Thermo LTQ Orbitrap, Bruker Maxis, Waters SYNAPT, Agilent QTOF, AB SCIEX TripleTOF Low resolution ion trap e.g. Bruker HCT, Bruker HCT Ultra, Thermo LTQ XL Thermo FTI-CR
Choose an experiment folder Brow Create experiment Can	cel

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

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

Then locate your data files using Add Files...

🔼 LC-MS Tuto	rial - Progenesis	LC-MS									
<u>F</u> ile											
Data Import	Reference Run Selection	Alignment	Filtering	Experiment Design Setup	View Results	Progenesis Stat	s Peptide Sean	ch Peptide Filter	Protein View	Report	nonlinear
•		0						0	0		D Y N A M I C S
Import Dat	a		Data pr	ocessing me	ethods:						
mzXML files	•	Add Files	Featur	e detection i	nethod: De	fault					
Agilent Q-TOF			Pea	k processing i	method: Pr	ofile data					
Bruker Maxis (.d)										
mzXML files	older	Include?									
Thermo .RAW f	iles		Sel	ect files							
NetCDF files					-MS Tutorial 3	0	- 4	Search			
					s-ivis ratonars	.0	• • 7	Search	~		
			🤚 C	Irganize 👻 📗	Views 🔻 📘	New Folder			0		
			Name	<u>^</u>	Date m	nodified	Туре	Size			
				.mzxml	20/05/	2008 08:23	MZXML File	349,372	КВ		
				2.mzxml	20/05/	2008 08:27	MZXML File	362,602	KB		
				s.mzxml	20/05/	2008 08:29	MZXML File	313,521	KB		
				2.mzxml	20/05/	2008 11:50	MZXML File	405.650	KB		
				3.mzxml	20/05/	2008 08:25	MZXML File	380,594	КВ		
				Fil	e name: "A1.mz	xml" "A2.mzxml"	"A3.mzxml" "(🔻	mzXML files (*.m:	zxml) 🔻		
								Open <	Cancel		
🗸 Include rur	in analysis										
	-										
X Don't inclu	de run in analysis										
Exclude an	eas from selected	run								[Section Complete (>)
										l	

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

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

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

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 LC-MS		- • •
Eile Data Import Selection Alignment	Experiment Filtering Design Setup View Results Progenesis Stats Peptide Search Peptide Filter Protein View Report	nonlinear
Import Data mzXML files Add Files Image: About this data format Include?	Data processing methods: Feature detection method: Default Peak processing method: Profile data No problems found	ĺ
A1 Image: Constraint of the second seco		E
	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 Don't include run in analysis		
	Ser	ction Complete 🤿

Note: as each data file is loaded the progress is reported in the **Import Data** list. The dialog below the run 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 user guide) by clicking Section Complete.

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 screen.

Drag out an area over the noisy part of the run 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 reoptimise the chromatography to improve the levels of noise in your data

Import Data	
Thermo .RAW files 🔹	Add Files
About this data format	
	Include?
No problems found	
х	
Y	
Z	V X
✓ Include run in analysis	
X Don't include run in analysis	
BEXCLUDE AREAS FROM SELECTED R	un

Appendix 3: Licensing runs (Stage 3)

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



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

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

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

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

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

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

🔼 LC-MS Tuto	orial - Progenesis I	LC-MS											- • ×
File													
2.02	Reference Run				Experiment								
Data Import	Selection	Licensing	Alignment	Filtering	Design Setup	View Results	Progenesis Stats	Peptide Search	Peptide Filter	Protein View	Report	non	linear
		•										DYN	AMICS
Dongle Li	cense Runs												
This installa	ation is currently	restricted to										Licence	License
analyse lice	ensed runs only.		Run name									state	this run
Tolicense	vour runs, vou n	eed an	C:\Users\andy.b	orthwick\Deskt	op\LC-MS Tutorial	2.6\A1.mznld						Unlicensed	
evaluation l	icence code wh	nich can be	C:\Users\andy.b	orthwick\Deskt	op\LC-MS Tutorial	2.6\A2.mznld						Unlicensed	
obtained fro	om a sales repre	esentative.	C:\Users\andy.b	orthwick\Deskt	op\LC-MS Tutorial	2.6\A3.mznld						Unlicensed	
Once licens	ed, vour runs ca	an be	C:\Users\andy.b	:\Users\andy.bothwick\Desktop\LC-MS Tutorial 2.6\C1.mznld Unli								Unlicensed	V
analysed o	n any installatio	n of the	C:\Users\andy.b	orthwick\Deskt	op\LC-MS Tutorial	2.6\C2.mznld						Unlicensed	V
included wh	he licence is aut tien archiving an	tomatically experiment	C:\Users\andy.b	orthwick\Deskt	op\LC-MS Tutorial	2.6\C3.mznld						Unlicensed	
If your runs	have been licens	sed on											
another com licences ava	nputer, <u>click here</u> ailable on this co	to make the mouter.											
file to install	one, you can <u>ope</u>	en a licence											
If you have j	just installed a d	ongle, <u>click</u>											
here.													
								Run lice	nce code: xxx-x	00-000-000		Use Licer	ice Code
								-					
												Section C	omplete 🏵

A message confirming successful installation of your licences will appear.



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

Appendix 4: Manual assistance of Alignment

Approach to alignment

To place manual alignment vectors on a run (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.



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.

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



5. As you are holding down the left mouse button drag the green feature over the corresponding magenta feature of the reference run. The red circle will appear as shown below 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.

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

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

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



8. 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.



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

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

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



11. Then select Automatic Alignment to bring up the Automatic Alignment dialog and click **OK**. The automatic alignment process will begin, using the manual vectors you have added to aid in automatic vector placement.

Automatic Al	gnment	X
Select the ru	ns for automatic alignment vector generation	
Add Run	Notes	Vectors
🔳 A1	this run does not need to be aligned as it is the alignment reference	Ref
📝 A2	run has user vectors	7
🗹 A3	run has user vectors	7
🗹 C1	run has user vectors	7
🗹 C2	run has user vectors	7
🗹 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.

To review the vectors, automatic and manual return to page 12

Appendix 5: Within-subject Design

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

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

e Reference Image Mask Of age QC Selection Disinterest Alignm	ent	Filtering	Experiment Design Setup	View Results	Progenesis Stats	Spot Picking	Report			ņo	nline
ew hich experiment design type do you wa	int to i	ise for th	is experimer	nt?		rubiest Dec	ian				
Do samples from a given subject appear in only one condition? Then use the between- subject design.	Drug A		gett p	Delete Remove	Have you taken subject under di use the within-su	samples from a fferent conditi ubject design.	a given ions? Then		Drug A	Drug B	Drug C
To set up this design, you simply group the images according to the condition (factor level) of the samples. The ANOVA calculation assumes that the conditions are independent and therefore gives a statistical test of	Drug B	Progen	gel2 gel3 g gel3 g gel4 g esis SameSpots	lemove lemove lemove	Note: you must h subject for every	ave a sample f condition to us	rom every se a within- nis type of	Person 1	gel1	gel5	gel9
equal.		Create Name:	e a new exp Before and A	o eriment d	l esign ent		nt where t each time software	Person 2	gel2	gel6	gel 10
	Drug C	Start Copy	with an empty layout from:	y layout			evel) each ubject it en perform a	Person 3	gel3	gel7	gel11
					Create desig	differences ca uced as a sources (which hele	nption of peasures n be ce of between ps to create a	Person 4	gel4	gel8	gel12
					The within-subject as an extension of to include compa	ct design can b f the paired-sa rison between	e thought of mples t-test more than two				

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

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

My_Timecourse - Progenesis LC-MS					
Eile					
Reference Run E Data Import Selection Alignment Filtering D	xperiment esign Setup View Results	Progenesis Stats Pepti	de Search Peptide Filte	r Protein View	Report DYNAMIC
Patients Progress I × 🖸 New					
Setup the conditions and subjects for your experiment design on the right, and then assign each of your samples to the correct subject/condition cell in the grid.		Before	During	After	Add Condition
 Add a column for each condition. Add a row for each subject. Drag each of your samples to the correct location in the grid. 	Patient X	X1	X2	X3	
Samples 🔎			(SEC)	1890) -	
Z2	Patient Y	Y1	Y2	Y3	
	Patient Z	Z1	Steet Sample	Select Sample	
	Add Subject				
					Section Complete →

You can create additional Experimental Designs using the New tab

All of these Experimental Designs are available at all the following stages in the LC-MS workflow

Appendix 6: Power Analysis (Progenesis Stats)

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

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

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

Select the option

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

It answers this question by informing you:

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

Using the Significant p<0.05 features (7847), as an example, view the power analysis.



This is displayed graphically showing that 79.7% of the 7847 features have a power of 80% or that 4 replicates would give you 96.5% 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 7 (a): Search engine parameters (Stage 9) Mascot

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

MASCOT	MS/MS lons	Search		
Your name	Andy_B		Email	andy.borthwick@nonlinear.com
Search title	Tutorial 3			
Database(s)	MSDB		Enzyme	Trypsin 🔹
	IPI_human		Allow up to	1 🔻 missed cleavages
	SwissProtVersion55		Quantitation	None 🔹
Taxonomy	Firmicutes (gram	-positive ba	cteria)	•
Fixed modifications	none selected	_	> <	mTRAQ (Y) mTRAQ:13C(3)15N(1) (K) mTRAQ:13C(3)15N(1) (N-term) mTRAQ:13C(3)15N(1) (Y)
	Display all modifie	cations 📃		Oxidation (HW)
Variable modifications	Carbamidomethyl (C) Oxidation (M) Phospho (ST) Phospho (Y)		> <	Pyridylethyl (C) Pyrocarbamidomethyl (N-term C) Sulfo (S) Sulfo (T)
Peptide tol. ±	9 ppm 🔻 ;	# 13C 0 ▼	MS/MS tol. ±	0.6 Da 🔻
Peptide charge	2+ •		Monoisotopic	Average
Data file	torial 3.0\Version 3 Ma	ascot.mgf 🗌	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 10/10) 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

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Appendix 7 (b): Search engine parameters (Stage 9) Phenyx

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

IDs	60629
Title	
File(s)	C:\Users\Andy.Borthwick\Desktop\LCMS Tutorial\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