

# Skyline Live Reports

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“Live Reports” is the name given to a group of windows available in Skyline which present information in rows and columns. There is a great deal of customizability in terms of which columns are displayed and how the rows are filtered.

This tutorial will give an overview of several of these windows.

## Getting Started

To start this tutorial, download the following ZIP file:

<https://skyline.ms/tutorials/LiveReports.zip>

Extract the files in it to a folder on your computer, like:

C:\Users\brendanx\Documents

This will create a new folder:

C:\Users\brendanx\Documents\LiveReports

To begin processing the data collected for the Differentiation phase of this method refinement study:

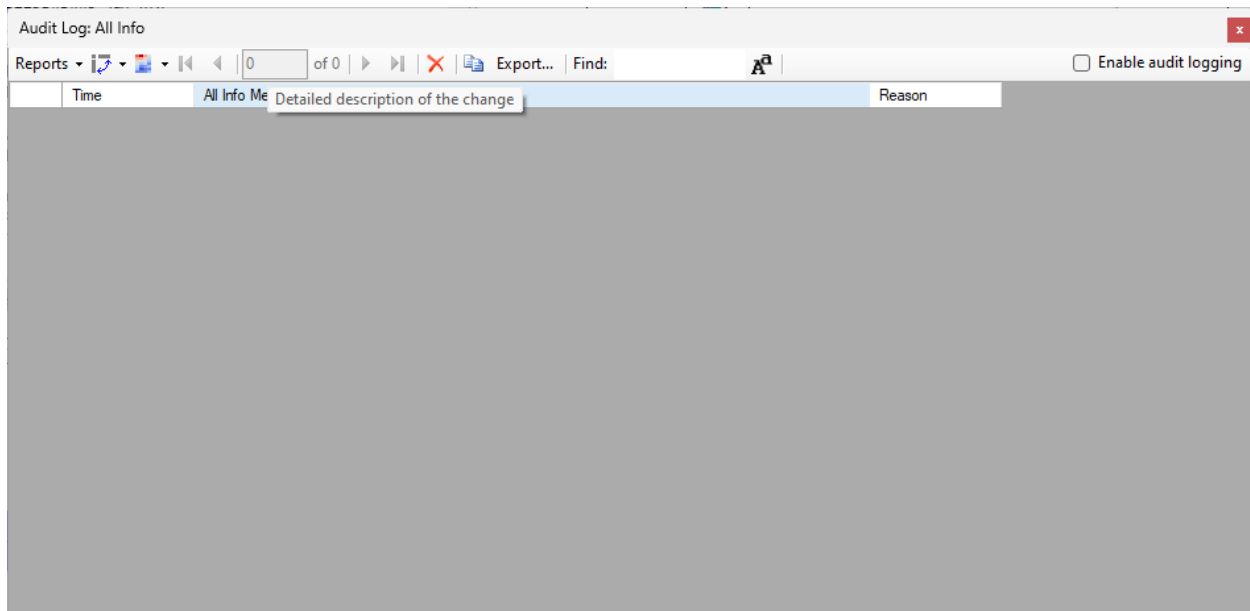
- Open the “Rat\_plasma.sky” file in the “LiveReports” folder.

## Enable Audit Logging

The first thing to do in this document is to enable audit logging. The Audit Log in Skyline keeps track of all of the changes that have been made to the document. When you create a new document in Skyline, the audit log is usually enabled, but in this much older document, the Audit Log has not been enabled.

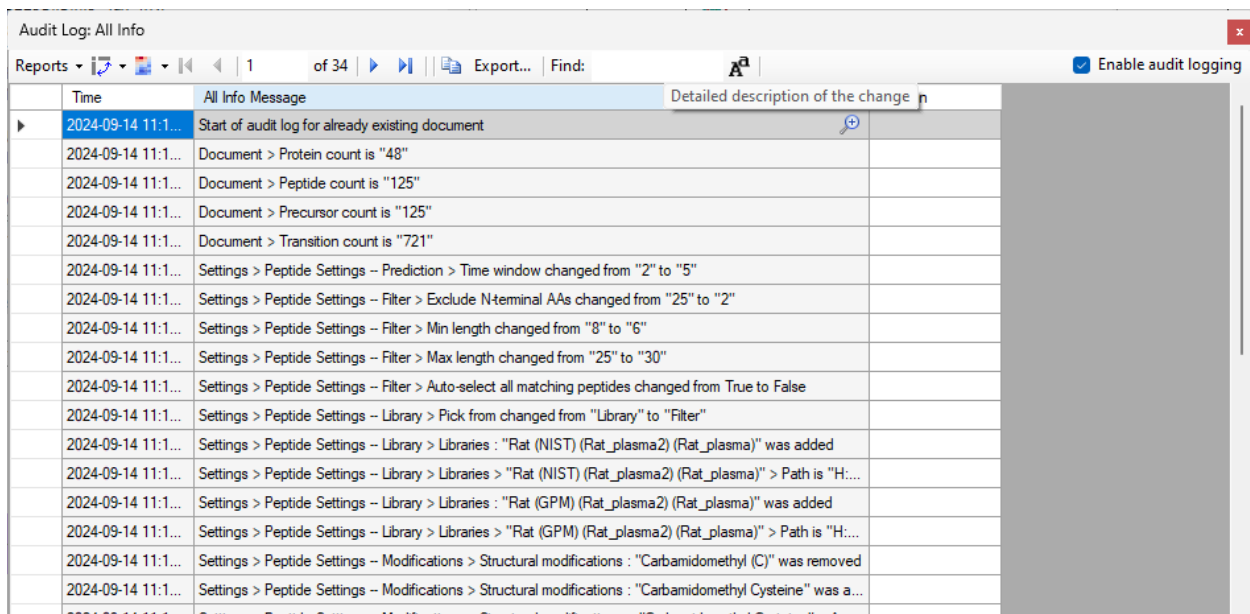
- On the View menu, click **Live Reports** and then click **Audit Log**

This brings up the Audit Log form



- Click the **Enable audit logging** checkbox

The Audit Log grid becomes populated with a set of entries which represent the difference between the current document and Skyline's default blank document



- Close the Audit Log window

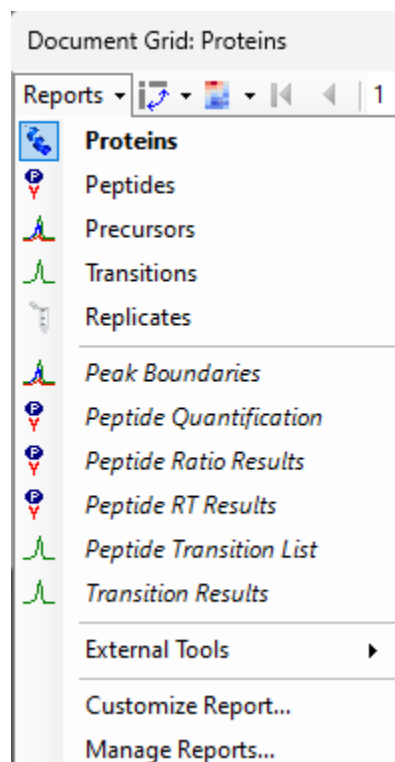
At the end of this tutorial we will return to the Audit Log and inspect its contents

By inspecting the indicators at the bottom right corner of the Skyline window, you will see that the file you opened contains 48 proteins, 125 peptides and 722 transitions.

## Showing the Document Grid

- On the **View** menu, click **Live Reports** and then click **Document Grid**.
- Click the **Reports** button in the top left corner of the **Document Grid**

This shows the list of reports that are available to be shown in the Document Grid.



The first five items in the list, **Proteins**, **Peptides**, **Precursors**, **Transitions**, and **Replicates** are the “built-in” reports which cannot be modified. Each of these reports show essential information from basic units in the Skyline document. The set of columns that these reports have may change based on the state of the document. In particular, if there are any user-defined annotations, those annotations will be added to the appropriate built-in report.

Below the built-in report are the user-defined reports which can be modified. Skyline ships with a small set of user-defined reports that are designed to be helpful. In addition, it is expected that Skyline users will define their own reports and share them with colleagues.

- On the **Reports** menu in the **Document Grid**, choose **Proteins** if it is not already chosen

This displays the built-in “Proteins” report, which shows the list of proteins in the document, in the same order that they appear in the Targets tree.

Protein	Protein Description	Protein Accession	Protein Preferred Name	Protein Gene	Protein Species	Protein Sequence	Protein Note
<a href="#">NP_036629</a>	Tax_id=10116 G...	P09117	ALDOC_RAT	Aldoc	Rattus norvegicu...	MPHSYPALSAE...	
<a href="#">NP_037030</a>	Tax_id=10116 G...	P24090	FETUA_RAT	Ahsg Fetua	Rattus norvegicu...	MKSLVLLLCFAQ...	
<a href="#">NP_602308</a>	Tax_id=10116 G...	P01015	ANGT_RAT	Agt Serpina8	Rattus norvegicu...	MTPTGAGLKATI...	
<a href="#">NP_001012027</a>	Tax_id=10116 G...	Q5M7T5	Q5M7T5_RAT	Serpinc 1	Rattus norvegicu...	MYSPIGIGSAVA...	
<a href="#">NP_036870</a>	Tax_id=10116 G...	P04639	APOA1_RAT	Apoa1	Rattus norvegicu...	MKAAVLAVALVF...	
<a href="#">NP_037244</a>	Tax_id=10116 G...	P04638	APOA2_RAT	Apoa2	Rattus norvegicu...	MKLLAMVALLV...	
<a href="#">NP_001010968</a>	Tax_id=10116 G...	Q6Q3E8	Q6Q3E8_RAT	Eng rCG_45763	Rattus norvegicu...	MDRSMPLVITL...	
<a href="#">NP_444180</a>	Tax_id=10116 G...	P05371	CLUS_RAT	Clu	Rattus norvegicu...	MKILLCCVALLT...	

The first column in the report is displayed as a hyperlink, and clicking on text in those cells will navigate to the particular protein in Skyline.

- Click on the fourth item in the “Protein” column (“NP\_001012027”)

This causes the fourth item in the Targets tree to be selected:

Targets

Replicates: D\_102\_REP1

- NP\_036629
  - R.CSLPRPWALTFSYGR.A [290, 304]
- NP\_037030
  - R.LGGEEVSVACK.L [238, 248]
  - K.VGQPGDAGAAGPVAPLCPGR.V [327, 346]
- NP\_602308
  - R.GSYNLQDLLAQAK.L [379, 391]
- NP\_001012027**
  - K.TSDQIHFFFAK.L [148, 158]
  - K.LQPLDFK.E [203, 209]
  - K.SQLPGIIAEGR.D [382, 392]

The Status Bar at the bottom of the Skyline window also indicates that the fourth protein in the document is selected.

4/48 prot 5/125 pep 5/125 prec 24/721 tran

The record navigator at the top of the Document Grid also indicates that the focus is in the fourth row.

Reports | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | of 48

The Document Grid can be used to inspect many types of data.

- Choose **Replicates** from the **Reports** dropdown at the top of the **Document Grid**

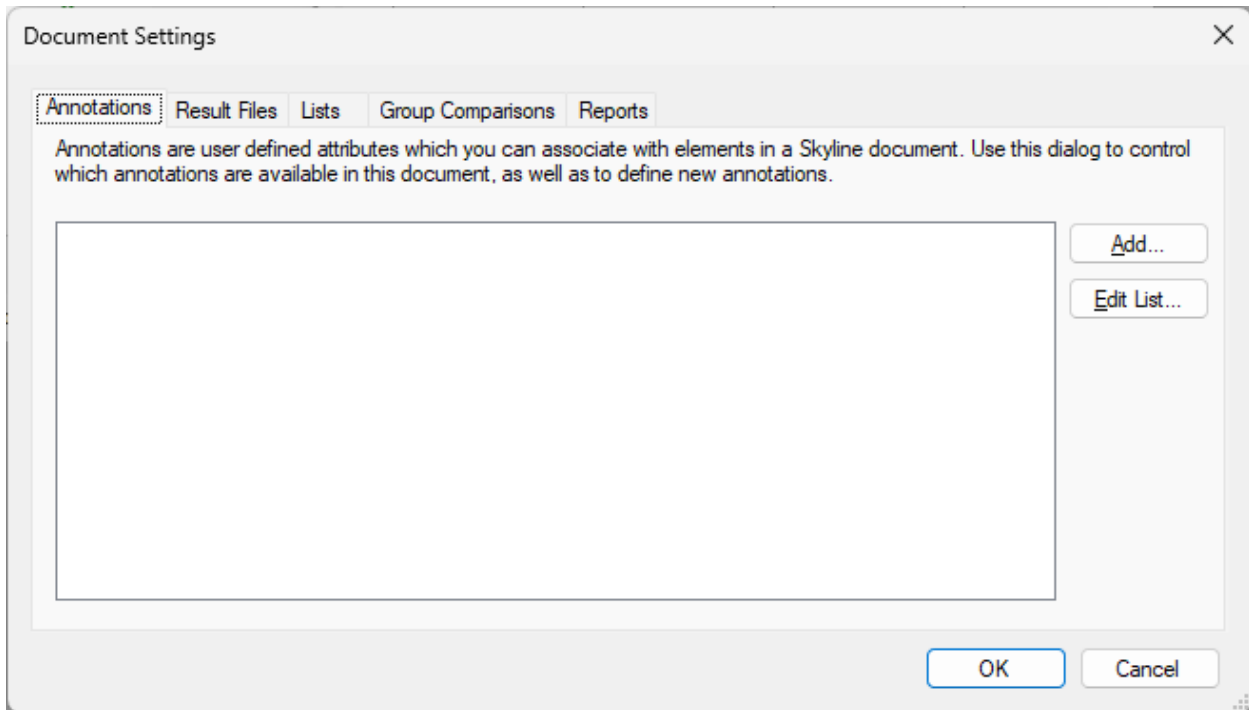
Replicate	Sample Type	Analyte Concentration
D_102_REP1	Unknown	
D_102_REP2	Unknown	
D_102_REP3	Unknown	
D_103_REP1	Unknown	
D_103_REP2	Unknown	
D_103_REP3	Unknown	
D_108_REP1	Unknown	
D_108_REP2	Unknown	
D_108_REP3	Unknown	

This document contains 42 Replicates. The names of the Replicates are all in three parts. The first letter of the replicate name is a “D” or “H” indicating whether the sample came from a diseased or a healthy rat. That is followed by an underscore, and then three digits which are the subject identifier of the rat. That is followed by “\_REP” and then one more digit which identifies which technical replicate it is.

## Defining some Replicate Annotations

Before using features such as “Group Comparisons”, it is necessary to tell Skyline more about these replicates. The way to do that is by defining some “Replicate Annotations”.

- On the **Settings** menu, choose **Document Settings**



- Click the **Add** button

This brings up the “Define Annotation” dialog.

Define Annotation

Name:

Editable Calculated

Type:  
Text

Values:

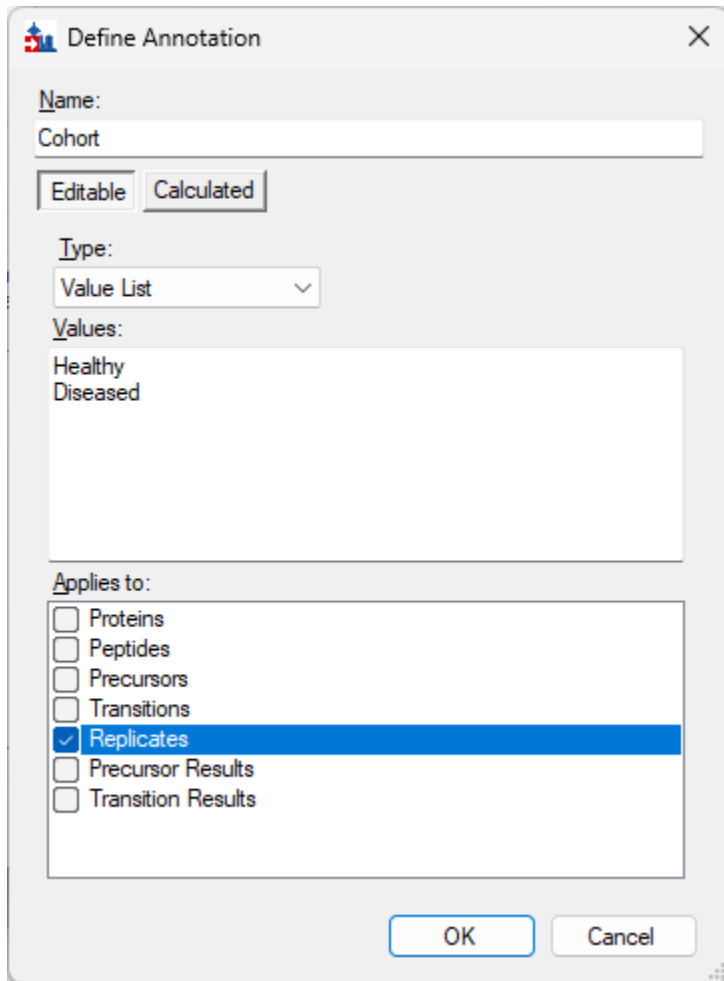
Applies to:

- Proteins
- Peptides
- Precursors
- Transitions
- Replicates
- Precursor Results
- Transition Results

OK Cancel

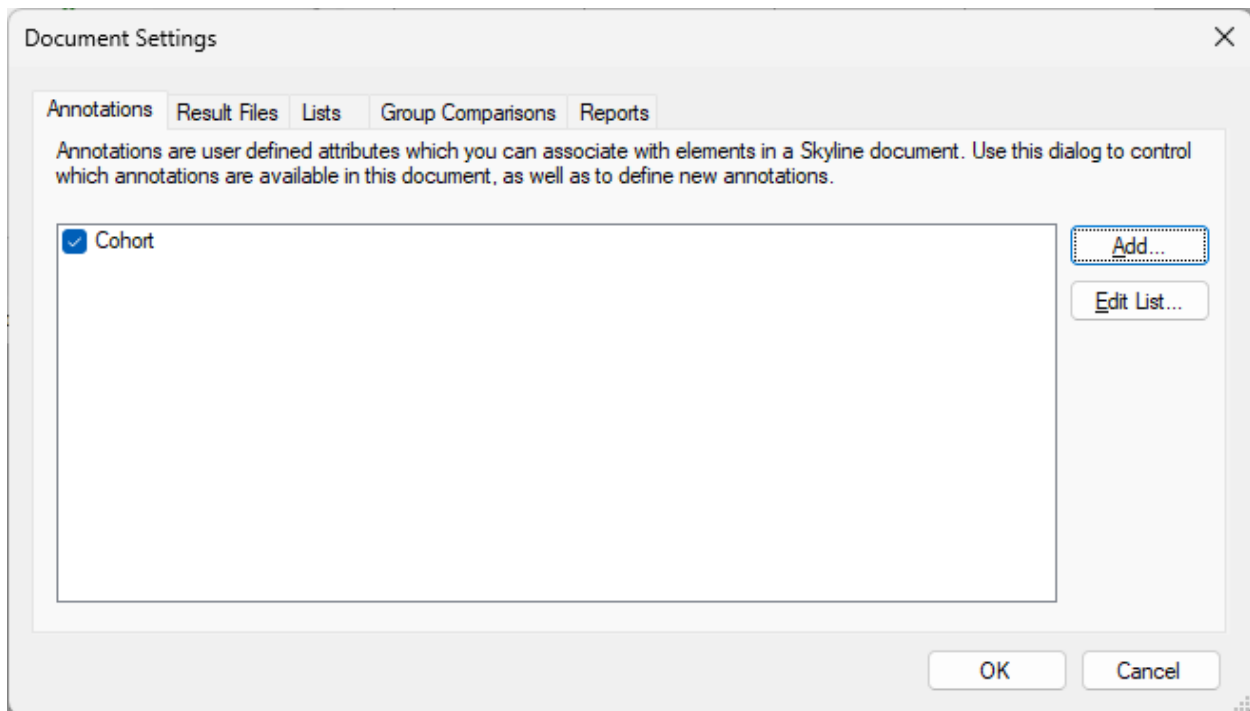
Annotations in Skyline must be given a unique name. There are three fundamental value types for annotations: text, numeric, and true/false. In addition, an annotation can be a “value list”, which is treated as a text annotation whose value is restricted to those in a specified list.

- Fill in the form as follows so that the name of the Annotation is “Cohort”, the type is “Value List”, the values are Healthy and Diseased and the checkbox next to “Replicates” is checked.



- Press the OK button

The new annotation now shows up in the Document Settings form



- Click the **Add** button
- Fill in the form so that the name is "SubjectID", the type is "Text" and the checkbox next to "Replicates" is checked



Define Annotation

Name:  
SubjectID

Editable Calculated

Type:  
Text

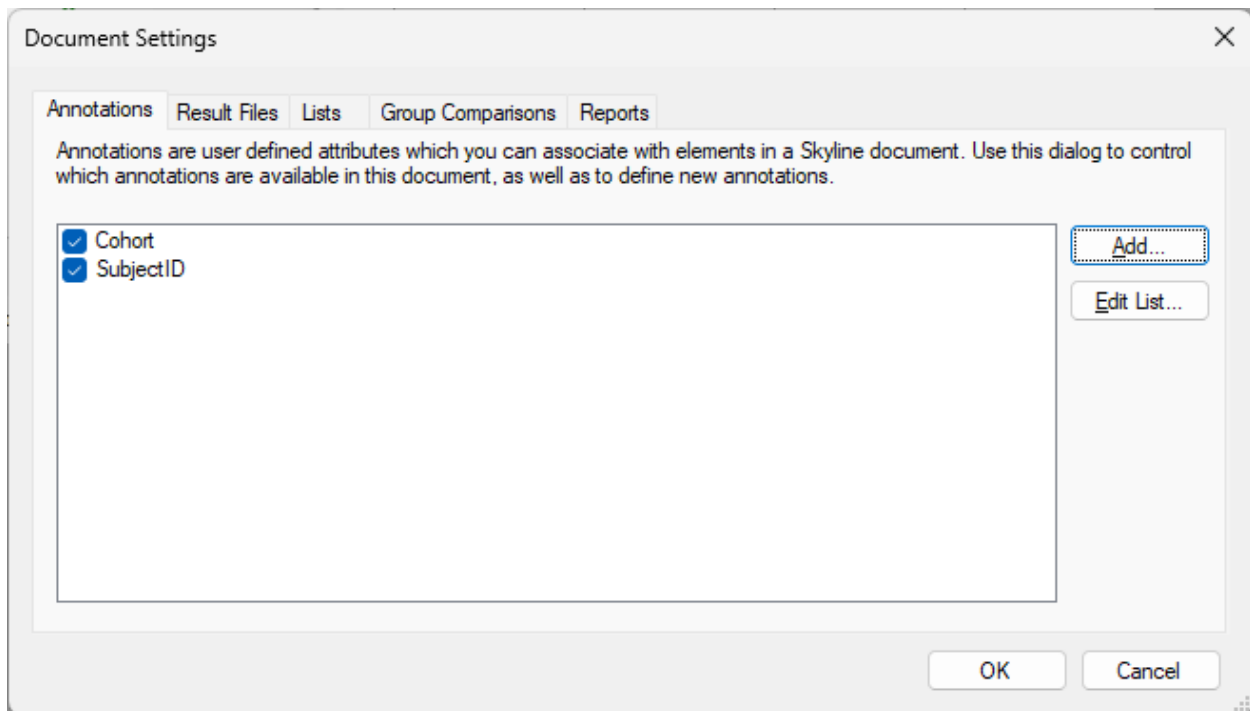
Values:

Applies to:

- Proteins
- Peptides
- Precursors
- Transitions
- Replicates
- Precursor Results
- Transition Results

OK Cancel

- Click the **OK** button



Now, two annotations have been defined and the checked checkbox next to each of them means that they are available in the current document.

- Click the OK button

The Document Grid which is displaying the “Replicates” report will show the new “Cohort” and “SubjectID” annotations which have been added to the document. If you click in a cell in the “Cohort” column you will see that it is a dropdown allowing you to choose the one of the two allowable values “Healthy” or “Diseased” (or blank).

Document Grid: Replicates

Reports | 1 of 42 | Export... Actions | Find:

Replicate	Sample Type	Analyte Concentration	Cohort	SubjectID
<a href="#">D_102_REP1</a>	Unknown			
<a href="#">D_102_REP2</a>	Unknown			
<a href="#">D_102_REP3</a>	Unknown			
<a href="#">D_103_REP1</a>	Unknown			
<a href="#">D_103_REP2</a>	Unknown			
<a href="#">D_103_REP3</a>	Unknown			
<a href="#">D_108_REP1</a>	Unknown			
<a href="#">D_108_REP2</a>	Unknown			
<a href="#">D_108_REP3</a>	Unknown			

It certainly would be possible to fill in the values in the “Cohort” and “SubjectID” columns by either typing into those cells, or pasting a column of data into them.

## Creating Result File Rules

In this case, because the values of the annotations are so closely tied to the names of the result files, there is another feature “Result File Rules” which can be used to set the values.

- On the **Settings** menu, click **Document Settings**
- Click on the **Result Files** tab
- Click the **Add** button

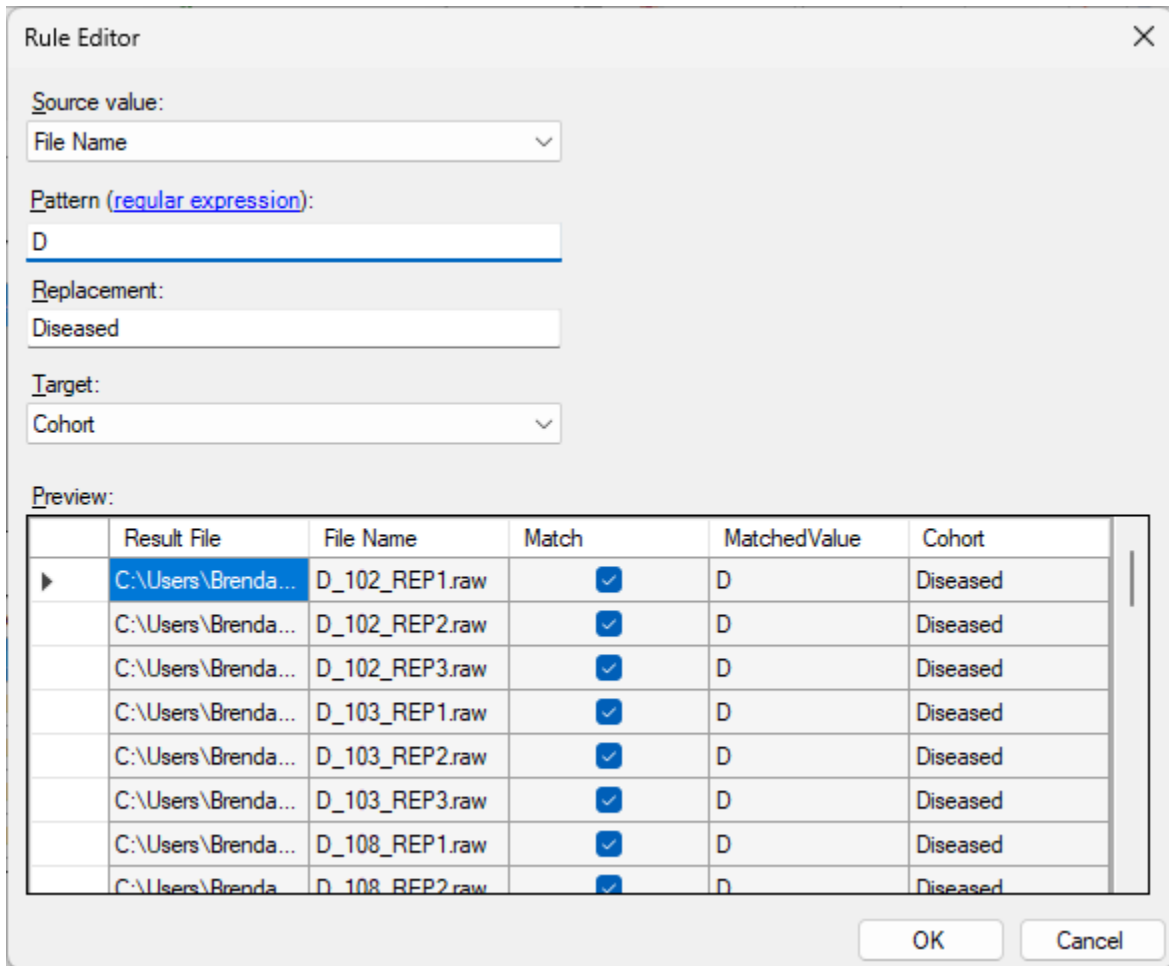
This brings up the Rule Set Editor which allows you to specify ways of populating values in the Skyline document from values from the Result Files.

- Click somewhere in the first row in the Rules list
- Click the “...” button located to the right of the Rules list

This brings up the Rule Editor

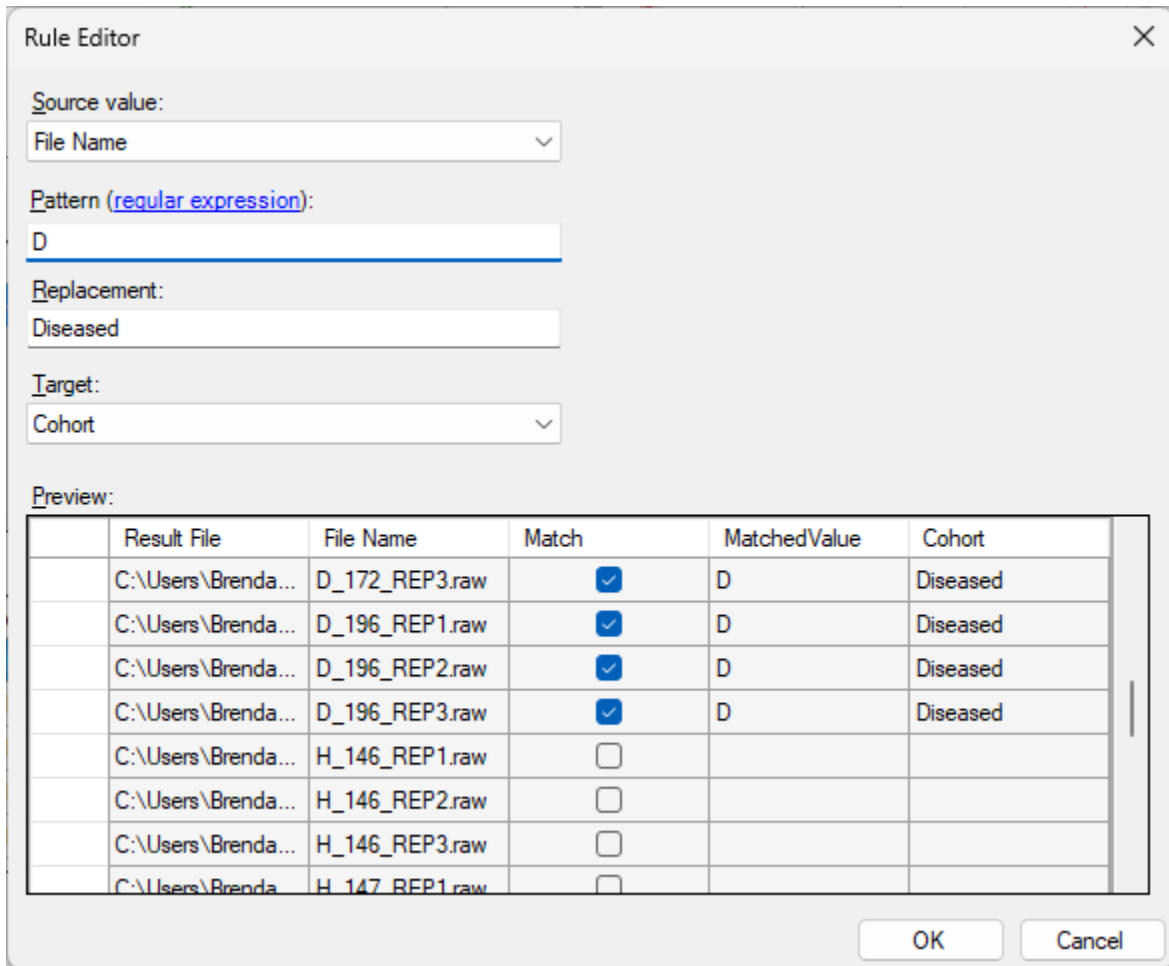
	Result File	File Name
▶	C:\Users\Brenda...	D_102_REP1.raw
	C:\Users\Brenda...	D_102_REP2.raw
	C:\Users\Brenda...	D_102_REP3.raw
	C:\Users\Brenda...	D_103_REP1.raw
	C:\Users\Brenda...	D_103_REP2.raw
	C:\Users\Brenda...	D_103_REP3.raw
	C:\Users\Brenda...	D_108_REP1.raw
	C:\Users\Brenda...	D_108_REP2.raw

- In the **Pattern** textbox, enter the letter **D**
- In the **Replacement** textbox, enter the word **Diseased**
- In the **Target** dropdown, choose **Cohort**



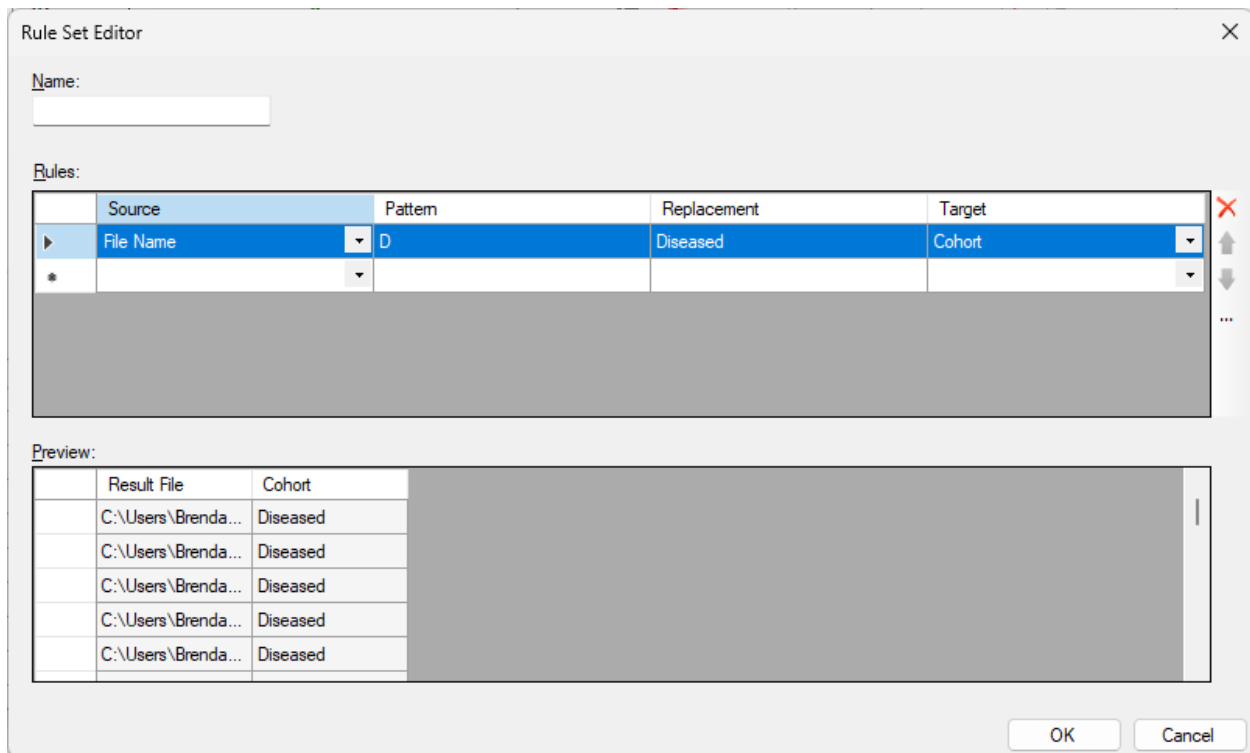
The Preview grid will show the effect that this rule will have. The **File Name** values in the first 21 rows all do contain the letter **D** so that the **Match** checkbox is checked and the word **Diseased** is filled in for the **Cohort**

If you move the scrollbar thumb down halfway you will see that the file names which start with “H” are not being matched.

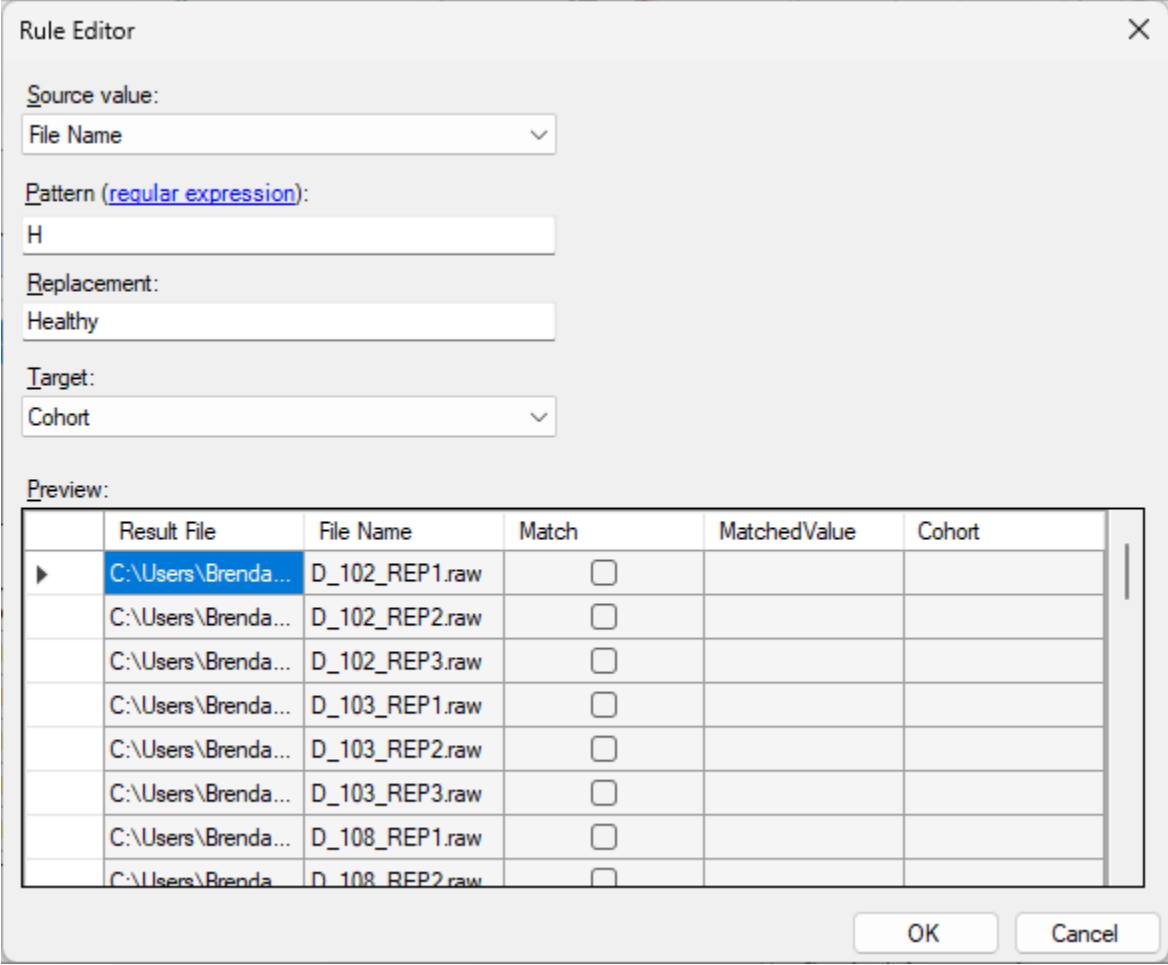


- Press the **OK** button

This brings you back to the **Rule Set Editor** where it is showing the results of that first rule

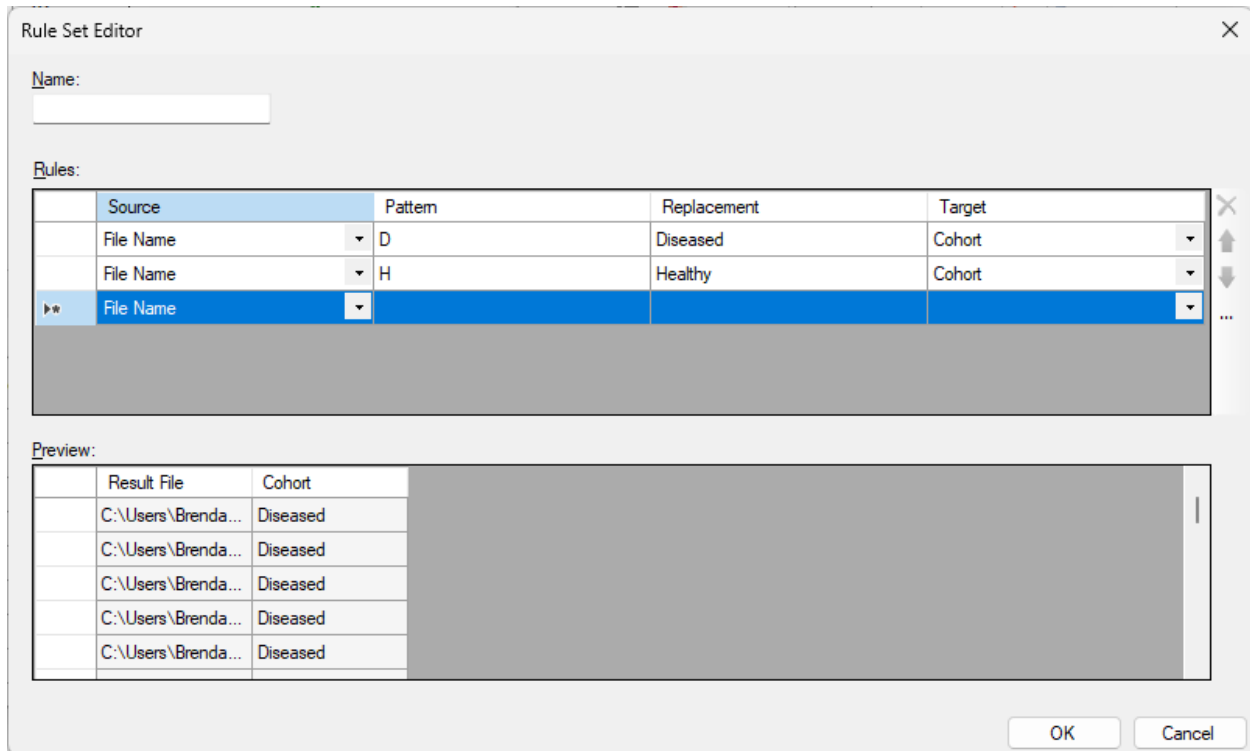


- Click somewhere in the second row of the **Rules** grid and then click the ... button to the right of the grid
- Fill in the Rule Editor with the letter **H** as the pattern, the word **Healthy** as Replacement and **Cohort** for **Target**



- Click OK

This brings you back to the **Rule Set Editor**



- Click in the third row of the **Rules** grid, and then click the ... button to the right of the grid

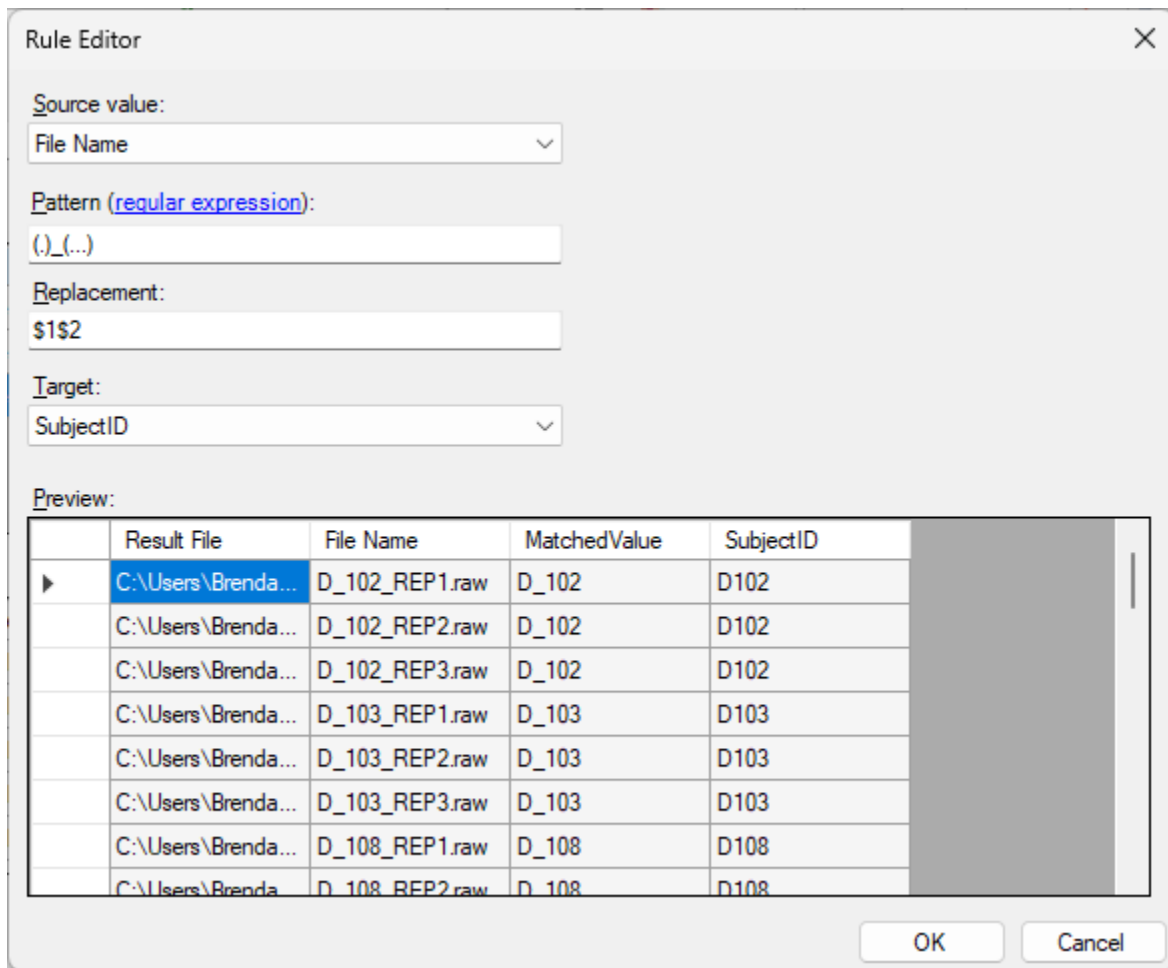
The purpose of the final rule that we create is to set the SubjectID values to D102, H162, etc. by combining the first letter of the file name with the 3 digits that follow the first underscore.

To accomplish this, the pattern that we are going to use is **(.)\_(...)** The parentheses denote regular expression groups, which are parts of the matched expression which can be referred back to in the Replacement text with “\$1” and “\$2”. The period character (dot) will match any character, and the underscore character will only match an underscore

- In the **Pattern** textbox, enter **(.)\_(...)**
- In the **Replacement** textbox, enter **\$1\$2**
- In the **Target** dropdown, choose **SubjectID**.

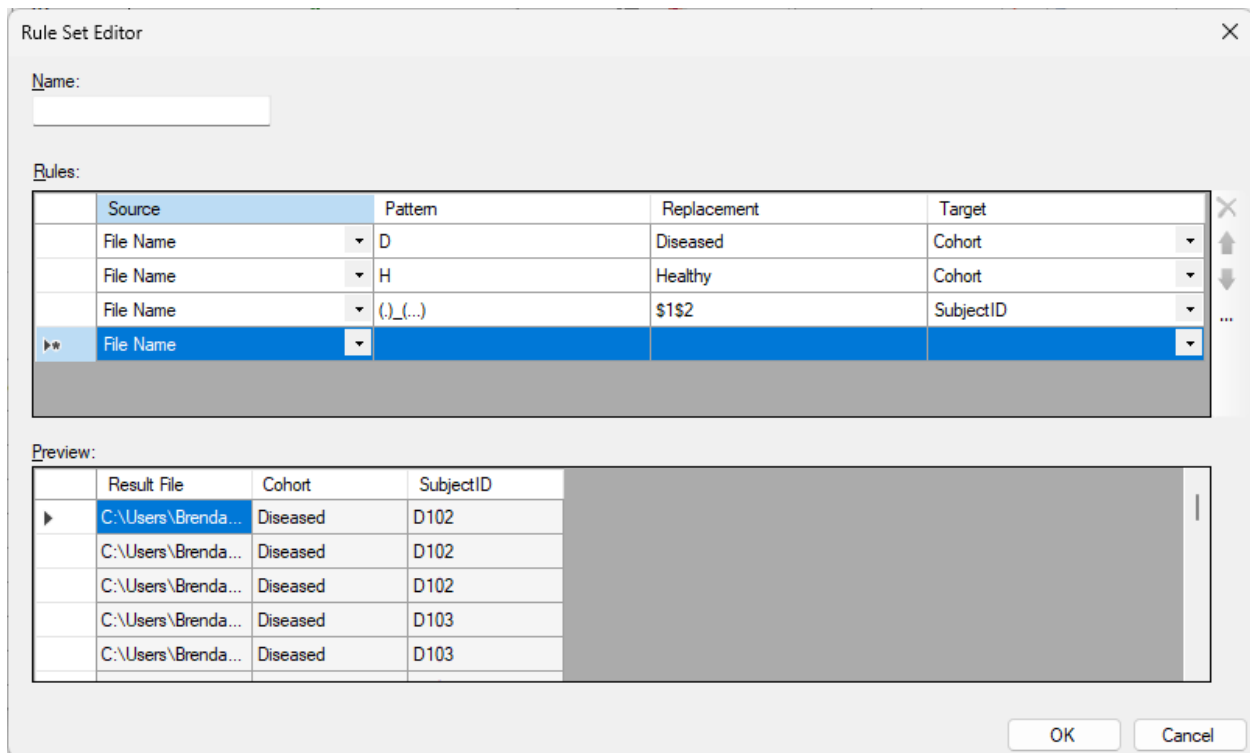
It should now look like this:





- Press the **OK** button

This returns you to the Rule Set Editor which should now look like this:



- In the **Name** textbox enter “Cohort and SubjectID”
- Press the **OK** button

This returns you to the Document Settings form where the “Cohort and SubjectID” rule has been added and has a checkmark next to it.

- Press the **OK** button in the **Document Settings** dialog

The **Cohort** and **SubjectID** values in the **Replicates** report will be filled in from the file names.

Document Grid: Replicates

Reports | 2 of 42 | Export... | Find:

Replicate	Sample Type	Analyte Concentration	Cohort	SubjectID
<a href="#">D_102_REP1</a>	Unknown		Diseased	D102
<a href="#">D_102_REP2</a>	Unknown		Diseased	D102
<a href="#">D_102_REP3</a>	Unknown		Diseased	D102
<a href="#">D_103_REP1</a>	Unknown		Diseased	D103
<a href="#">D_103_REP2</a>	Unknown		Diseased	D103
<a href="#">D_103_REP3</a>	Unknown		Diseased	D103
<a href="#">D_108_REP1</a>	Unknown		Diseased	D108
<a href="#">D_108_REP2</a>	Unknown		Diseased	D108
<a href="#">D_108_REP3</a>	Unknown		Diseased	D108

## Creating a list of samples

- On the **Settings** menu, click **Document Settings**
- Click on the **Lists** tab
- Click the **Add** button

This brings up the List Designer

List Designer

List name:

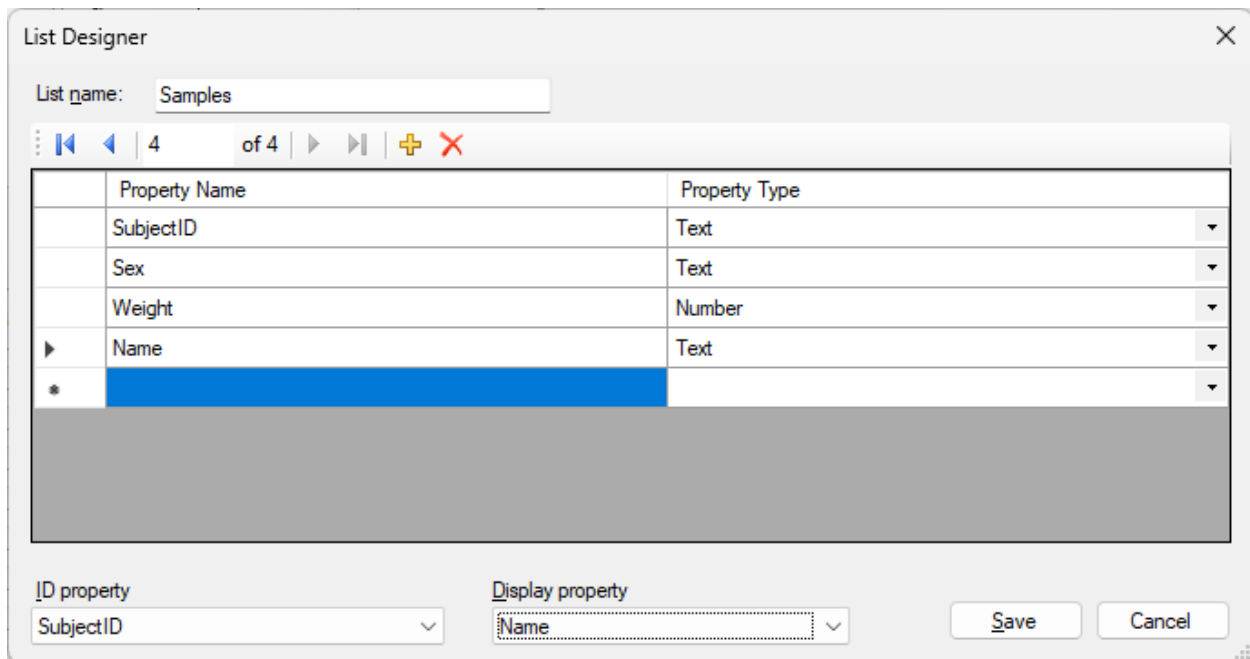
0 of 0

	Property Name	Property Type
*		

ID property  Display property

- In the **List name** textbox, enter “Samples”
- In the first row of the grid, enter “SubjectID” in the **Property Name** column
- In the second row of the grid, enter “Sex” in the **Property Name** column
- In the third row of the grid, enter “Weight” in the **Property Name** column and choose **Number** in the **Property Type** column
- In the fourth row of the grid, enter “Name” in the **Property Name** column
- In the ID Property dropdown, choose “SubjectID” and in the Display Property dropdown choose Name

The List Designer should now look like this:

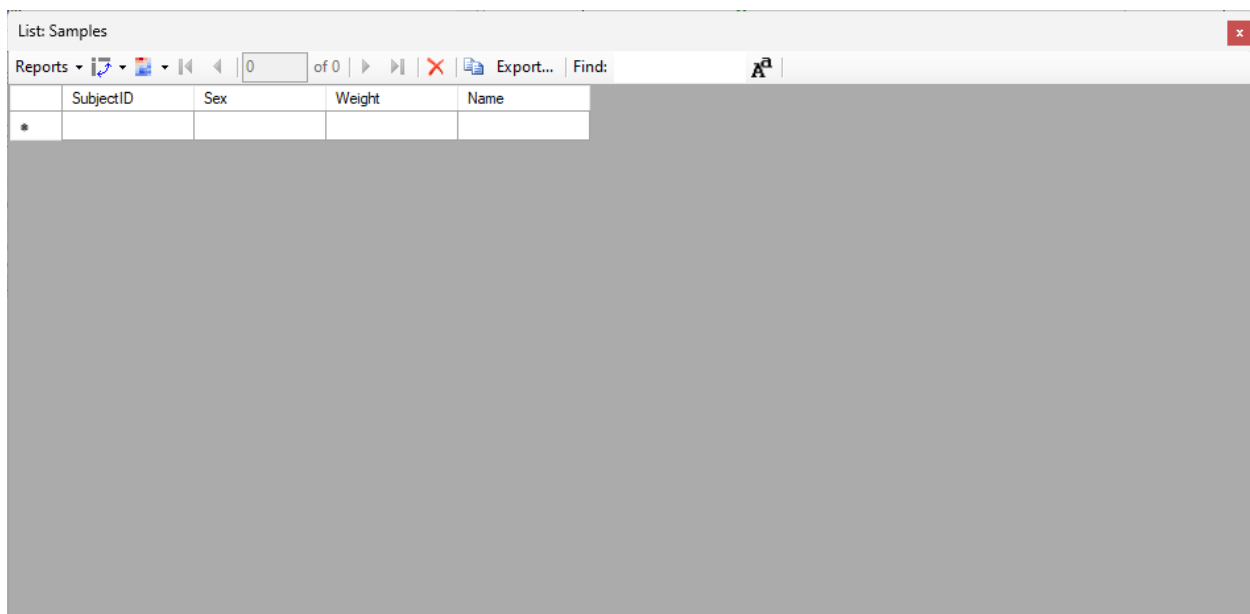


- Press the **Save** button
- Press the **OK** button in the **Document Settings** dialog

This brings you back to the main Skyline window.

- On the **View** menu choose **Live Reports** and then **Lists** and then **Samples**

This brings up a grid which shows the list that we just defined. There are no rows in the list, but it has the four columns that we defined: SubjectID, Sex, Weight and Name



It would be possible to fill in values by typing into the grid, but we have already prepared a text file with data that can be used.

You can navigate to that text file using the “File > Open Containing Folder” menu item in Skyline.

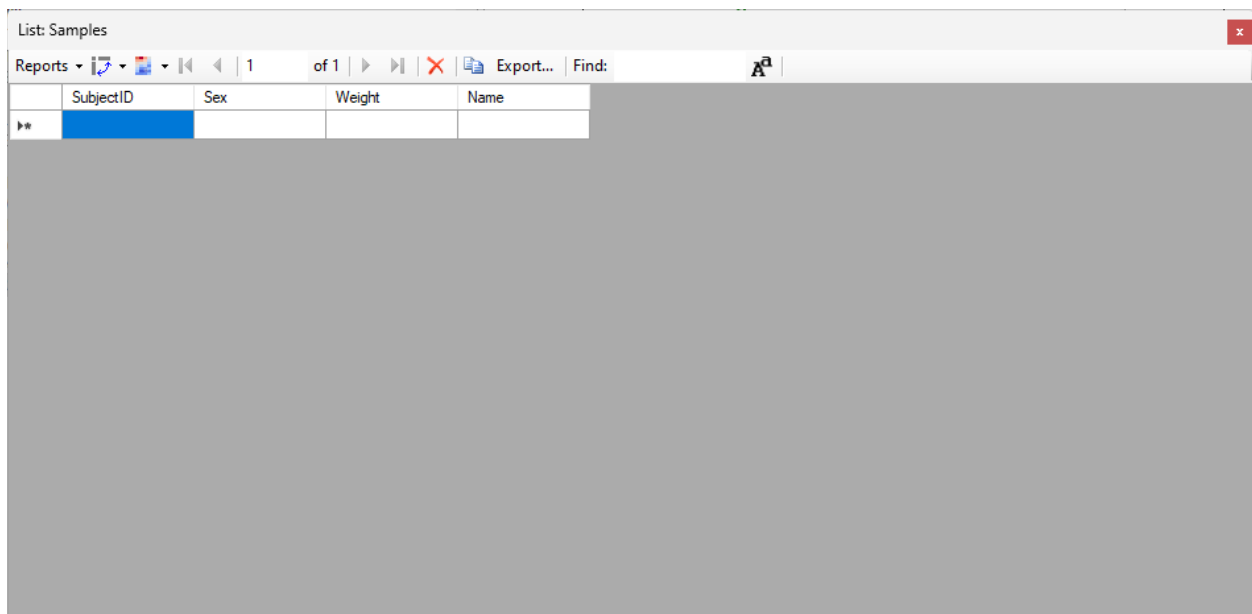
- Click on the **File** menu and choose **Open Containing Folder**

This will bring up the Windows Explorer on the folder containing the Skyline document. Find the file there called “SampleInfo.txt”. Double-click on that file in Windows Explorer.

This will most likely bring up Notepad, a simple text editor. The first line of the text editor has the column names “SubjectID”, “Sex”, “Weight” and “Name”.

- Select all the text in the document except for the first line and copy it to the clipboard.

Go back to Skyline and make it so that the first column of the first row in the **List: Samples** is the current cell.



- Paste the text from the clipboard (**Ctrl+V**)

This should add 14 rows to the grid.

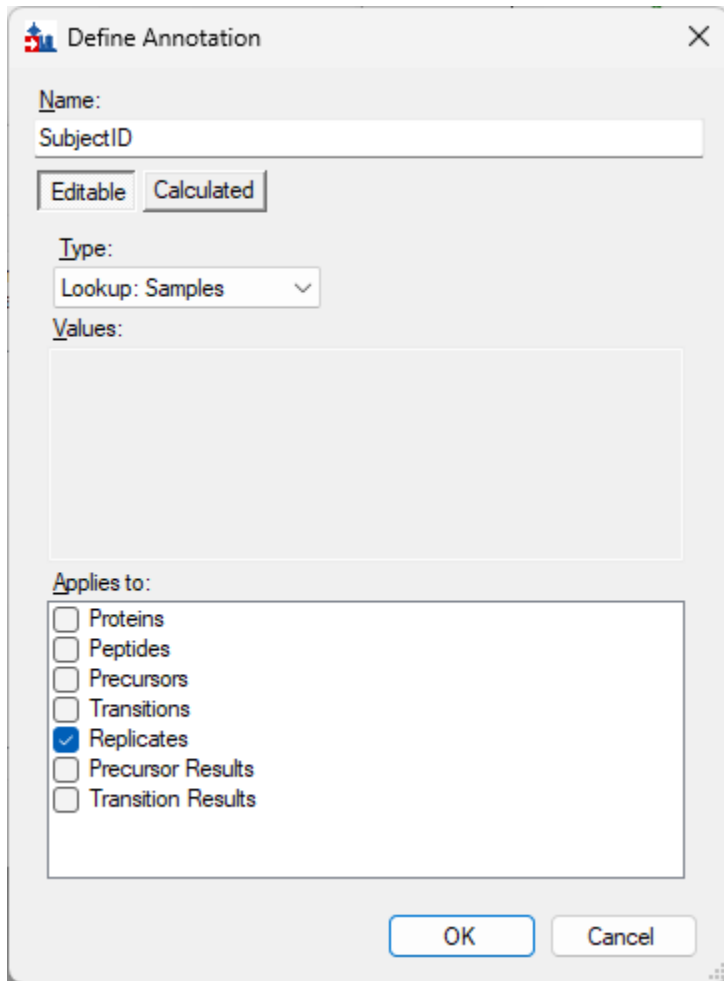
List: Samples

Reports ▾ | 14 of 14 | Export... | Find:

SubjectID	Sex	Weight	Name
D102	M	190	Drizzle
D103	F	245	Sniffles
D108	F	182	Mumbles
D138	M	210	Rattle
D154	M	228	Fumble
D172	F	175	Wheezy
D196	F	195	Wobbles
H146	M	250	Squeaks
H147	F	240	Nibbles
H148	M	202	Zippy
H159	F	220	Whiskers
H160	M	215	Skipper
H161	F	255	Pippin
H162	M	235	Scamper

## Changing annotation type

- On the **Settings** menu choose **Document Settings**
- On the **Annotations** tab of the **Document Settings** dialog, click the **Edit List** button
- Click on **SubjectID** in the **Define Annotations** dialog
- Click the **Edit** button
- In the **Type** dropdown, choose **Lookup: Samples**



- Click the **OK** button on the **Define Annotation** dialog
- Click the **OK** button on the **Define Annotations** dialog

Currently there is a bug in Skyline-daily that prevents lookup annotations from working with Result File Rules. The following two steps will not be necessary in the future

- Select the **Result Files** tab in the **Document Settings** dialog
- Uncheck the checkbox next to **Cohort and SubjectID**

After that has been done you can continue with:

- Click the **OK** button in the **Document Settings** dialog

Notice that in the Document Grid, the values displayed in the "SubjectID" column are now the names of rats from the Samples list. For each row where the SubjectID annotation has matched something in the ID Property column from the list, Skyline displays the value from the Display Property of the list.

Replicate	Sample Type	Analyte Concentration	Cohort	SubjectID
<a href="#">D_102_REP1</a>	Unknown		Diseased	Drizzle
<a href="#">D_102_REP2</a>	Unknown		Diseased	Drizzle
<a href="#">D_102_REP3</a>	Unknown		Diseased	Drizzle
<a href="#">D_103_REP1</a>	Unknown		Diseased	Sniffles
<a href="#">D_103_REP2</a>	Unknown		Diseased	Sniffles
<a href="#">D_103_REP3</a>	Unknown		Diseased	Sniffles
<a href="#">D_108_REP1</a>	Unknown		Diseased	Mumbles
<a href="#">D_108_REP2</a>	Unknown		Diseased	Mumbles
<a href="#">D_108_REP3</a>	Unknown		Diseased	Mumbles
<a href="#">D_138_REP1</a>	Unknown		Diseased	Rattle
<a href="#">D_138_REP2</a>	Unknown		Diseased	Rattle
<a href="#">D_138_REP3</a>	Unknown		Diseased	Rattle
<a href="#">D_154_REP1</a>	Unknown		Diseased	Fumble
<a href="#">D_154_REP2</a>	Unknown		Diseased	Fumble
<a href="#">D_154_REP3</a>	Unknown		Diseased	Fumble
<a href="#">D_172_REP1</a>	Unknown		Diseased	Whoozy

## Looking at results in the Document Grid

- Choose **Peptides** in the **Reports** dropdown at the top of the Document Grid
- Choose **Customize Report** in the **Reports** dropdown at the top of the Document Grid

This brings up the Customize Report dialog where the columns from the Peptides report have been selected.

- Click on the name “First Position” in the listbox on the right
- While holding down the shift key, click on the name “Peptide Note” in that same listbox

This causes all the column names between “First Position” and “Peptide Note” to become selected.

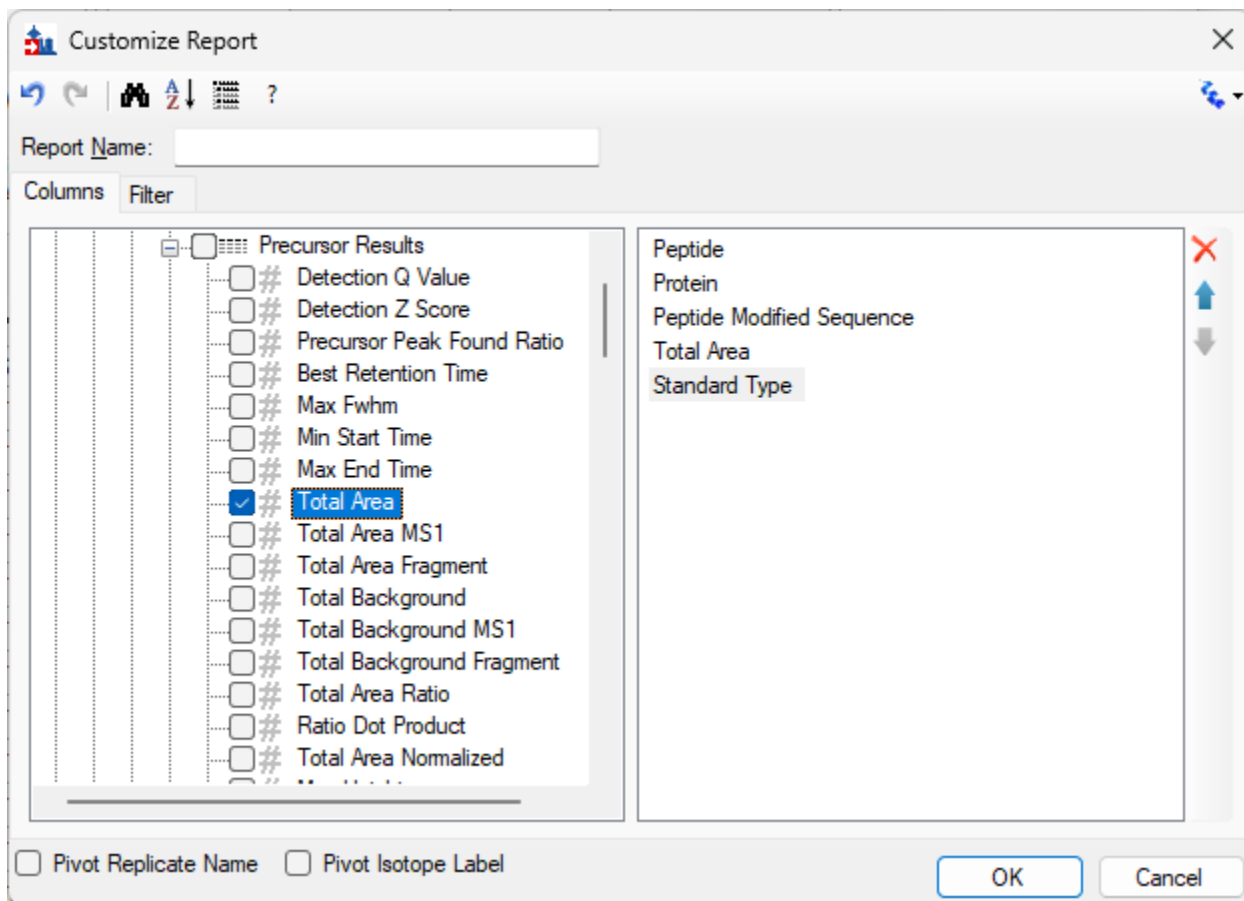
- Click on the red X to the right of the column list

This removes the selected columns from the column list, so that only “Peptide”, “Protein”, “Peptide Modified Sequence” and “Standard Type” remain.

- In the column tree on the left, expand the item “Peptides” and then expand “Precursors” and then “Precursor Results”
- Check the checkbox next to “Total Area”



This adds the “Total Area” column to the list of chosen columns.



When a column is added to a report, it is usually inserted ahead of whatever column may be selected in the column list. If no column is selected in the column list then newly added columns are added to the end.

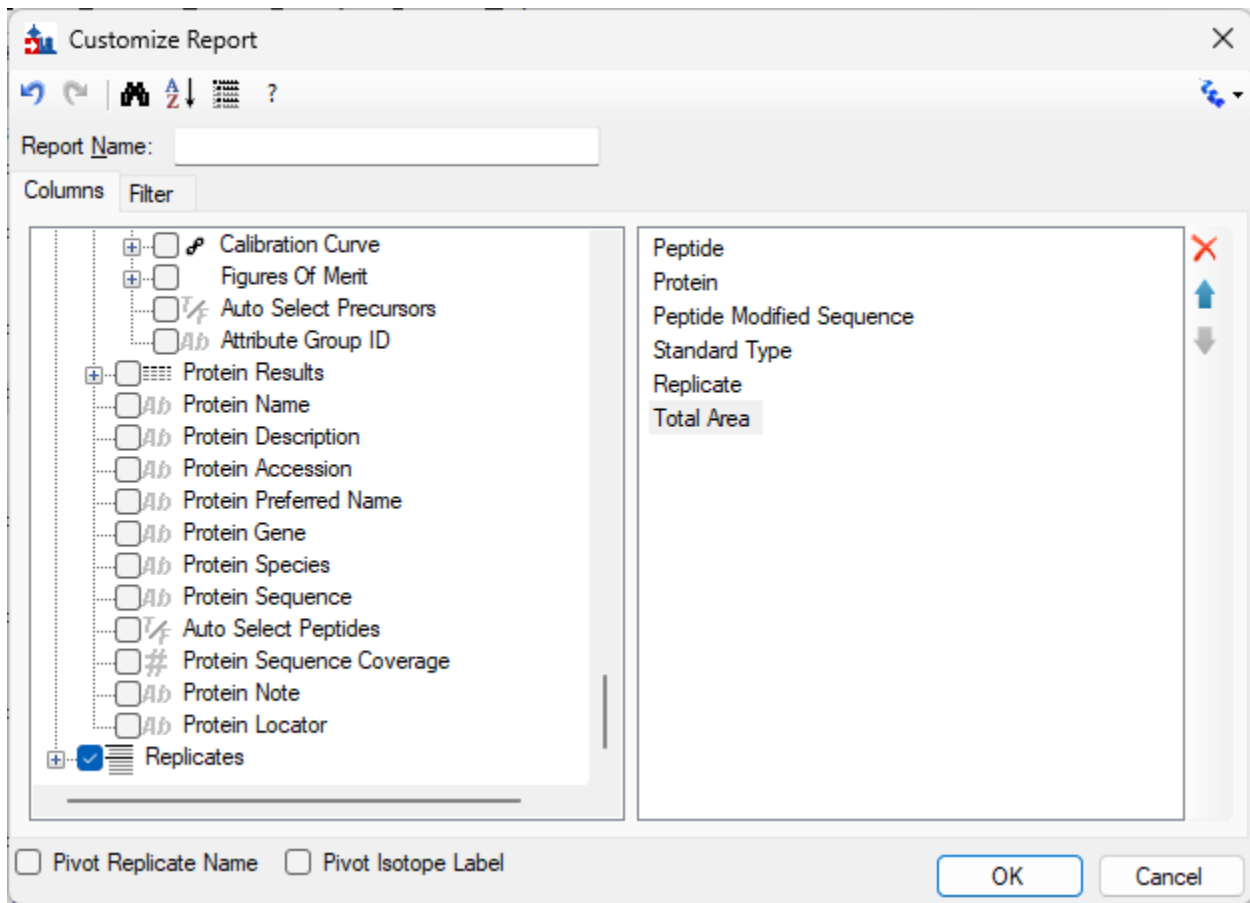
We would prefer to have the “Total Area” column appear after “Standard Type”

- Click the up arrow button to the right of the column list so that **Standard Type** moves before **Total Area**

We would like to add another column to the report, and we would like that column to appear before **Total Area**

- Click on **Total Area** in the column list on the right
- In the tree view on the left, scroll all the way to the bottom
- Click the checkbox next to **Replicates** in the tree view

The Customize Report dialog should now look like this:



- Type “Peptide Areas” into the **Report Name** textbox
- Press the **OK** button

The Document Grid should now look like this:

Document Grid: Peptide Areas

Reports ▾ | 1 of 5250 | Export... | Find: A<sup>1</sup>

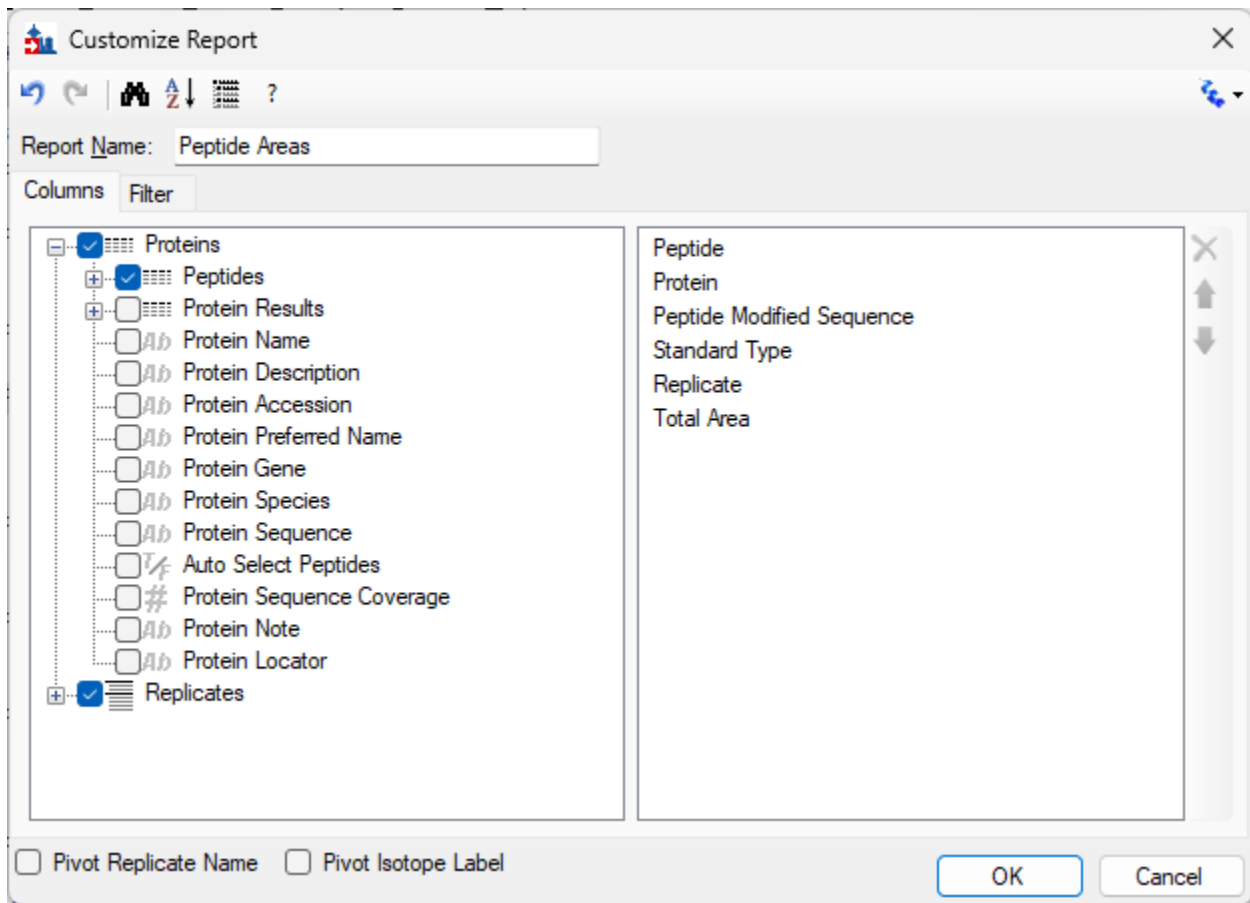
	Peptide	Protein	Peptide Modified Sequence	Standard Type	Replicate	Total Area
▶	<a href="#">CSLPRPWALTE...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_102_REP1</a>	137463
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_102_REP2</a>	96271
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_102_REP3</a>	94935
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_103_REP1</a>	60306
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_103_REP2</a>	53572
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_103_REP3</a>	2513
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_108_REP1</a>	104768
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_108_REP2</a>	1067
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_108_REP3</a>	63505
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_138_REP1</a>	88238
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_138_REP2</a>	71761
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_138_REP3</a>	68037
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_154_REP1</a>	109990
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_154_REP2</a>	74480
	<a href="#">CSLPRPWALTF...</a>	<a href="#">NP_036629</a>	C[+57]SLPRPW...		<a href="#">D_154_REP3</a>	76174

This report has 42 rows for each peptide.

## Pivoting on Replicate Name

- In the **Reports** dropdown at the top of the Document Grid choose **Edit Report**

This brings up the Customize Report dialog again



- Click the **Pivot Replicate Name** checkbox
- Press the **OK** button

The Document Grid is now displaying one row per peptide.

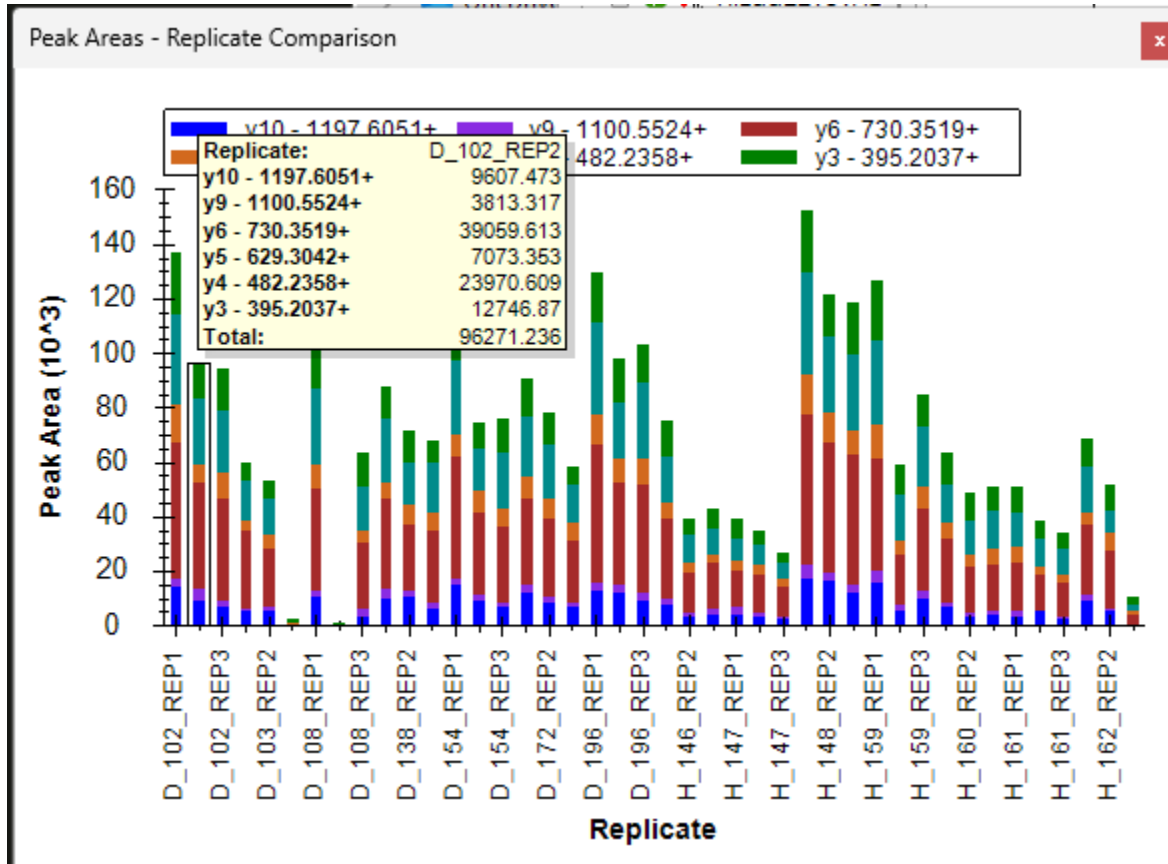
- Make the **Document Grid** window as wide as you can

Peptide	Protein	Peptide Modified Sequence	Standard Type	D_102_REP1 Replicate	D_102_REP1 Total Area	D_102_REP2 Replicate	D_102_REP2 Total Area	D_102_REP3 Replicate	D_102_REP3 Total Area	D_103_REP1 Replicate	D_103_REP1 Total Area	D_103_REP2 Replicate
CSLRPRPWALTE...	NP_036629	C[+57]SLRPPW...		D_102_REP1	137463	D_102_REP2	96271	D_102_REP3	94935	D_103_REP1	60306	D_103_REP2
LGGEVSVACK	NP_037030	LGGEVSVAC[+...		D_102_REP1	44348	D_102_REP2	30437	D_102_REP3	23316	D_103_REP1	38642	D_103_REP2
VGQPGDAGAA...	NP_037030	VGQPGDAGAAG...		D_102_REP1	9615704	D_102_REP2	6632949	D_102_REP3	6325865	D_103_REP1	6333859	D_103_REP2
GSYNLQDLLAQ...	NP_602308	GSYNLQDLLAQ...		D_102_REP1	102349	D_102_REP2	88045	D_102_REP3	87100	D_103_REP1	37012	D_103_REP2
TSDOIHFFFAK	NP_001012027	TSDOIHFFFAK		D_102_REP1	144712	D_102_REP2	141005	D_102_REP3	155442	D_103_REP1	48834	D_103_REP2
LQPLDFK	NP_001012027	LQPLDFK		D_102_REP1	3890327	D_102_REP2	2695215	D_102_REP3	3001110	D_103_REP1	1447657	D_103_REP2
SQLPGIIAEGR	NP_001012027	SQLPGIIAEGR		D_102_REP1	384659	D_102_REP2	358221	D_102_REP3	296176	D_103_REP1	122332	D_103_REP2
DFATVYVDAVK	NP_036870	DFATVYVDAVK		D_102_REP1	1882523	D_102_REP2	1518331	D_102_REP3	1430196	D_103_REP1	4480907	D_103_REP2
DYVSQFESSTL...	NP_036870	DYVSQFESSTL...		D_102_REP1	788727	D_102_REP2	654249	D_102_REP3	611045	D_103_REP1	1504408	D_103_REP2
TGTNLMDFLSR	NP_037244	TGTNLMDFLSR		D_102_REP1	282895	D_102_REP2	159183	D_102_REP3	233907	D_103_REP1	192569	D_103_REP2
LMSPEEKPAAPA...	NP_037244	LMSPEEKPAAPA...		D_102_REP1	364297	D_102_REP2	255979	D_102_REP3	152243	D_103_REP1	267682	D_103_REP2
GTITTSIALDDPK	NP_001010968	GTITTSIALDDPK		D_102_REP1	188041	D_102_REP2	156887	D_102_REP3	149429	D_103_REP1	128381	D_103_REP2
TQTPVQSGCHLE...	NP_001010968	TQTPVQSGC[+57]...		D_102_REP1	297921	D_102_REP2	235554	D_102_REP3	216155	D_103_REP1	181697	D_103_REP2
IFPENNIK	NP_001010968	IFPENNIK		D_102_REP1	1314304	D_102_REP2	1114572	D_102_REP3	1085910	D_103_REP1	430986	D_103_REP2

The “Replicate” and “Total Area” columns are repeated horizontally, showing values from the different replicates.

- Click on the first cell in the Peptide column where it says “CSLPRPWALTFSYGR”. This will select that peptide in the Targets tree
- On the **View** menu choose **Peak Areas** and then **Replicate Comparison**

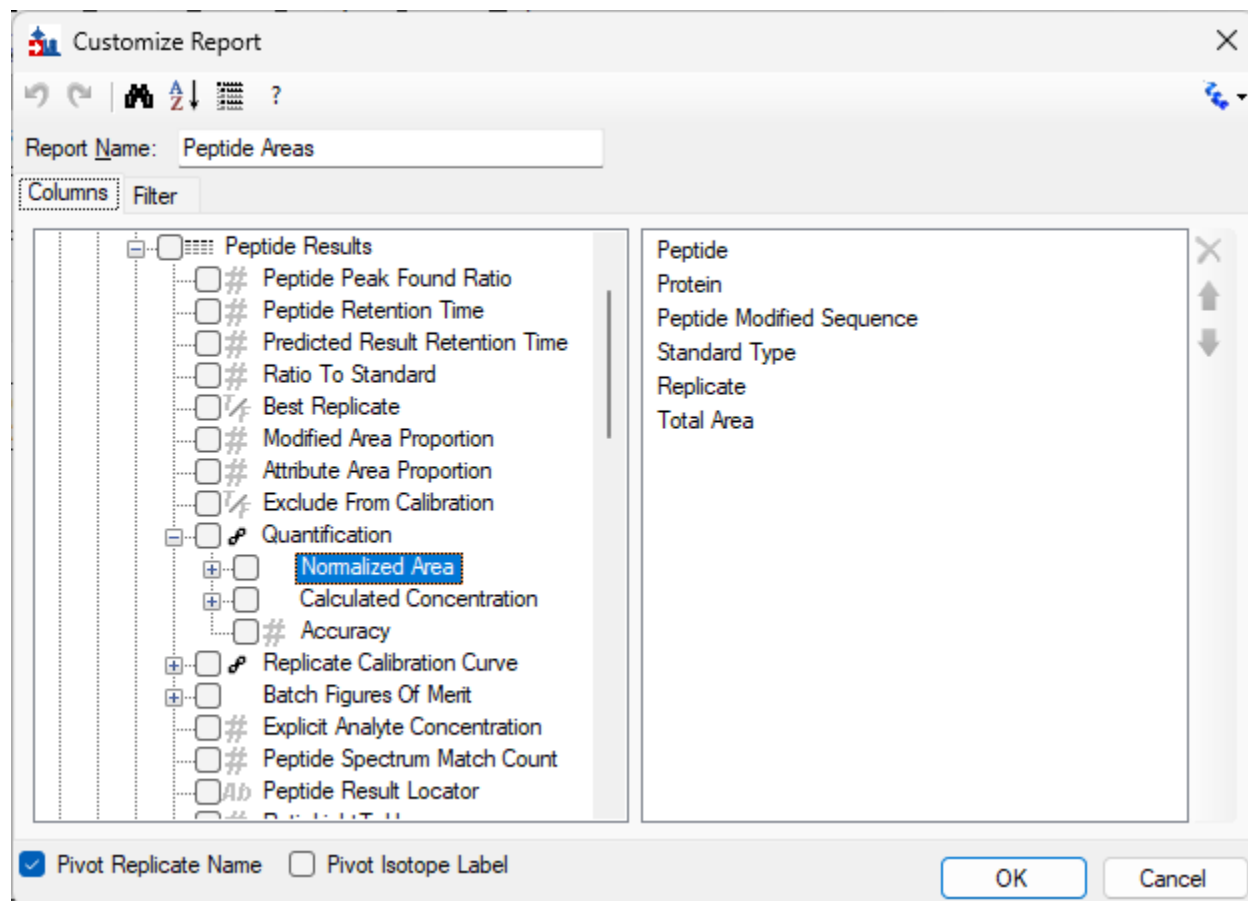
This shows the peak areas for the currently selected peptide across all the replicates. The order of the replicates in the Peak Areas Replicate Comparison graph is the same as the horizontal order of the replicates in the Document Grid. You can visually verify that the Total Area values displayed in the Document Grid are the same as the values displayed in the Peak Areas Replicate Comparison graph.



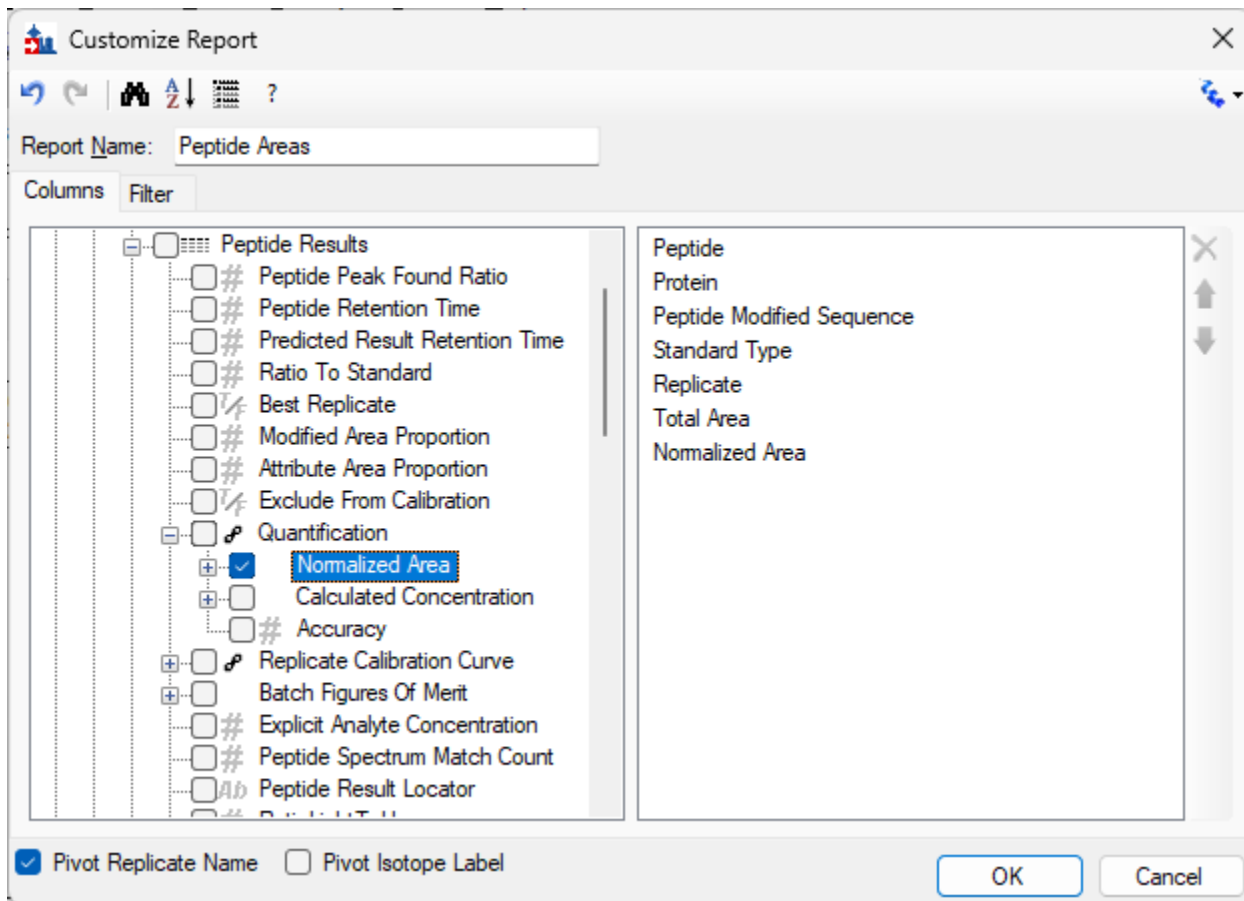
## The “Normalized Area” column

- On the **Reports** dropdown at the top of the Document Grid choose **Edit Report**
- Click the binoculars button on the tool strip at the top of the Customize Report dialog
- Type “Normalized Area” into the textbox in the **Find Column** dialog
- Click **Find Next**
- Click the **Close** button on the **Find Column** dialog

The Normalized Area column is now selected in the column tree on the left



- Click the checkbox next to **Normalized Area**



- Click the **OK** button in the Customize Report dialog

The Document Grid now shows the values from the “Normalized Area” column next to each of the “Total Area” values.

Peptide	Protein	Peptide Modified Sequence	Standard Type	D_102_REP1 Replicate	D_102_REP1 Total Area	D_102_REP1 Normalized Area	D_102_REP2 Replicate	D_102_REP2 Total Area	D_102_REP2 Normalized Area	D_102_REP3 Replicate
ISLEIENVALTF	NP_036629	C(+57)SLPRPW...		D_102_REP1	137463	1.3746E+5	D_102_REP2	96271	9.6271E+4	D_102_REP3
LGGEVSVACK	NP_037030	LGGEVSVAC[+...		D_102_REP1	44348	4.4348E+4	D_102_REP2	30437	3.0437E+4	D_102_REP3
VGQPGDAGAA...	NP_037030	VGQPGDAGAAG...		D_102_REP1	9615704	9.6157E+6	D_102_REP2	6632949	6.6329E+6	D_102_REP3
GSYNLQDLLAQ...	NP_602308	GSYNLQDLLAQ...		D_102_REP1	102349	1.0235E+5	D_102_REP2	88045	8.8045E+4	D_102_REP3
TSDQIHFFFAK	NP_001012027	TSDQIHFFFAK		D_102_REP1	144712	1.4471E+5	D_102_REP2	141005	1.4100E+5	D_102_REP3
LQPLDFK	NP_001012027	LQPLDFK		D_102_REP1	3890327	3.8903E+6	D_102_REP2	2695215	2.6952E+6	D_102_REP3
SQLPGIIAAGR	NP_001012027	SQLPGIIAAGR		D_102_REP1	384659	3.8466E+5	D_102_REP2	358221	3.5822E+5	D_102_REP3
DFATVYVDAVK	NP_036870	DFATVYVDAVK		D_102_REP1	1882523	1.8825E+6	D_102_REP2	1518331	1.5183E+6	D_102_REP3
DYVSQFESSTL...	NP_036870	DYVSQFESSTLGK		D_102_REP1	788727	7.8873E+5	D_102_REP2	654249	6.5425E+5	D_102_REP3
TGTLNMDFLSR	NP_037244	TGTLNMDFLSR		D_102_REP1	282895	2.8290E+5	D_102_REP2	159183	*1.5918E+5	D_102_REP3
LMSPEEKPAAP...	NP_037244	LMSPEEKPAAPAK		D_102_REP1	364297	3.6430E+5	D_102_REP2	255979	2.5598E+5	D_102_REP3
GTITSIAALDDPK	NP_001010968	GTITSIAALDDPK		D_102_REP1	188041	1.8804E+5	D_102_REP2	156887	1.5689E+5	D_102_REP3
TQTPVGGCHLE...	NP_001010968	TQTPVGGC(+57)...		D_102_REP1	297921	2.9792E+5	D_102_REP2	235554	2.3555E+5	D_102_REP3
IFPENNIK	NP_001010968	IFPENNIK		D_102_REP1	1314304	1.3143E+6	D_102_REP2	1114572	1.1146E+6	D_102_REP3

The values shown in the “Total Area” columns are the same as the values in the “Normalized Area” columns although they are formatted differently.

## Changing the format on a column

It may be easier to see that the values if you change the format of one of the columns to be the same.

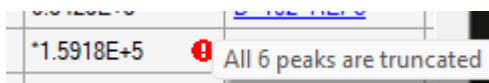
- Right click on the column header where it says “D\_102\_REP1 Total Area” and choose **Number Format**
- In the **Choose Format** dialog, choose **Scientific 3.1416E+0** in the dropdown.
- Press the **OK** button on the **Choose Format** dialog

Peptide	Protein	Peptide Modified Sequence	Standard Type	D_102_REP1 Replicate	D_102_REP1 Total Area	D_102_REP1 Normalized Area	D_102_REP2 Replicate	D_102_REP2 Total Area	D_102_REP2 Normalized Area	D_102_REP3 Replicate
<a href="#">GRPNPWALTC...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_102_REP1</a>	1.3746E+5	1.3746E+5	<a href="#">D_102_REP2</a>	96271	9.6271E+4	<a href="#">D_102_REP3</a>
<a href="#">LGGEEVSVACK</a>	NP_037030	LGGEEVSVACJ+...		<a href="#">D_102_REP1</a>	4.4348E+4	4.4348E+4	<a href="#">D_102_REP2</a>	30437	3.0437E+4	<a href="#">D_102_REP3</a>
<a href="#">VGQPGDAGAA...</a>	NP_037030	VGQPGDAGAAAG...		<a href="#">D_102_REP1</a>	9.6157E+6	9.6157E+6	<a href="#">D_102_REP2</a>	6632949	6.6329E+6	<a href="#">D_102_REP3</a>
<a href="#">GSYNLQDLLAQ...</a>	NP_602308	GSYNLQDLLAQ...		<a href="#">D_102_REP1</a>	1.0235E+5	1.0235E+5	<a href="#">D_102_REP2</a>	88045	8.8045E+4	<a href="#">D_102_REP3</a>
<a href="#">TSDQIHFFFAK</a>	NP_001012027	TSDQIHFFFAK		<a href="#">D_102_REP1</a>	1.4471E+5	1.4471E+5	<a href="#">D_102_REP2</a>	141005	1.4100E+5	<a href="#">D_102_REP3</a>
<a href="#">LQPLDFK</a>	NP_001012027	LQPLDFK		<a href="#">D_102_REP1</a>	3.8903E+6	3.8903E+6	<a href="#">D_102_REP2</a>	2695215	2.6952E+6	<a href="#">D_102_REP3</a>
<a href="#">SQLPGIIAAGR</a>	NP_001012027	SQLPGIIAAGR		<a href="#">D_102_REP1</a>	3.8466E+5	3.8466E+5	<a href="#">D_102_REP2</a>	358221	3.5822E+5	<a href="#">D_102_REP3</a>
<a href="#">DFATVYVDAVK</a>	NP_036870	DFATVYVDAVK		<a href="#">D_102_REP1</a>	1.8825E+6	1.8825E+6	<a href="#">D_102_REP2</a>	1518331	1.5183E+6	<a href="#">D_102_REP3</a>
<a href="#">DYVSQFESSTL...</a>	NP_036870	DYVSQFESSTLGK		<a href="#">D_102_REP1</a>	7.8873E+5	7.8873E+5	<a href="#">D_102_REP2</a>	654249	6.5425E+5	<a href="#">D_102_REP3</a>
<a href="#">TGTNLMDFLSR</a>	NP_037244	TGTNLMDFLSR		<a href="#">D_102_REP1</a>	2.8290E+5	2.8290E+5	<a href="#">D_102_REP2</a>	159183	*1.5918E+5	<a href="#">D_102_REP3</a>
<a href="#">LMSPEEKPAAP...</a>	NP_037244	LMSPEEKPAAPAK		<a href="#">D_102_REP1</a>	3.6430E+5	3.6430E+5	<a href="#">D_102_REP2</a>	255979	2.5598E+5	<a href="#">D_102_REP3</a>
<a href="#">GTITSIAALDDPK</a>	NP_001010968	GTITSIAALDDPK		<a href="#">D_102_REP1</a>	1.8804E+5	1.8804E+5	<a href="#">D_102_REP2</a>	156887	1.5689E+5	<a href="#">D_102_REP3</a>
<a href="#">TQTPVGGCHLE...</a>	NP_001010968	TQTPVGGC(+57)...		<a href="#">D_102_REP1</a>	2.9792E+5	2.9792E+5	<a href="#">D_102_REP2</a>	235554	2.3555E+5	<a href="#">D_102_REP3</a>
<a href="#">IFPENNIK</a>	NP_001010968	IFPENNIK		<a href="#">D_102_REP1</a>	1.3143E+6	1.3143E+6	<a href="#">D_102_REP2</a>	1114572	1.1146E+6	<a href="#">D_102_REP3</a>

It is now much easier to see that the values in the “Total Area” and “Normalized Area” columns are the same.

Notice that in the tenth row in the “D\_102\_REP2 Normalized Area” column there is a red exclamation mark next to the value.

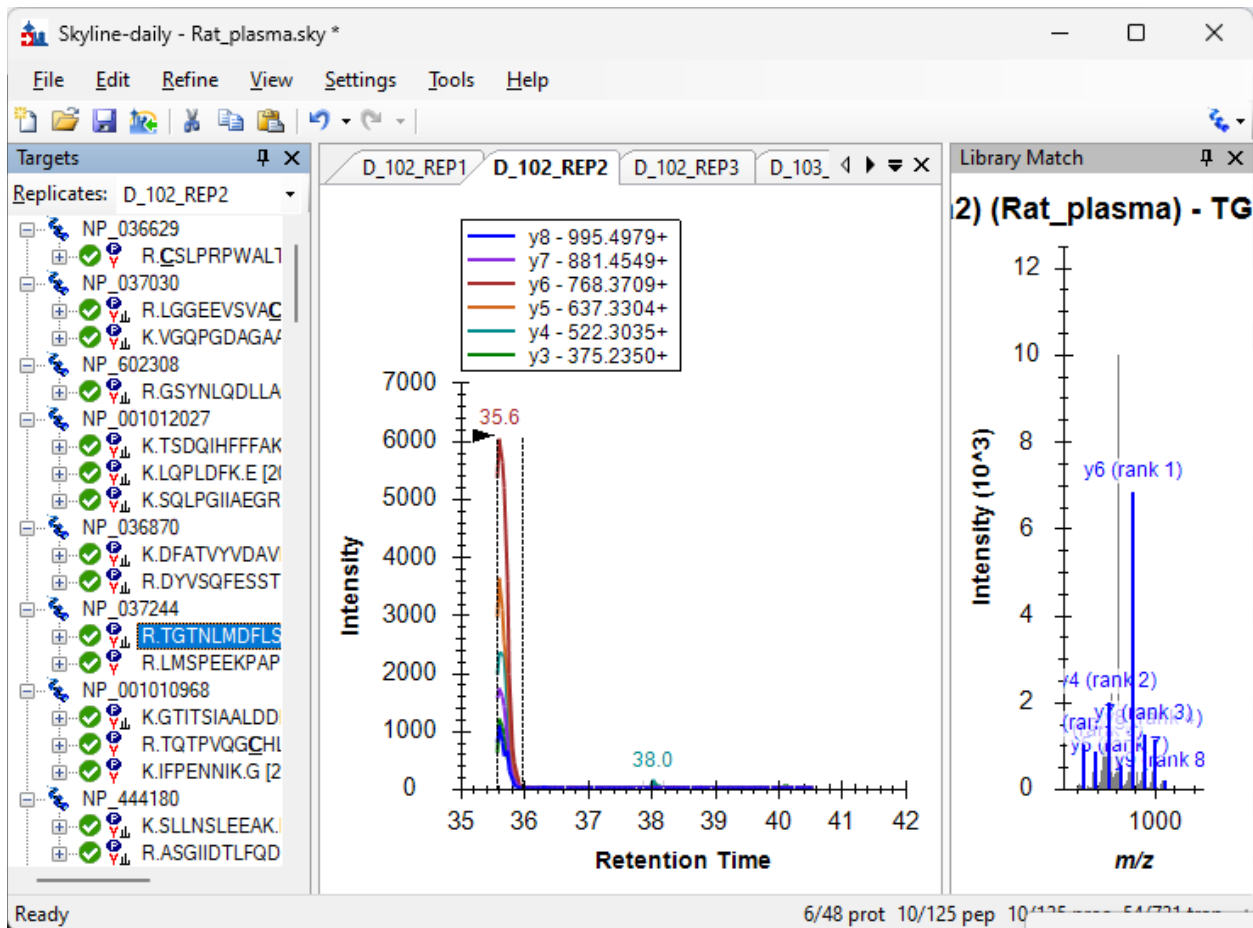
If you hover the mouse over the exclamation mark you will see a tooltip.



In order to investigate that

- Click on the blue underlined text in the first column of the tenth row in the grid (“TGTNLMDFLSR”). This will select that peptide in the Targets tree.
- Click on one of the blue underlined “D\_102\_REP2” to select that replicate
- Move the Document Grid and other windows out of the way so that you can see the chromatograms in the main window.





The red exclamation mark is warning that the integration boundaries of the chosen peak coincide with the edit of the time range of the acquired SRM chromatogram. This could be a problem when comparing peak areas between replicates, as replicates with truncated peaks will tend to have lower peak areas than non-truncated peaks.

## Sub-properties of the Normalized Area column

- In the **Reports** dropdown at the top of the **Document Grid** choose **Edit Report**
- Double click in the **Normalized Area** in the list on the right

The **Normalized Area** in the tree on the left will become selected when the column is double clicked in the list box.

- Click the plus button to the left of the **Normalized Area** node in the tree to expand it

This reveals three additional columns which could be added to the report.

- Click the checkbox next to **Normalized Area Raw**, **Normalized Area Strict** and **Normalized Area Message**
- Click OK in the **Customize Report** dialog



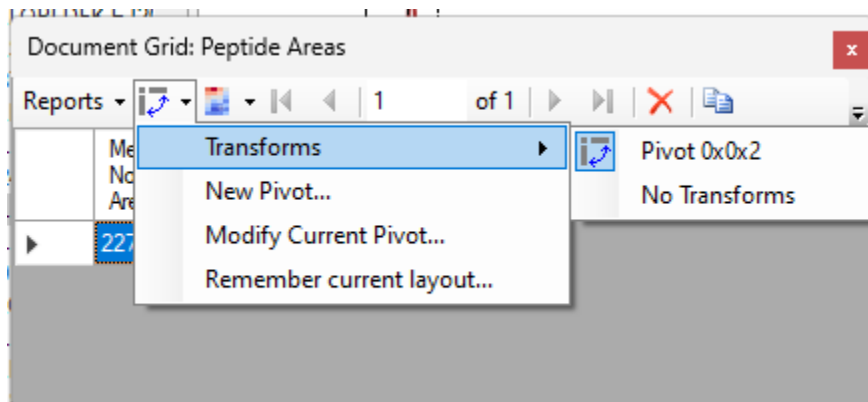
	Mean Normalized Area Raw	Mean Normalized Area Strict
	2271607.772094...	2323741.928744...

The Document Grid now shows only a single row where the two columns show the average value from the Normalized Area Raw and Normalized Area Strict.

The average value in the Normalized Area Strict cell is a higher number than the value in the Normalized Area Raw cell. This is to be expected because truncated peaks tend to have smaller than expected areas and excluding them from a list would tend to result in a higher mean.

## Pivoting with row headers and column headers

- Click on the inverted triangle in the right portion of the second button from the left on the toolbar at the top of the Document Grid.
- Hover the mouse over the Transforms item



This shows a list of the operations which have been performed on the report.

- Click on the **No Transforms** item

This restores the Document Grid to the way that it looked before

Document Grid: Peptide Areas

Reports ▾ | of 5250 | Export... | Actions ▾ | Find:

	Peptide	Protein	Peptide Modified Sequence	Standard Type	Replicate	Total Area	Normalized Area Raw	Normalized Area Strict	Normalized Area Message	Normalized Area
▶	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_102_REP1</a>	137463	137462.7866210...	137462.7866210...		1.3746E+5
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_102_REP2</a>	96271	96271.23559570...	96271.23559570...		9.6271E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_102_REP3</a>	94935	94935.19567871...	94935.19567871...		9.4935E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_103_REP1</a>	60306	60305.82891845...	60305.82891845...		6.0306E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_103_REP2</a>	53572	53572.298828125	53572.298828125		5.3572E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_103_REP3</a>	2513	2512.730072021...	2512.730072021...		2.5127E+3
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_108_REP1</a>	104768	104768.4890136...	104768.4890136...		1.0477E+5
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_108_REP2</a>	1067	1066.721562504...	1066.721562504...		1.0667E+3
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_108_REP3</a>	63505	63504.994140625	63504.994140625		6.3505E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_138_REP1</a>	88238	88238.40747070...	88238.40747070...		8.8238E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_138_REP2</a>	71761	71761.22314453...	71761.22314453...		7.1761E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_138_REP3</a>	68037	68037.25854492...	68037.25854492...		6.8037E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_154_REP1</a>	109990	109989.6794433...	109989.6794433...		1.0999E+5
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_154_REP2</a>	74480	74479.84594726...	74479.84594726...		7.4480E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_154_REP3</a>	76174	76173.55432128...	76173.55432128...		7.6174E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_172_REP1</a>	90538	90537.76586914...	90537.76586914...		9.0538E+4
	<a href="#">CSLPRPWALTF...</a>	NP_036629	C(+57)SLPRPW...		<a href="#">D_172_REP2</a>	78634	78633.87207031...	78633.87207031...		7.8634E+4

- In the **Reports** dropdown at the top of the Document Grid choose **Edit Report**
- Click the “+” button next to “Replicates” at the bottom to expand it and then click the checkbox next to SampleID

Customize Report

Report Name: Peptide Areas

Columns Filter

- Protein Preferred Name
- Protein Gene
- Protein Species
- Protein Sequence
- Auto Select Peptides
- Protein Sequence Coverage
- Protein Note
- Protein Locator
- Replicates
  - Replicate Name
  - Files
    - Sample Type
    - Analyte Concentration
    - Sample Dilution Factor
    - Batch Name
    - Replicate Locator
    - Cohort
    - SampleID

Peptide  
Protein  
Peptide Modified Sequence  
Standard Type  
Replicate  
Total Area  
Normalized Area Raw  
Normalized Area Strict  
Normalized Area Message  
Normalized Area  
SampleID

Pivot Replicate Name  Pivot Isotope Label

OK Cancel

- Click the **OK** button in the Customize Report dialog

This adds the SampleID column to the report

Document Grid: Peptide Areas

Reports | 1 of 5250 | Export... | Actions | Find: A

Peptide	Protein	Peptide Modified Sequence	Standard Type	Replicate	Total Area	Normalized Area Raw	Normalized Area Strict	Normalized Area Message	Normalized Area	SampleID
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_102_REP1	137463	137462.7866210...	137462.7866210...		1.3746E+5	Drizzle
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_102_REP2	96271	96271.23559570...	96271.23559570...		9.6271E+4	Drizzle
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_102_REP3	94935	94935.19567871...	94935.19567871...		9.4935E+4	Drizzle
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_103_REP1	60306	60305.82891845...	60305.82891845...		6.0306E+4	Sniffles
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_103_REP2	53572	53572.298828125	53572.298828125		5.3572E+4	Sniffles
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_103_REP3	2513	2512.730072021...	2512.730072021...		2.5127E+3	Sniffles
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_108_REP1	104768	104768.4890136...	104768.4890136...		1.0477E+5	Mumbles
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_108_REP2	1067	1066.721562504...	1066.721562504...		1.0667E+3	Mumbles
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_108_REP3	63505	63504.994140625	63504.994140625		6.3505E+4	Mumbles
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_138_REP1	88238	88238.40747070...	88238.40747070...		8.8238E+4	Rattle
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_138_REP2	71761	71761.22314453...	71761.22314453...		7.1761E+4	Rattle
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_138_REP3	68037	68037.25854492...	68037.25854492...		6.8037E+4	Rattle
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_154_REP1	109990	109989.6794433...	109989.6794433...		1.0999E+5	Fumble
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_154_REP2	74480	74479.84594726...	74479.84594726...		7.4480E+4	Fumble
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_154_REP3	76174	76173.55432128...	76173.55432128...		7.6174E+4	Fumble
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_172_REP1	90538	90537.76586914...	90537.76586914...		9.0538E+4	Wheezy
CSLPRPWALTE...	NP_036629	C[+57]SLPRPW...		D_172_REP2	78634	78633.87207031...	78633.87207031...		7.8634E+4	Wheezy

- Click the second button from the left on the tool strip at the top of the Document Grid to bring up the Pivot Editor

Pivot Editor

Peptide

Protein

Peptide Modified Sequence

Standard Type

Replicate

Total Area

Normalized Area Raw

Normalized Area Strict

Normalized Area Message

Normalized Area

SampleID

Add Row Header >>

Add Column Header >>

Add Value >>

Count

Row Headers

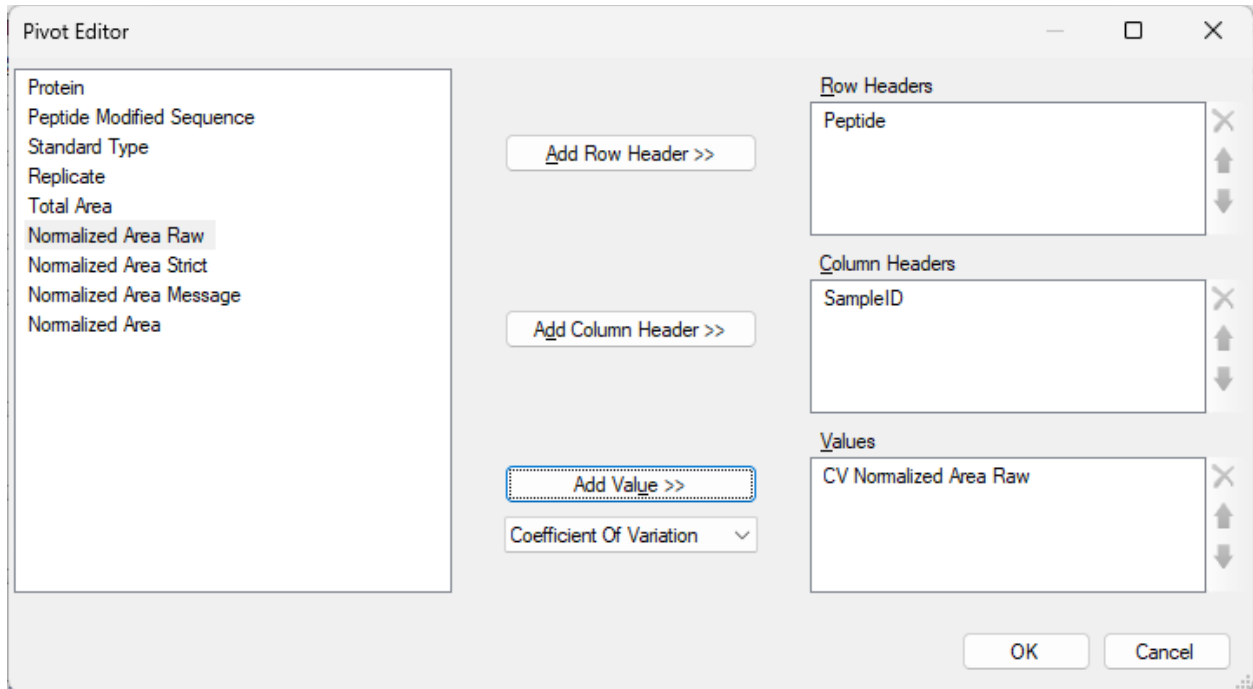
Column Headers

Values

OK Cancel

- Click on the **Peptide** item in the list box on the left and then press the **Add Row Header** button
- Click on the **SampleID** item in the list box on the left and press the **Add Column Header** button
- Click on the **Normalized Area Raw** button on the left
- Choose **Coefficient of Variation** in the dropdown below the **Add Value** button
- Click on the **Add Value** button

The Pivot Editor should now look like this:



The report now shows the Coefficients of Variations for each peptide between each rat's technical replicates

Peptide	Drizzle CV Normalized Area Raw	Sniffles CV Normalized Area Raw	Mumbles CV Normalized Area Raw	Rattle CV Normalized Area Raw	Funble CV Normalized Area Raw	Wheezy CV Normalized Area Raw	Wobbles CV Normalized Area Raw	Squeaks CV Normalized Area Raw	Nibbles CV Normalized Area Raw	Zppy CV Normalized Area Raw	Wh No Arc
CSLPRIPWALTE	22.1%	81.5%	92.5%	14.1%	23.1%	21%	15.5%	37.7%	18.3%	14.5%	37.1
LGGEVSVACK	32.7%	25.7%	53.7%	37.3%	32%	23.6%	60.4%	24.2%	26.2%	19.8%	21.1
VGQPGDAGAA	24.1%	19.7%	25.5%	22.5%	30.2%	18.7%	27.1%	17.3%	16.6%	10.4%	10.1
GSYNLQDLLAQ	9.2%	71.9%	50.8%	7.9%	16.9%	7.6%	15.4%	42.6%	41.4%	44.2%	97.1
TSDQIHFFFAK	5.1%	11.8%	17.3%	5.6%	11.2%	6.4%	18.9%	25%	36.5%	9.6%	4%
LQPLDFK	19.4%	14.4%	20.8%	6%	15.4%	10.6%	19.7%	15.2%	3.6%	14.2%	13.1
SQPLGIIAEGR	13.1%	12.1%	20.3%	23.6%	23.8%	18.2%	14.7%	21.1%	13.1%	8.1%	14.1
DFATVYVDVAVK	14.9%	84.9%	89.2%	17.9%	18.6%	21.2%	34.5%	88.1%	26.1%	36.2%	80.1
DYVSQFESSTL	13.5%	76.3%	152.9%	18.4%	23.8%	15.7%	146.1%	151.6%	146.1%	152.9%	157
TGTLNMDFLSR	27.6%	86.5%	104.6%	19.4%	22%	10.9%	87.5%	169.9%	159.6%	92.4%	36.1
LMSPEEKAPA	41.2%	24.2%	35.1%	36.9%	45.3%	28.8%	21.7%	33%	24.6%	10.7%	24.1
GTITSIALLDDPK	12.4%	83.4%	44.2%	16.9%	18.8%	85.2%	64.8%	86%	14.5%	46%	72.1
TQTPVQGCHLE	17.1%	11.1%	16.6%	19.1%	21.6%	12.6%	22.4%	15.7%	15.3%	8.4%	6.6
IPEPNNIK	10.6%	87.2%	88.5%	16.5%	19%	14.5%	17.1%	86.6%	90.7%	19.6%	91.1
SLLNSLEEAK	10.9%	12.2%	15.5%	19%	12.6%	8.8%	14.7%	14.7%	14.9%	11.5%	12.1
ASGIHDTLFGDR	15.2%	86.7%	44.3%	20.8%	20.8%	8.2%	12.2%	13.5%	16.3%	16.6%	26.1

- Click on the column header which says "Drizzle CV Normalized Area Raw" and choose **Sort Ascending**
- Click on the peptide **SVVDIGLIK** which is in the first column of the first row

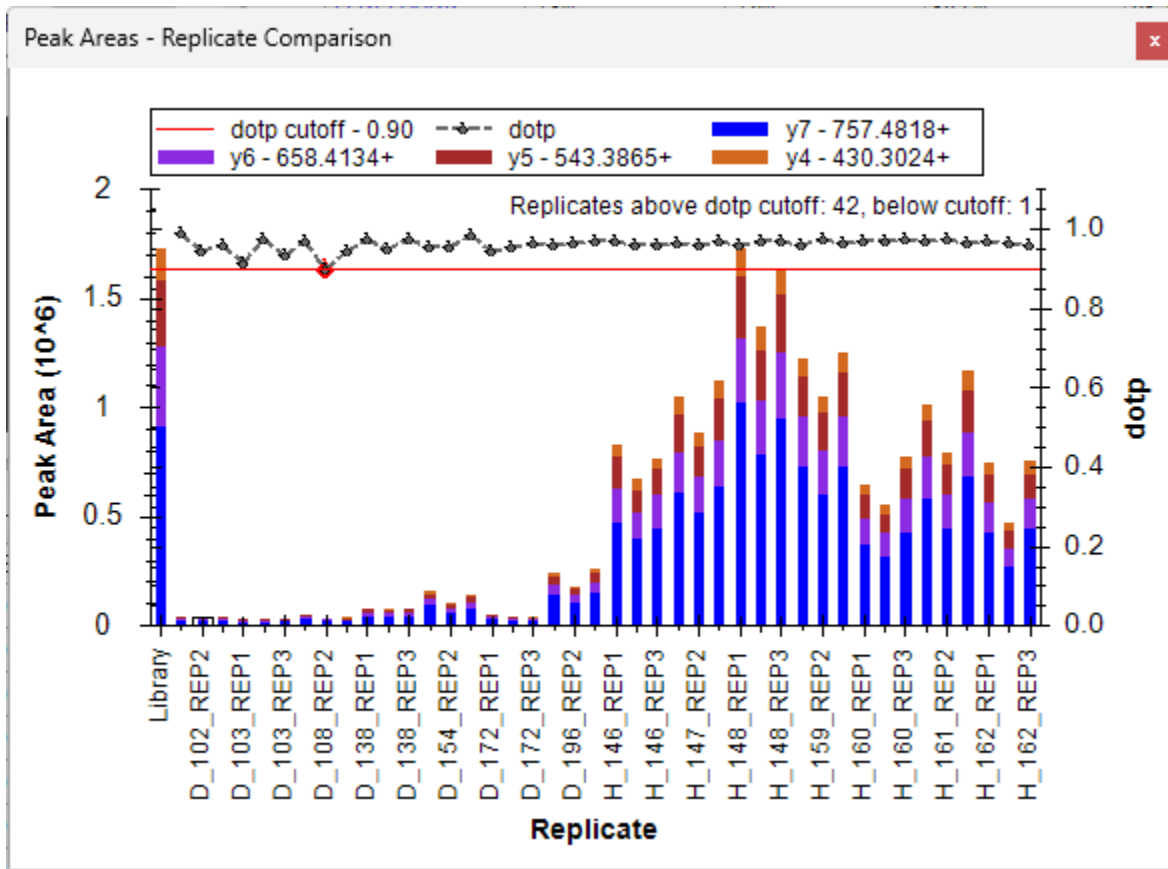
Document Grid: Peptide Areas

Reports 1 of 125 Export... Actions Find:

Peptide	Drizzle CV Normalized Area Raw	Sniffles CV Normalized Area Raw	Mumbles CV Normalized Area Raw	Rattle CV Normalized Area Raw	Fumble CV Normalized Area Raw	Wheezy CV Normalized Area Raw	Wobbles CV Normalized Area Raw	Squeaks CV Normalized Area Raw	Nibbles CV Normalized Area Raw	Zppy CV Normalized Area Raw	Wh No Are
<b>SVVDTGILK</b>	4.1%	9%	19.9%	2.8%	17.6%	13%	17.3%	10.6%	12%	11.9%	9.2
TSDQIHFFFAK	5.1%	11.8%	17.3%	5.6%	11.2%	6.4%	18.9%	25%	36.5%	9.6%	4%
AAPITQYLK	5.3%	12.8%	18.1%	20.1%	13.3%	18.5%	20.6%	11.9%	15.9%	16%	18.
LFDELVVDK	7.1%	7.6%	19.5%	21.4%	15.2%	9.4%	13.2%	11.4%	7.6%	12.9%	8.8
YHGVVTGLVMDK	7.2%	7.1%	26.4%	22.6%	16.3%	12%	21.2%	20%	14.9%	7.8%	7.8
VLVVEPEGIK	7.2%	11.1%	21.5%	9.9%	12%	17.8%	14.6%	12.2%	4.1%	13.3%	10.
DGAFTLYSFK	7.2%	16.6%	19.4%	14.6%	22%	13.4%	14.9%	24.5%	8.7%	10.8%	10.
EVLPELGIK	7.6%	6.2%	13.3%	15.8%	17%	8.7%	14.9%	14.1%	18.9%	14.8%	17.
TLFVSLPGLK	7.9%	7.4%	20.4%	9.2%	10.4%	13%	11.2%	6.5%	8.4%	14%	13.1
TFYQIGDSWEK	8.1%	9.4%	16.8%	25.8%	20.4%	21.6%	18%	22.2%	15.1%	9%	6.7
GSYNLQDLLAQ...	9.2%	71.9%	50.8%	7.9%	16.9%	7.6%	15.4%	42.6%	41.4%	44.2%	97.
DGGPDLVLLAK	9.3%	10.2%	18.4%	20%	20%	18.4%	9.3%	17.6%	8.6%	7.9%	9.2
IITGNALFDK	9.7%	19.6%	18.3%	29.7%	26.3%	19.6%	16.9%	22.2%	14.9%	20.7%	6.8
YGFYTHVFR	10.2%	15.5%	28.2%	28.5%	28.4%	25.8%	37.1%	23.1%	34.5%	13.9%	15.
VNTLPLNFDK	10.3%	19.6%	18.1%	25.1%	15.5%	11.4%	13%	10.8%	7.1%	17.3%	14.
IEPENNIK	10.6%	87.2%	88.5%	16.5%	19%	14.5%	17.1%	86.6%	90.7%	19.6%	91.

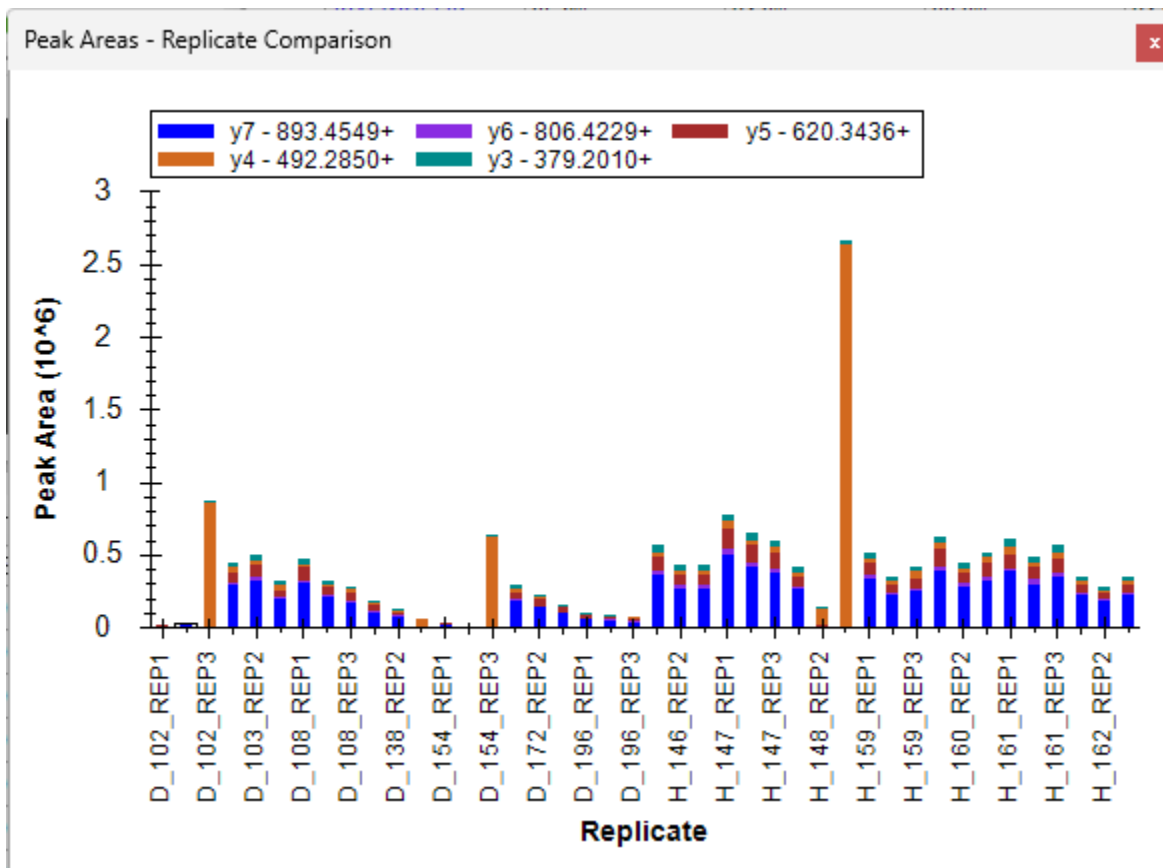
The currently selected peptide now has the lowest CV for the rat named Drizzle of all of the peptides in the document.

Look at the Peak Areas Replicate Comparison window



The very large bar at the left edge shows the relative intensities from the spectral library. The three bars immediately to the right of that belong to the rat named “Drizzle”. Their heights are very similar to each other.

- In the Document Grid, click on the **Drizzle CV Normalized Area Raw** column and choose **Sort Descending**
- The peptide AGSWQITMK now sorts to the top. Click on that peptide to select it in the Targets tree and look at the Peak Area Replicate Comparison graph



The currently selected peptide has the most variation in peak areas across the replicates associated with the rat named Drizzle. There is a great deal of variation in the heights of the three bars at the left end of the graph.

## Changing the Normalization Method in the document

The “Normalized Area” column shows areas normalized according to the normalization method specified on the Quantification tab at “Settings > Peptide Settings”

- On the **Settings** menu choose **Peptide Settings**



- On the **Peptide Settings** dialog, choose **Ratio to Global Standards** in the **Normalization method** dropdown

The image shows a screenshot of the 'Peptide Settings' dialog box, specifically the 'Quantification' tab. The dialog has a title bar with a close button (X) and a tabbed interface with the following tabs: Digestion, Prediction, Filter, Library, Modifications, and Quantification. The 'Quantification' tab is active. The settings are as follows:

- Regression fit:** None (dropdown)
- Normalization method:** Ratio to Global Standards (dropdown)
- Simple precursor ratios**
- Regression weighting:** None (dropdown)
- MS level:** All (dropdown)
- Units:** (empty text box)
- Figures of merit:**
  - Calculate LOD by:** None (dropdown)
  - Max LOQ CV:** (empty text box) %
  - Max LOQ bias:** (empty text box) %
- Qualitative ion ratio threshold:** (empty text box) %

At the bottom right, there are two buttons: 'OK' and 'Cancel'.

- Press **OK** on the Peptide Settings dialog

The Normalized Area values are now the Total Area values divided by the area of the Global Standard peptide.

- Click on the **Drizzle CV Normalized Area Raw** column header and choose **Sort Ascending**

Document Grid: Peptide Areas

Reports | 1 of 125 | Export... | Actions | Find:

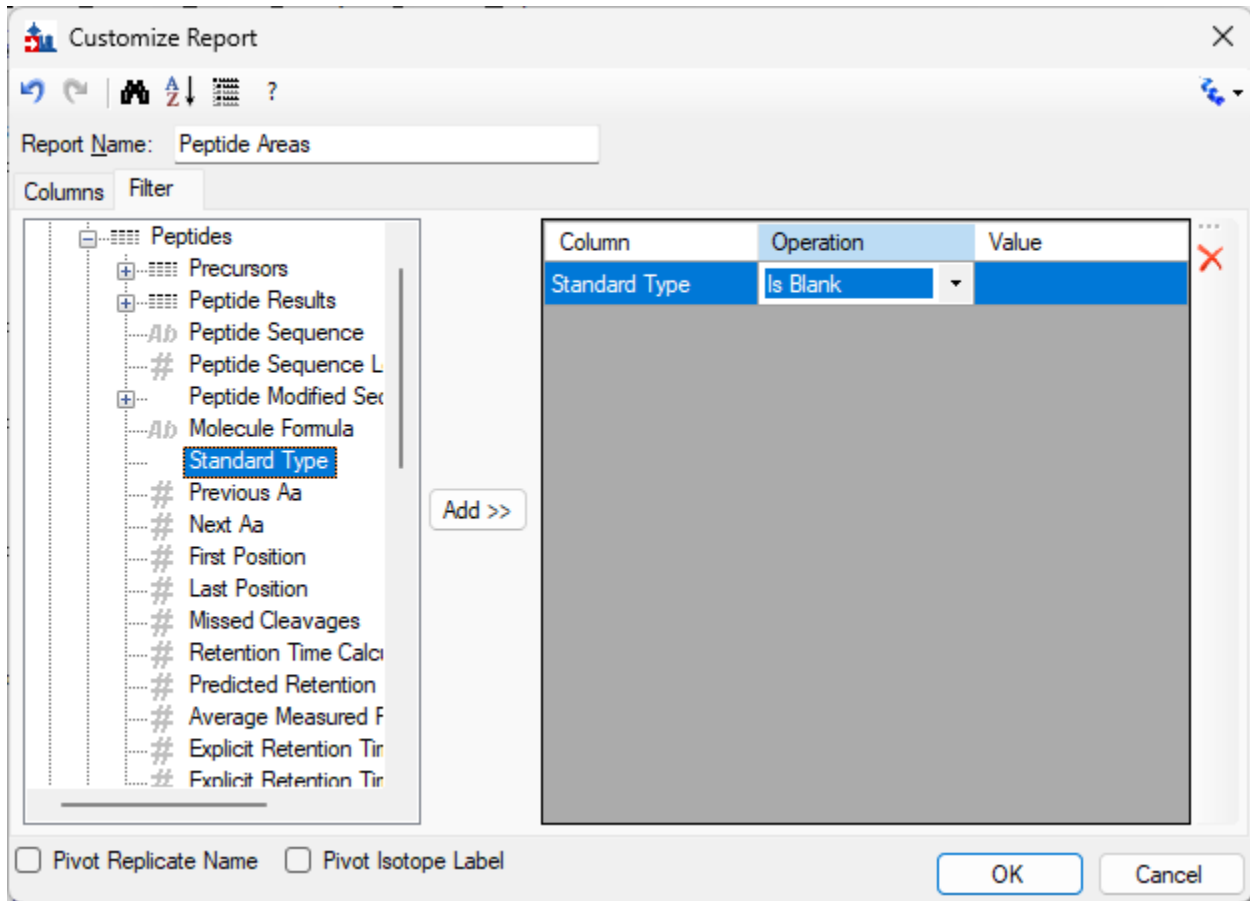
Peptide	Dizzle CV Normalized Area Raw	Sniffles CV Normalized Area Raw	Mumbles CV Normalized Area Raw	Rattle CV Normalized Area Raw	Funble CV Normalized Area Raw	Wheezy CV Normalized Area Raw	Wobbles CV Normalized Area Raw	Squeaks CV Normalized Area Raw	Nibbles CV Normalized Area Raw	Zippy CV Normalized Area Raw	Wh No Are
WVLSGSDATLS	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
IAWESPOGQVSR	2.6%	9.5%	13%	22.1%	12%	2.6%	2.4%	9.3%	14.8%	9%	13%
FAEDHFAHEATK	3.3%	2.6%	7.9%	11%	18.1%	16.7%	5.8%	9.4%	3.9%	3.9%	19%
ALYQAEAFVAD	4.1%	10.8%	5.8%	12.1%	14.7%	12.7%	7.6%	8.8%	4.2%	1.8%	11%
SLVQKPSEEN	4.2%	23.3%	1.6%	10.5%	9%	59.8%	13.8%	4.9%	17.8%	12.2%	24%
LNHQMEGLAF	4.5%	10.9%	0.3%	7.5%	21.7%	22.5%	16%	15%	1.7%	15.1%	11%
IARLFSDLER	4.8%	4.9%	3.7%	13%	15.8%	8%	11%	8%	2.8%	4.3%	9.8%
IARLFSLEDER	4.9%	5.1%	3.6%	12.7%	15.1%	7.7%	10.9%	7.9%	2.5%	4.3%	9.7%
FSISTDYSLK	5.2%	11.4%	11.2%	11.5%	5.8%	1.3%	6.1%	1.5%	2.4%	10.6%	7.6%
AAVFNHFISDGVK	5.4%	6.4%	11.5%	8%	17.8%	16.9%	14%	8.2%	19.6%	14.3%	15%
ATIDQNLEDLR	6%	7.8%	6.4%	5.2%	11.3%	12%	1.8%	8.9%	11.1%	8.9%	10%
HEEEVERPAVEK	6%	4.8%	7.3%	1.4%	8.2%	8.6%	17.7%	4.4%	11.9%	13.8%	15%
ALYSEYTDGTE	6.1%	6.9%	5.5%	5.3%	9.3%	6.8%	7.7%	8.8%	10.9%	6.5%	8.2%
IGVHTGPPVAG	6.2%	21.2%	9.7%	6.8%	11.2%	9.8%	13.1%	3.7%	6.4%	14.3%	21%
CWAQDPTERP	6.4%	4.1%	5.3%	1.6%	13%	11.3%	9.8%	6.6%	4.6%	5.2%	9.4%
WYLVVHCFEGR	6.5%	6.7%	10.8%	15.8%	18.8%	18.8%	5.3%	10.3%	10.7%	17.1%	9%

The very first row shows “0%” as the CV for each of the samples. This is to be expected because this document has only one Global Standard normalization peptide. The area of that peptide normalized to itself will always be exactly 1, and therefore the CV of that value will always be zero.

## Adding a filter to a report definition

- In the **Reports** dropdown choose **Edit Report**
- Double click on **Standard Type** in the column list on the right so that it becomes selected in the tree
- Click on the **Filter** tab
- Click the **Add** button
- In the dropdown in the **Operation** column choose “Is Blank”

The Customize Report dialog should now look like this:



- Press the OK button

The first row with the global standard peptide VVLSGSDATLAYSFAFK has been removed

## Adding a Group Comparison

- On the **View** menu choose **Live Reports** then **Group Comparisons** then **Add**
- In the Edit Group Comparison dialog, set the following values:
  - Name: Peptide Group Comparison
  - Control group annotation: Cohort
  - Control group value: Healthy
  - Value to compare against: Diseased
  - Identity annotation: Sample ID
  - Normalization method: Default
- Click OK on the Edit Group Comparison dialog
- On the **View** menu choose **Live Reports** then **Group Comparisons** then **Peptide Group Comparison**

This shows the Peptide Group Comparison grid

Peptide Group Comparison:Grid

Volcano Plot Bar Graph Settings

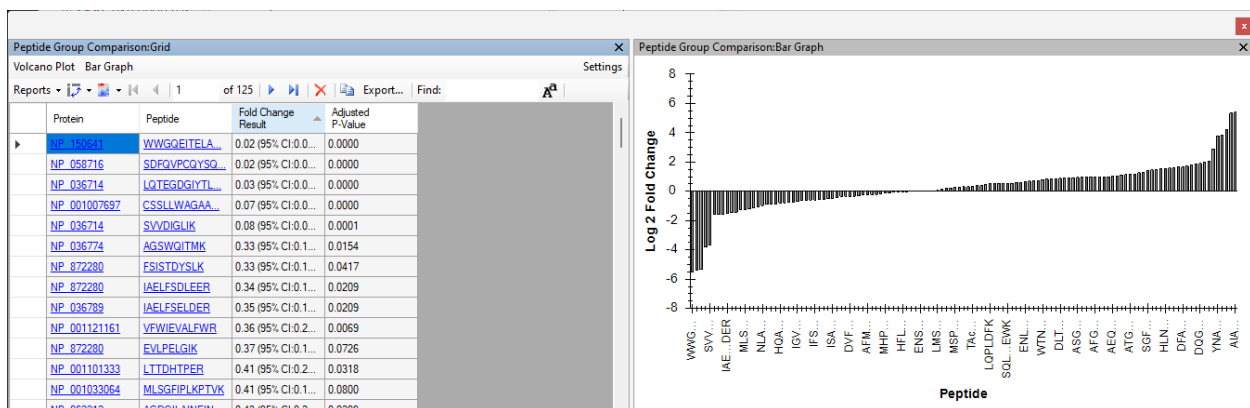
Reports 1 of 125 Export... Find:

Protein	Peptide	Fold Change Result	Adjusted P-Value
NP_036529	CSLPRPWALTF...	0.96 (95% CI:0.4...	0.9276
NP_037030	LGEEVSVACK	1.01 (95% CI:0.7...	0.9276
NP_037030	VGQPGDAGAA...	0.74 (95% CI:0.4...	0.1761
NP_602308	GSYNLQDLLAQ...	1.53 (95% CI:0.7...	0.2725
NP_001012027	TSQIHFFFAK	1.3 (95% CI:0.84 ...	0.2725
NP_001012027	LQPLDFK	1.42 (95% CI:0.9 ...	0.1751
NP_001012027	SQLPGIIAEGR	1.8 (95% CI:1.12 ...	0.0417
NP_036870	DFATVYVDAVK	3.19 (95% CI:0.8...	0.1279
NP_036870	DYVSQFESSTL...	2.2 (95% CI:0.84 ...	0.1445
NP_037244	TGTLNMDFLSR	40.93 (95% CI:10...	0.0006
NP_037244	LMSPEEKAPA...	1.04 (95% CI:0.8 ...	0.8028
NP_001010968	GTITSIAALDDPK	1.97 (95% CI:0.6...	0.2940
NP_001010968	TQTPVQGCHLE...	0.79 (95% CI:0.5...	0.2948
NP_001010968	IFPENNIK	18.37 (95% CI:2....	0.0269

- Click the **Bar Graph** button at the top of the **Group Comparison: Grid**

The bar graph plot shows the fold change values for each of the peptides that is displayed in the grid.

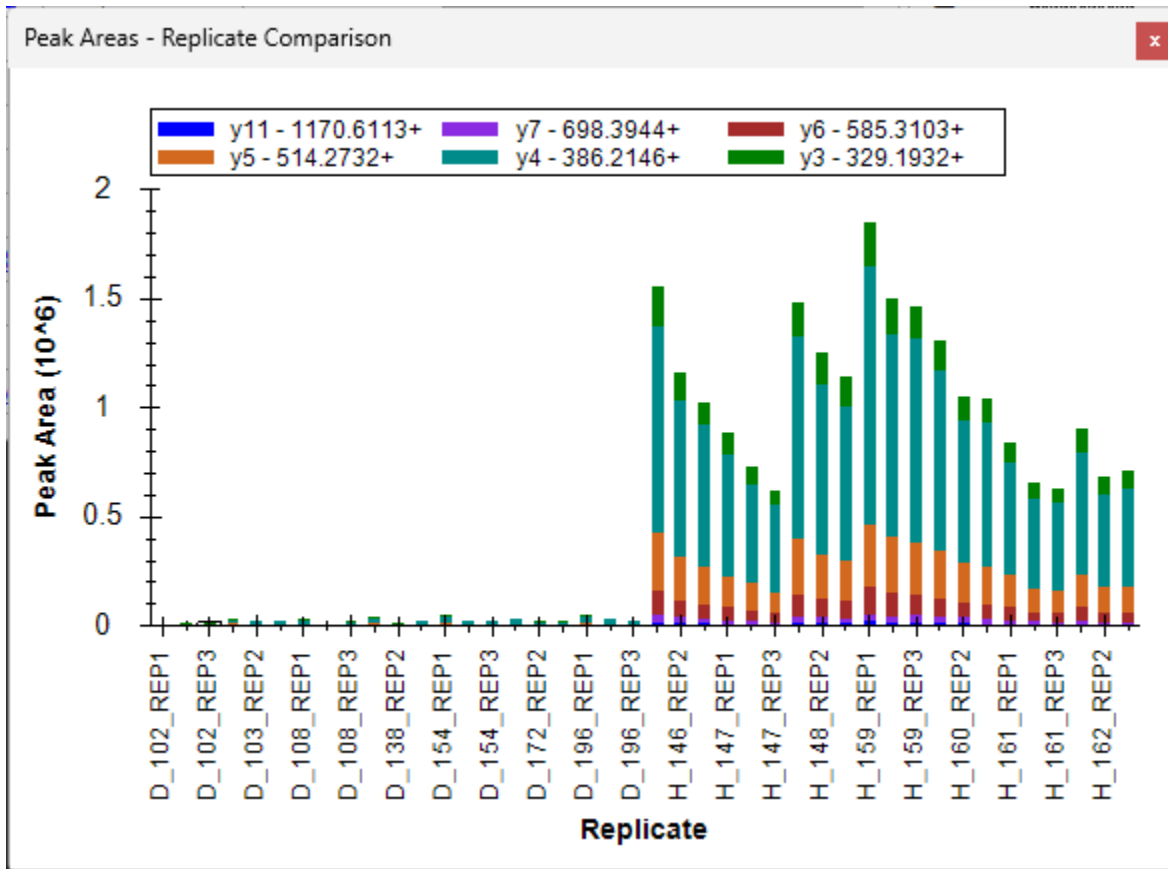
- Click on the **Fold Change Result** column header and choose “Sort Ascending”



The data is now sorted so that the peptides with the smallest fold change are displayed first and the peptides with the largest fold changes are displayed last.

- Click on the first peptide in the grid (WWGQEITELAQGPR)

- Look at the Peak Area Replicate Comparison graph



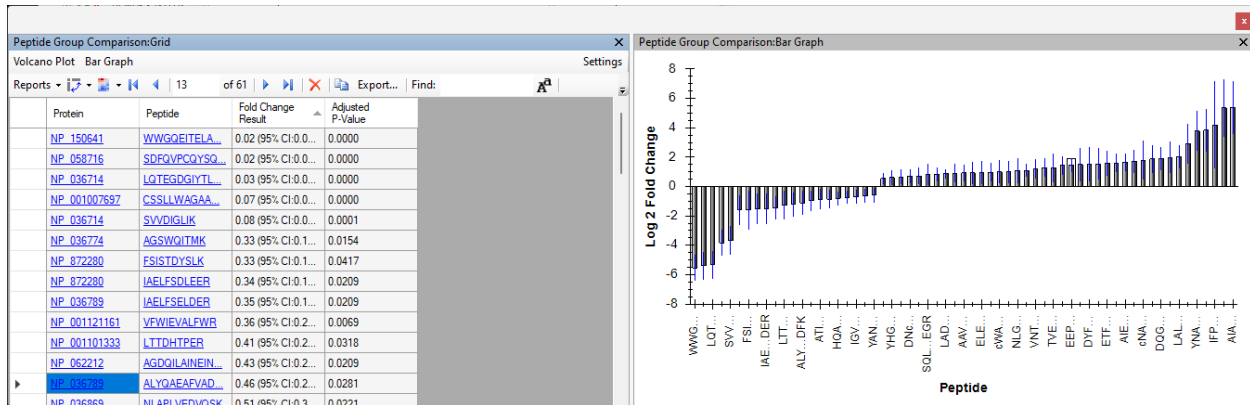
This peptide clearly has lower abundance in the diseased replicates compared to the healthy replicates.

- Right-click on the Adjusted P-Value column and choose "Filter"
- Choose "Is Less Than" for the Filter Type and type "0.05" into the text box below that

The image shows a dialog box titled "Show rows where Adjusted P-Value...". The "Filter type:" dropdown is set to "Is Less Than". The text input field contains "0.05". Below this, there is an "and:" section with an empty dropdown and text input field. At the bottom, there are four buttons: "OK", "Cancel", "Clear Filter", and "Clear All Filters".

- Click **OK** on the dialog box

The grid and the graph are now showing only the rows where the adjusted P-value is less than 0.05.



- Close the Peptide Group Comparison Bar Graph by clicking the gray X at the right edge of that window's title bar. Do not click the red X above it because that will close both the grid and the bar graph

## Showing abundances with the group comparison results

The group comparison grid can also show the per-replicate abundances which contributed to the fold change calculation

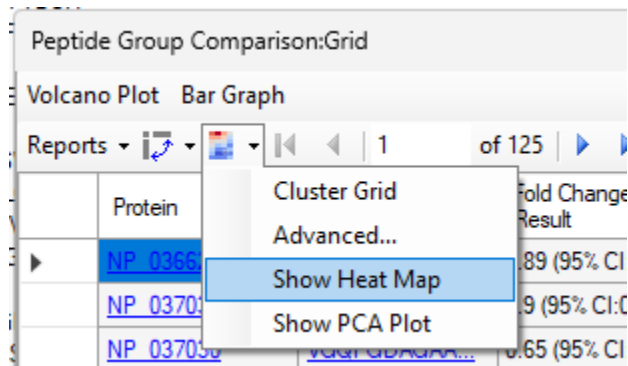
- On the Reports dropdown at the top of the **Peptide Group Comparison Grid** choose **Customize Report**
- Click the + button next to Replicate Abundances at the bottom of the tree in the Customize Report dialog to expand it
- Check the checkbox next to Abundance
- Type **Replicate Abundances** in the **Report Name** field
- Click OK in the Customize Report dialog

Protein	Peptide	Fold Change Result	Adjusted P-Value	D_102_REP1 Abundance	D_102_REP2 Abundance	D_102_REP3 Abundance	D_103_REP1 Abundance	D_103_REP2 Abundance	D_103_REP3 Abundance	D_108_REP1 Abundance	D_108_REP2 Abundance	D_108_REP3 Abundance
NP_036839	CSLFRPWALTE...	0.89 (95% CI:0.5...	0.6973	1.5559E+4	1.3173E+4	1.3768E+4	9.8768E+3	1.0781E+4	6.2223E+2	1.3163E+4	2.0569E+2	1.1512E+4
NP_037030	LGGEVSVACK	0.9 (95% CI:0.73...	0.3225	6.0233E+3	4.9978E+3	4.0577E+3	7.5946E+3	6.5206E+3	7.1586E+3	1.0843E+4	7.1815E+3	6.5677E+3
NP_037030	VSGPQDAGAA...	0.65 (95% CI:0.5...	0.0024	9.3286E+5	7.7796E+5	7.8636E+5	8.8916E+5	8.2535E+5	9.3533E+5	1.1761E+6	1.2882E+6	1.0503E+6
NP_602308	GSYNLQDLAQA...	1.51 (95% CI:0.9...	0.1341	1.3901E+4	1.4457E+4	1.5158E+4	7.2742E+3	8.5069E+3	1.2927E+3	8.8783E+3	4.1930E+3	1.0057E+4
NP_001012027	TSQIHFFFAK	1.15 (95% CI:0.9...	0.2072	1.6379E+4	1.9294E+4	2.2543E+4	7.9979E+3	1.0683E+4	1.5228E+4	9.4003E+3	1.4946E+4	1.0070E+4
NP_001012027	LQPLDFK	1.25 (95% CI:0.9...	0.0808	8.8064E+5	7.3760E+5	8.7048E+5	4.7419E+5	4.3949E+5	6.0240E+5	4.5016E+5	4.6957E+5	4.8684E+5
NP_001012027	SQLPGIIAAGR	1.59 (95% CI:1.2...	0.0081	3.7318E+4	4.2015E+4	3.6817E+4	1.7173E+4	1.6645E+4	2.2451E+4	1.4580E+4	1.5332E+4	1.5885E+4
NP_036870	DFATVYVDVAVK	2.79 (95% CI:0.7...	0.1392	1.8263E+5	1.7808E+5	1.7779E+5	6.2904E+5	2.0345E+4	6.3604E+5	1.2631E+4	6.2554E+5	
NP_036870	DYVSGFESSTL...	1.95 (95% CI:0.8...	0.1660	7.6518E+4	7.6739E+4	7.5958E+4	2.1119E+5	2.4075E+5	2.5803E+4	2.3649E+5	7.4485E+6	2.5298E+5
NP_037244	TGTNLMDFLSR	36.33 (95% CI:9...	0.0004	3.2019E+4			3.1539E+4			2.6645E+4	1.2482E+2	
NP_037244	LMSPEEKAPA...	0.92 (95% CI:0.8...	0.3028	4.1232E+4	3.5027E+4	2.2079E+4	4.3841E+4	3.9278E+4	4.1895E+4	3.9565E+4	3.7988E+4	2.9853E+4
NP_001010968	GTTISIAALDDPK	1.69 (95% CI:0.5...	0.3614	2.5540E+4	2.5761E+4	2.6006E+4	2.5231E+4		9.6028E+2	2.7979E+4	1.7231E+4	
NP_001010968	LQTPVGGCHLE...	0.7 (95% CI:0.56...	0.0084	3.3720E+4	3.2232E+4	3.1348E+4	2.9758E+4	2.9328E+4	3.9977E+4	4.0933E+4	4.6165E+4	4.6622E+4
NP_001010968	IFPFINNIK	16.12 (95% CI:2...	0.0126	7.2716E+5	7.2877E+5	7.3627E+5	1.0588E+6	1.1074E+6	7.9529E+2	8.2514E+4	1.7007E+2	9.4870E+4

The grid now shows abundances for each peptide and replicate. The values shown in the cells are equal to the **Normalized Area Strict** values that were seen in the Document Grid, except that the values in the group comparison grid have been divided by the number of transitions in the peptide.

## Showing a heat map with dendrograms

- Click the inverted triangle to the right of the third button from the left on the tool strip at the top of the Peptide Group Comparison Grid window
- Choose **Show Heat Map** from the drop down menu



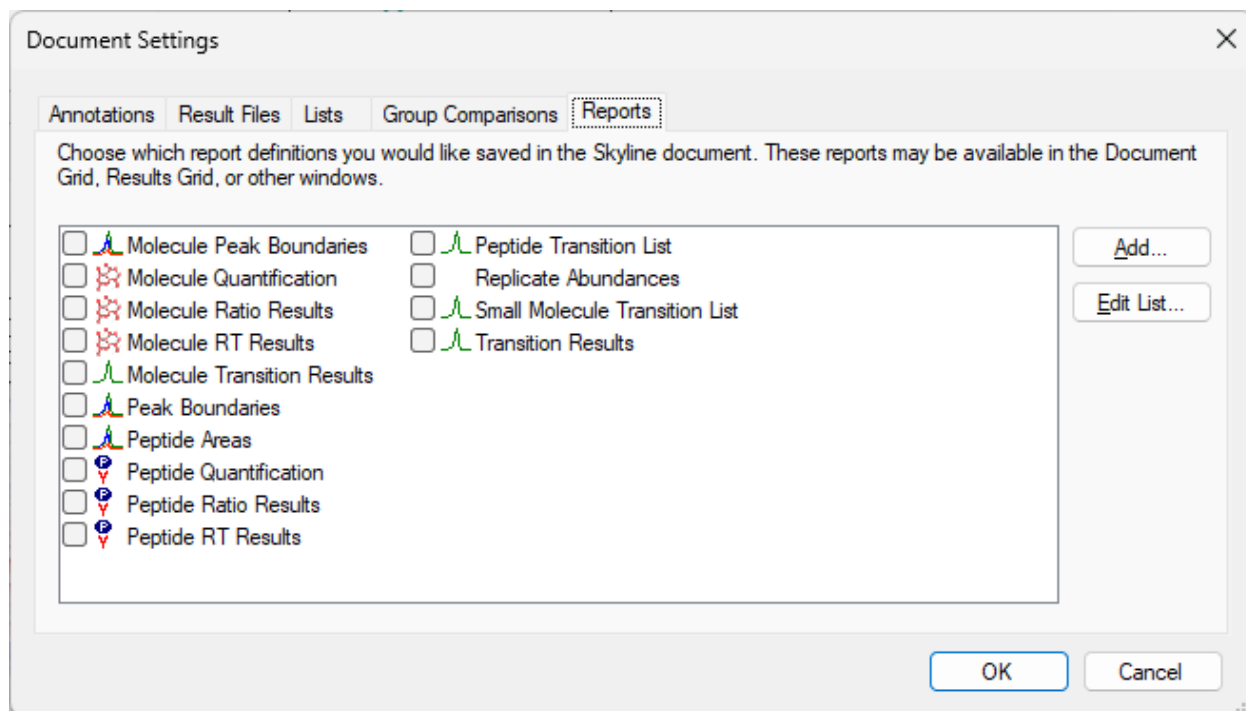
Skyline displays a heat map where the peptides and replicates have been reordered so that the dendrograms above and to the right of the graph can be drawn to indicate which rows and columns are most similar to each other

## Adding report definitions to the document

- On the View menu choose Document Settings
- Click the Reports tab in the Document Settings dialog

This shows all the custom reports that Skyline knows about. These reports include the “Peptide Areas” which we created in the Document Grid and the “Replicate Abundances” report from the Group Comparison grid. There are also some small molecule reports which would only show up in the Document Grid if this were a small molecule or mixed mode document instead of a proteomics

document.



- Check the checkbox next to Peptide Areas and Replicate Abundances
- Click OK in the Document Settings dialog

The Replicate Abundances and Peptide Areas report definitions are now part of this document.

If you were to use the “File > Share” menu item to create a .sky.zip file containing this document and send that document to someone else, when they opened that document the “Peptide Areas” and “Replicate Abundances” report definitions from this document would be added to the list of reports in their Skyline instance.

## Inspecting the audit log

- On the View menu choose Live Reports and then Audit Log



Audit Log: All Info			
Time	All Info Message	Reason	
2024-09-14 18:3...	Reports changed		
2024-09-14 18:3...	Settings > Document Settings > Reports : "Peptide Areas" was added		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Peptide"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Protein"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Modified Sequence"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Standard Type"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Replicate"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Total Area"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Raw"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Strict"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Message"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "Normalized Area"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Columns : contains "annotation_SampleID"		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Filters : contains { Column = "Standard Typ...		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Peptide Areas" > Default Layout Name is Missing		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Replicate Abundances" was added		
2024-09-14 18:3...	Settings > Document Settings > Reports > "Replicate Abundances" > Columns : contains "Protein"		

This shows in detail the operations that have modified this document.

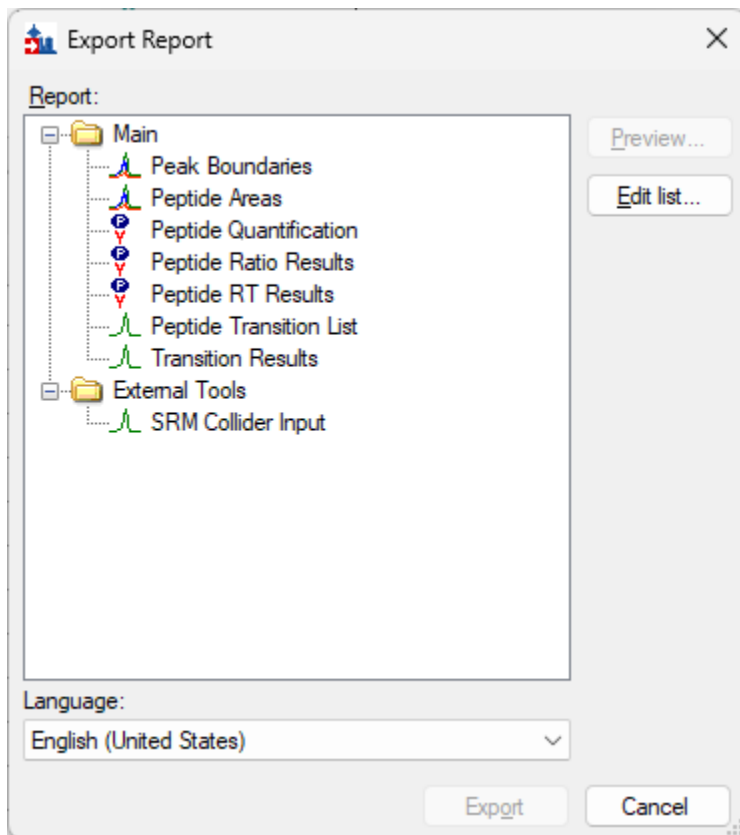
- In the Reports dropdown at the top of the Audit Log window choose Summary

This shows a more concise list of things that have happened to the document with one row per operation.

Audit Log: Summary			
Time	Summary Message	Reason	
2024-09-14 18:3...	Settings > Document Settings > Reports changed		
2024-09-14 18:1...	Settings > Peptide Settings – Quantification > Normalization method changed from "Ratio to Global Stand...		
2024-09-14 17:3...	Settings > Peptide Settings – Quantification > Normalization method changed from "Equalize Medians" ...		
2024-09-14 17:3...	Settings > Peptide Settings – Quantification > Normalization method changed from "None" to "Equalize ...		
2024-09-14 17:3...	Settings > Peptide Settings – Quantification > Normalization method changed from "Ratio to Global Stand...		
2024-09-14 17:2...	Settings > Document Settings – Group Comparisons : "Peptide Group Comparison" was added		
2024-09-14 17:1...	Settings > Peptide Settings – Quantification > Normalization method changed from "None" to "Ratio to G...		
2024-09-14 17:0...	Settings > Document Settings > Rule sets: removed all		
2024-09-14 17:0...	Settings > Document Settings – Lists > "Samples" > Rows changed		
2024-09-14 17:0...	Pasted 13 values into the document grid		
2024-09-14 17:0...	Settings > Document Settings – Lists : "Samples" was added		
2024-09-14 16:5...	Settings > Document Settings > Rule sets changed		
2024-09-14 16:5...	Settings > Document Settings > Rule sets changed		
2024-09-14 16:5...	Settings > Document Settings – Annotations changed		
2024-09-14 16:5...	Start of audit log for already existing document		

## Exporting a report

- On the **File** menu choose **Export** and then **Report**



This dialog allows exporting of reports from the Document Grid. The **Peptide Areas** that we designed in this tutorial is one of the choices. The “Replicate Abundances” report from the Group Comparison grid is not one of the choices because this dialog only allows exporting of Document Grid reports.