

Panorama Chromatogram Libraries

Targeted assay development for proteins or peptides is typically a time-consuming and lengthy process. Deciding the ideal set of peptides and transitions to measure for your proteins of interest requires careful experimental design and iterative optimization. Once an assay has been established, the assay parameters can be reused for designing future experiments. Chromatogram libraries in Panorama provide a way you can store targeted assays that have been curated in Skyline and reuse them in the future for measuring proteins and peptides in other samples, as well as share them with other researchers. Chromatogram libraries can also capture physiochemical and experimental properties such as the relative ion abundance, measured retention time, measured chromatograms, and in the future we expect to store iRT, ion mobility, and optimal instrument parameters such as collision energy. Empirical measurements for these parameters can be particularly relevant in guiding targeted experiment design.

In this tutorial we will

- Create a folder in Panorama for chromatogram library data.
- Add Skyline documents, with curated targeted results, to the chromatogram library folder.
- Download the chromatogram library from Panorama and use it in Skyline.

Getting started

To start this tutorial, download the following ZIP file:

<https://panoramaweb.org/tutorials/PanoramaChromatogramLibraries.zip>

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

```
C:\Users\vsharma\Documents
```

This will create a new folder:

```
C:\Users\vsharma\Documents\PanoramaChromatogramLibraries
```

It will contain all the files necessary for this tutorial.

The data used in this tutorial was published in Stergachis *et al.* 2011¹, where the authors described a high-throughput method for selecting optimal peptides and fragment ions for targeted proteomics applications. The Skyline files used in this tutorial contain assays for a subset of the 96 human transcription factors on which they demonstrated their approach.

Signing up for an account on panoramaweb.org

This tutorial assumes that you will be working in a sub-folder of the “Tutorials” project on panoramaweb.org, the Panorama server hosted by the MacCoss lab at the University of Washington. However, if you already have an existing account and project on panoramaweb.org, or have an account on another Panorama server you are welcome to use that, and skip to the next step.

To request a folder on panoramaweb.org for working through this tutorial, fill out the sign-up form on this page:

<https://panoramaweb.org/labkey/wiki/home/support/page.view?name=hosted-project-signup-form>

- Fill in the required fields, marked with a red asterisk, in the form shown below.
- Enter ‘Tutorials’ in the **Group name**.
- Click on the **Submit** button.

Sign Up for Hosted Project

Sign up here to request a project on PanoramaWeb.

You can own a free project for your lab on PanoramaWeb without needing to setup and maintain your own instance of LabKey Server. The LabKey Server security infrastructure will allow you to keep your data as private as you want or release it publicly when you are ready.

The information you provide will not be shared with any outside organizations.

First Name* Vagisha

Last Name* Sharma

Title

Organization* University of Washington

Email* vsharma@uw.edu

Group name Tutorials

We will try to create a project with the requested group name if it is available, or contact you, if it looks like someone else in your group has already started a project.

Submit

NOTE: You will receive email after your request has been reviewed and accepted.

DISCUSSION

Search

Pages

Welcome to Panorama support

Sign Up for Support

Sign Up for Hosted Project

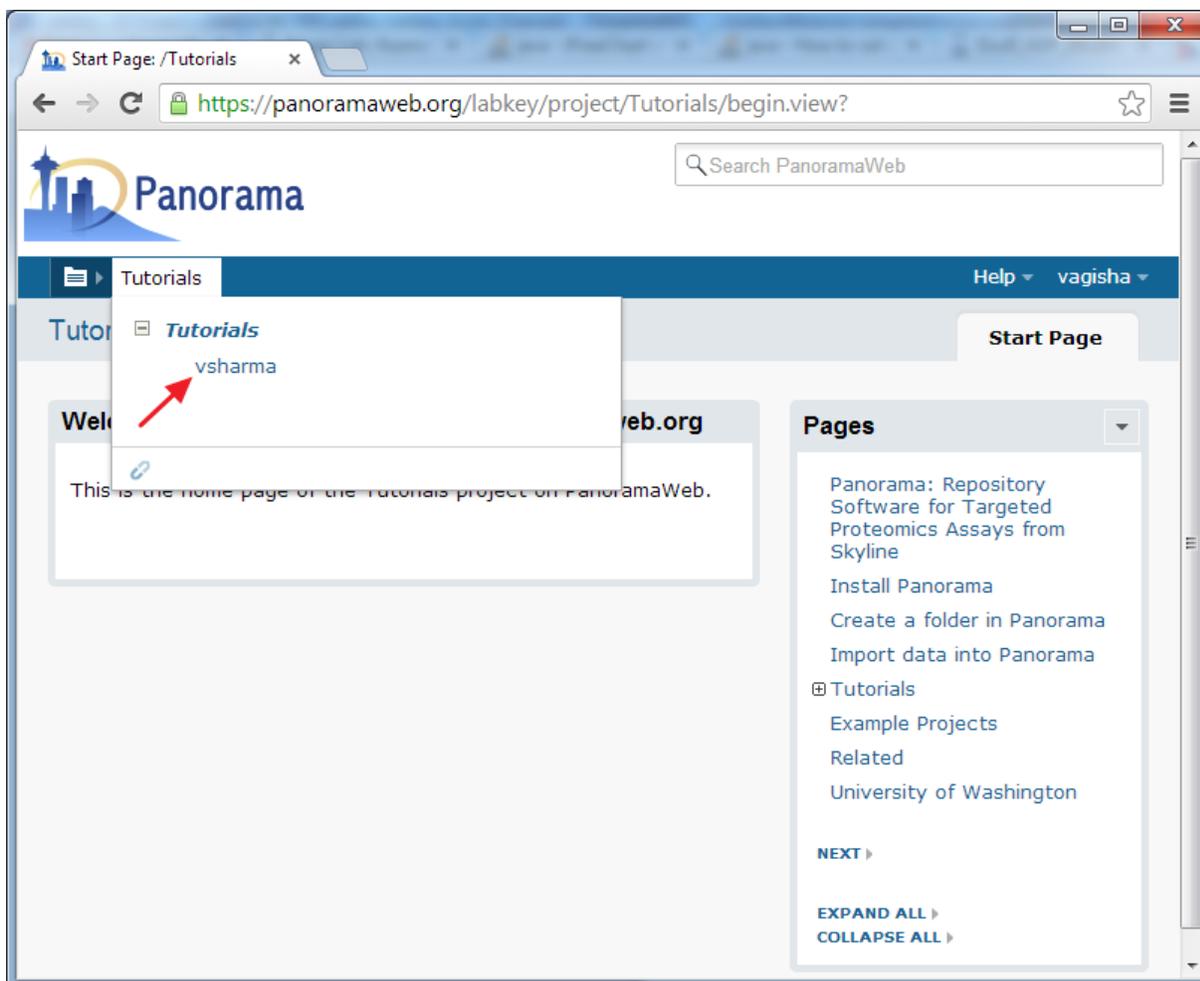
PREVIOUS

Once your request is approved, a folder with full administrative rights will be created for you in the “Tutorials” project on panoramaweb.org. You will also receive a welcoming email with a link to complete your account registration by choosing a password.

Creating a chromatogram library folder in Panorama

First sign in to the Panorama server and navigate to your folder. If you are working in the “Tutorials” project on panoramaweb.org follow these steps:

- Click the **Projects** tab to see a list of all the projects to which you have access on the server.
- Click the “Tutorials” project.
- On the project homepage, hover over the project name in the menu bar below the Panorama icon and click on the folder that was created for you (e.g. ‘vsharma’).

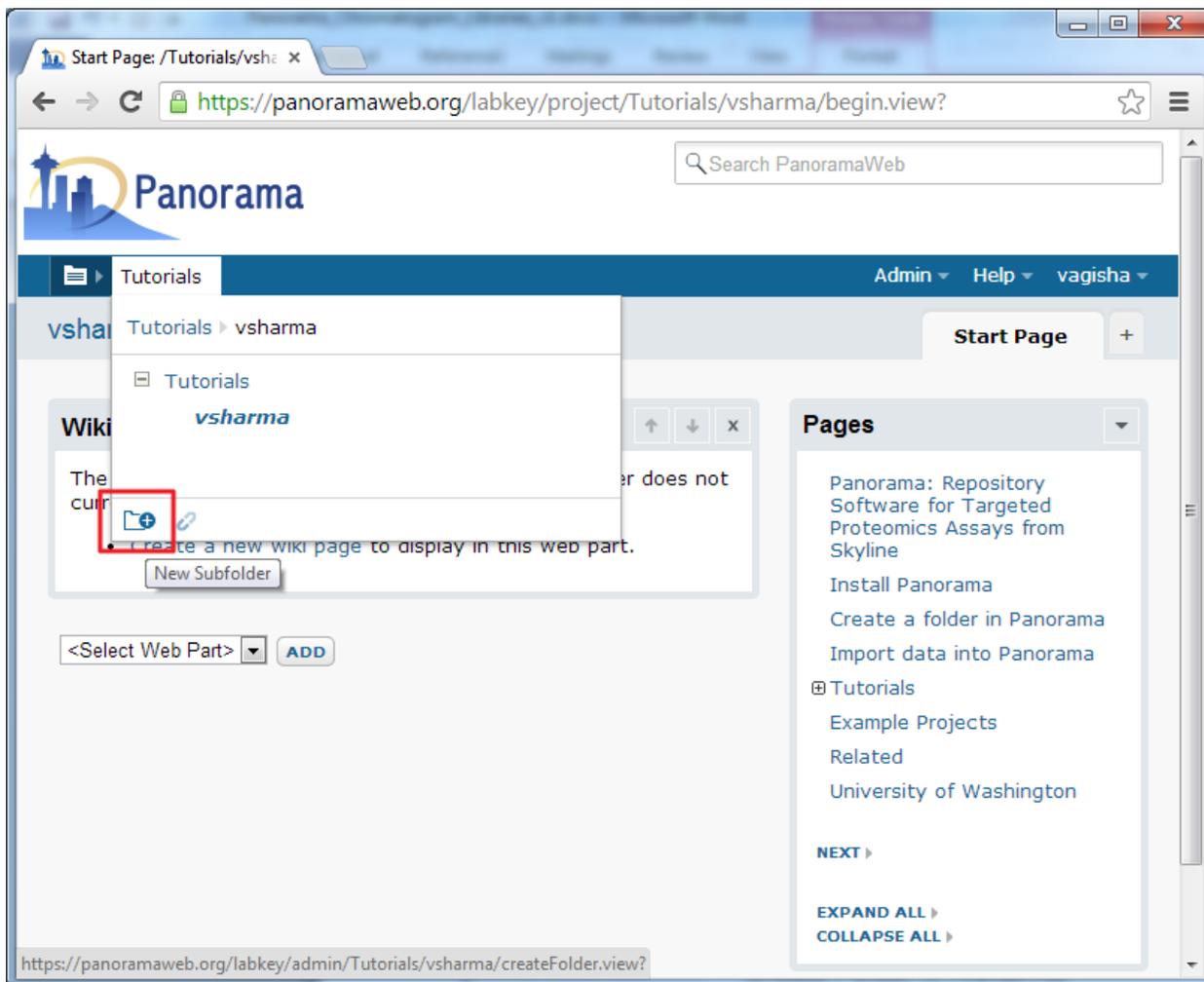


When organizing your data for chromatogram libraries, we recommend that you create a folder for each species that you study, and under each species folder you can create subfolders for the instrument type

where the data was collected, or any other experimental conditions for which it is appropriate to have separate chromatogram libraries. In this tutorial you will create a subfolder for building a chromatogram library for human transcription factors.

To create a sub-folder for this tutorial, do the following:

- Click the new folder icon shown in the image below.



- In the **Create Folder** form, enter 'Human' in the folder **Name** field.
- Select the **Panorama** option under **Folder Type**, as shown below.

1. Create Folder

2. Users / Permissions

Create Folder in /vsharma

Name:

Human

Folder Type:

Collaboration

Panorama

Create From Template Folder

NEXT CANCEL

- Click the **Next** button.
- On the **Users / Permissions** page accept the default selection.

1. Create Folder

2. Users / Permissions

3. Configure Panorama Folder

Users / Permissions

Inherit From Parent Folder

My User Only

Configure Later

NEXT

- Click the **Next** button.
- On the **Configure Panorama Folder** page choose the **Chromatogram library** option.
- Check **Rank peptides within proteins by peak area**.

1. Create Folder

2. Users / Permissions

3. Configure Panorama Folder

Configure Panorama Folder

Experimental data - a collection of published Skyline documents for various experimental designs

Chromatogram library - curated precursor and product ion expression data for use in designing and validating future experiments

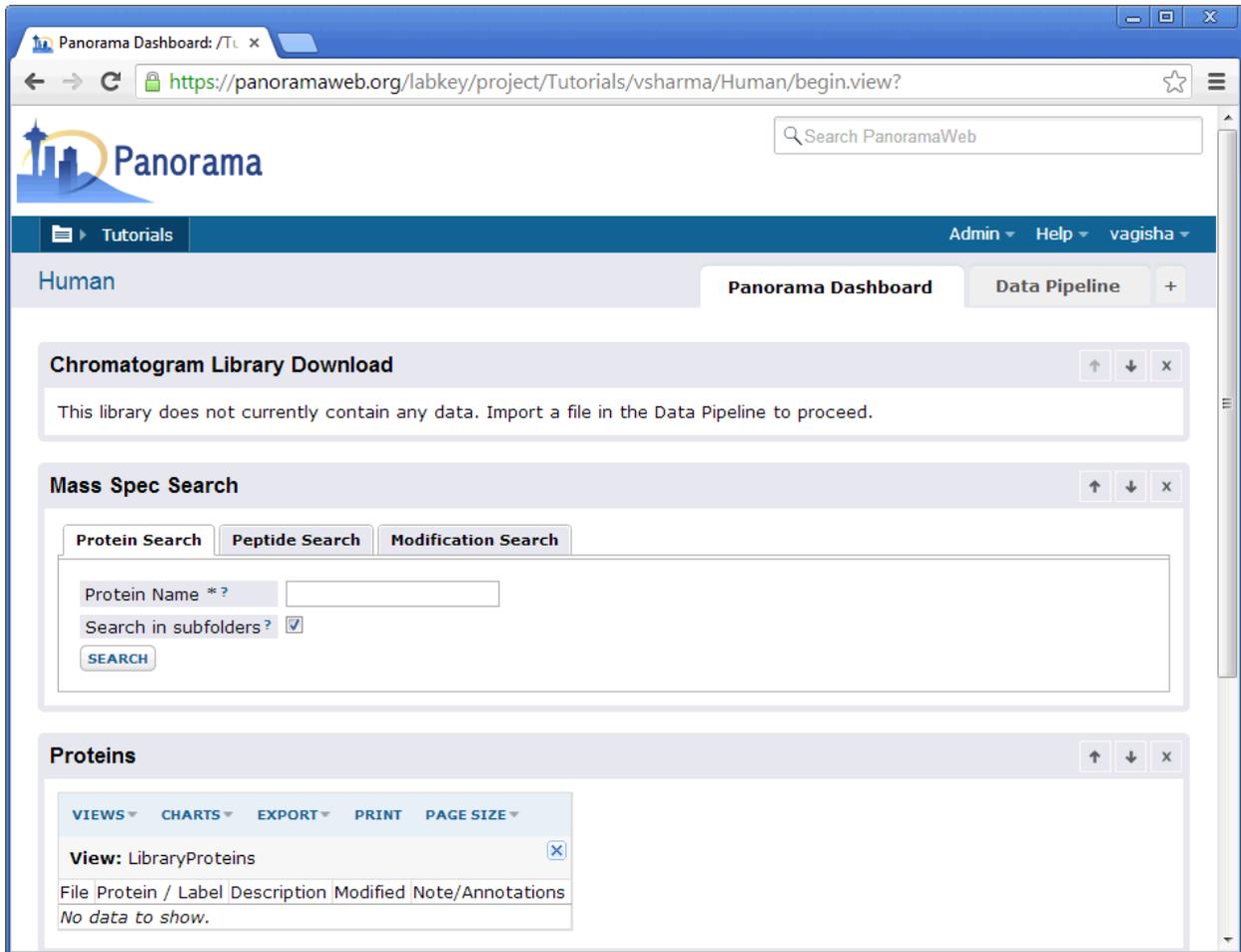
Rank peptides within proteins by peak area

FINISH

- Click the **Finish** button.

The **Rank peptides within proteins by peak area** box should be checked when you have targeted results containing relative peptide peak areas for a given protein. Leave this box unchecked if you do not yet have quantitative data from proteolytically digested proteins, such as when you are working with synthetic peptides.

After successful folder creation, you will be taken to the home page of the 'Human' chromatogram library folder that should look as follows:



You are now ready to upload Skyline documents to this folder.

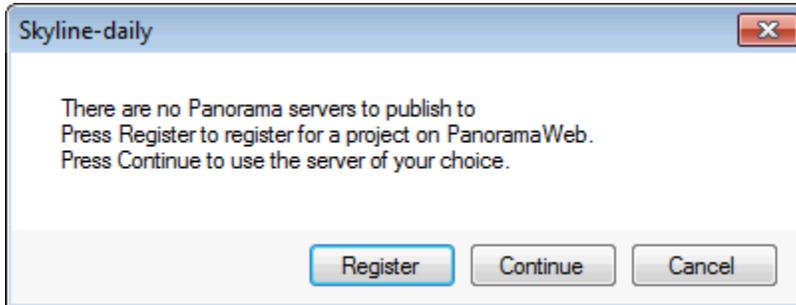
Publishing Skyline documents to a library folder in Panorama

Open the "Stergachis-SupplementaryData_2_a.sky" file in Skyline either by double-clicking on the file in Windows Explorer or in Skyline, on the **File** menu, click **Open** (Ctrl-O). This file contains peptides and fragment ions for 10 human transcription factors published in Stergachis *et al.* 2011¹. To publish this document to your library folder on Panorama, follow these steps:

- Click on the Publish to Panorama button in the Skyline toolbar, shown in the image below. Alternatively, on the **File** menu, click on **Publish to Panorama**.



If you have not yet set up a Panorama server in Skyline, you will see the following message.



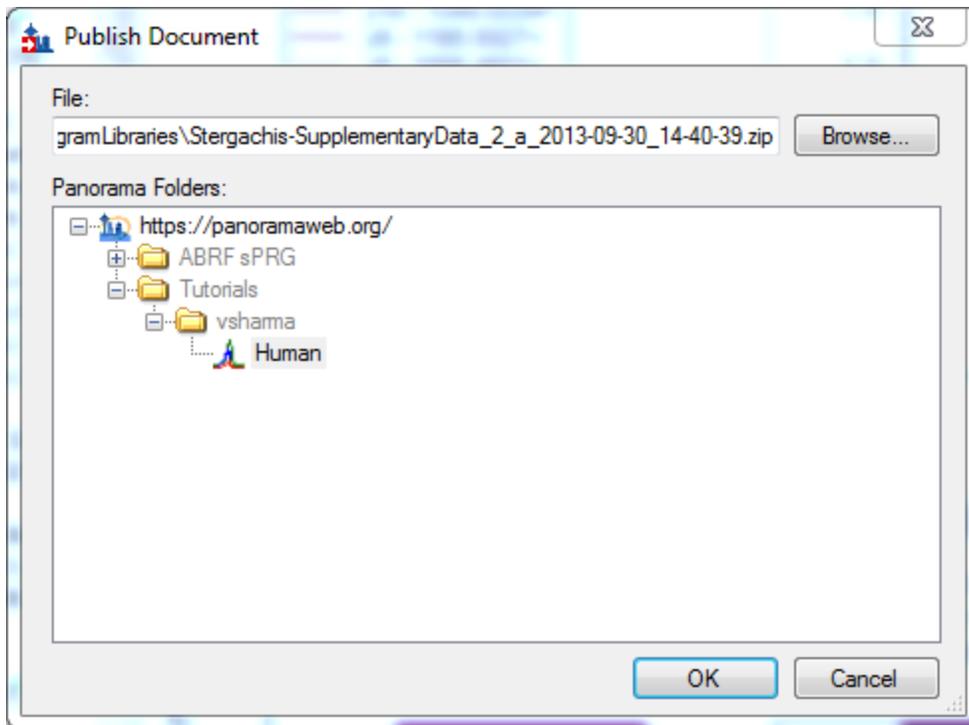
- Click the **Continue** button.

In the **Edit Server** form that appears, the default value in the **URL** field is <https://panoramaweb.org>. This is the URL of the server hosted at the University of Washington, and the server where you have an account if you followed the registration steps at the beginning of this tutorial.

- If you are working on a server other than panoramaweb.org, enter the URL of your server.
- Enter your email address and password on the Panorama server.
- Click the **OK** button.

Skyline will now display a form with the folders available to you on your Panorama server.

Chromatogram library folders will have the chromatogram  icon next to them.

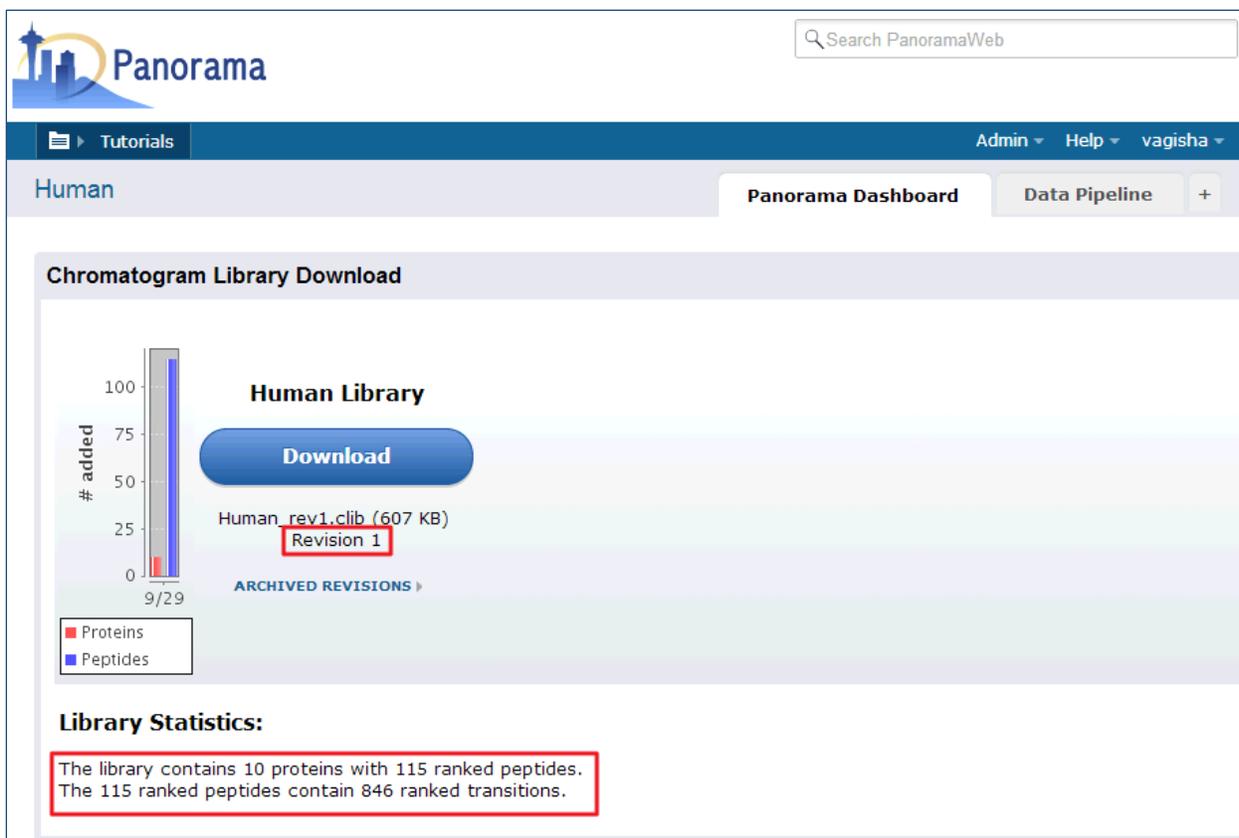


- Navigate to and select the 'Human' folder that you just created in Panorama.
- Click the **OK** button.

Skyline will upload a ZIP archive of your document to the selected library folder on the Panorama server.

Viewing chromatogram library data in Panorama

Once your document has been imported into Panorama, go back to the web-browser where the home page of the 'Human' library folder should still be open. Refresh the page (F5 in most browsers) and, in the **Chromatogram Library Download** box, you will see that the first version (Revision 1) of your 'Human Library' is now available for download. A brief summary of the contents of the library is also displayed under the **Library Statistics** heading.



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Human Panorama Dashboard Data Pipeline +

Chromatogram Library Download

Human Library

added

100
75
50
25
0

9/29

Proteins Peptides

Download

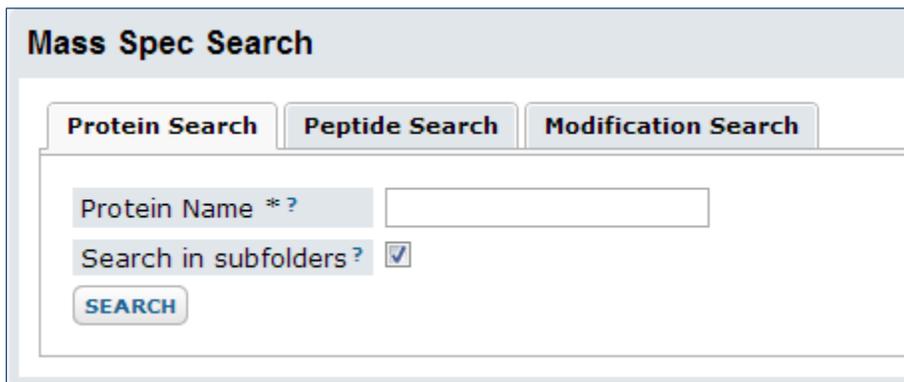
Human_rev1.clib (607 KB)
Revision 1

ARCHIVED REVISIONS >

Library Statistics:

The library contains 10 proteins with 115 ranked peptides.
The 115 ranked peptides contain 846 ranked transitions.

Further down on the page, in the **Mass Spec Search** box, you can search for proteins, peptide sequences or peptides with specific modifications in your library.



Mass Spec Search

Protein Search Peptide Search Modification Search

Protein Name *?

Search in subfolders?

SEARCH

The tables below the **Mass Spec Search** box display a list of the proteins and peptides in the library folder. Clicking on an entry in one of these tables will display more details for the selected protein or peptide. Click on 'CTCF' in the **Proteins** table to bring up a page with more details for this protein. The **Sequence Coverage** box displays the sequence, with colored rectangles highlighting the peptides for which there are measurements in the library.

Sequence Coverage

0	10	20	30	40	50	60	70	80	90
MEGDAVEAIVEESETFIKGRKTKYQRRREGQEEDACHLPQNQTGGGEVVDVNSSVQMVMEQLDPTLLQMKTEVMGTVAFEAEEAAVDDTQIITLQV									
1									
100	110	120	130	140	150	160	170	180	190
VNMEEQPINIGELQLVQVPVPTVTPVATTSVEELQGAYENEVSKEGLAESSEPMICHTLPLPEGFQVVKVGANGEVETLEQGELPPQEDPSWQKDFDYQPP									
1 >>									
200	210	220	230	240	250	260	270	280	290
AKTKTKTKSKLRYTEEGKDVDSVYDFEEQQEGLLSEVNAEKYVGNMKPPKPTKIKKKGVKKTFCQELCSYTCPRRSNLDHRHMKSHTERPHKCHLGG									
>									
300	310	320	330	340	350	360	370	380	390
RAFRTVILLRNHLNTHGTGRPHKCPDCDMAFVTSGELVRHRRYKHTHEKPFKCSMCDYASVEVSKLKRHRSHTGERPFQCSLCSYASRDYKLRHMRT									
1 1 1 1 1 1 1									
400	410	420	430	440	450	460	470	480	490
HSGEKPYECYICHARFTQSGTKMMHILQKHTENVAKFHCPHCDTVIARSDLVHLLRQKHSYIEQGKKCRYCDAVFHERYALIQHQKSHKNEKRFKCDQC									
<< 1 1 1 1 1 1 1 1 <									
500	510	520	530	540	550	560	570	580	590
DYACRQERHMIMHKRTHTGEKPYACSHCDKTFRQKQLDMHFHRYHDPNFVPAAFVCSKCGKTFTRRNTMARHADNCAGPDGVEGENGGETKKSRRGRKR									
<< 1 1 1 1 1									
600	610	620	630	640	650	660	670	680	690
KMRSKKEDSSDSENAEPDLDDNEDEEEPAVEIEPEPEPQPVTIPAPPAPAKKARGRPPGRINQPKQNQPTAIQVEDQNTGAIENIIVEVKKPEPDAEPAEGE									
700	710	720							
EEEAQPAATDAPNGDLTPEMILSMMDRL									

The **Peptides** table displays a list of these peptides along with some information that was contained in the uploaded Skyline document, such as the retention time.

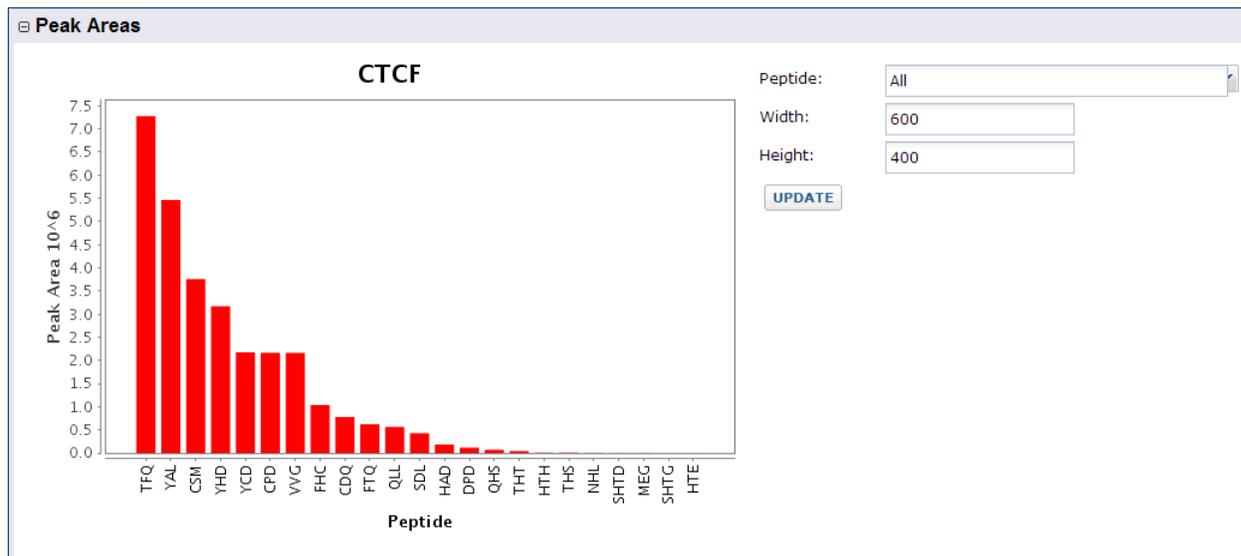
Peptides

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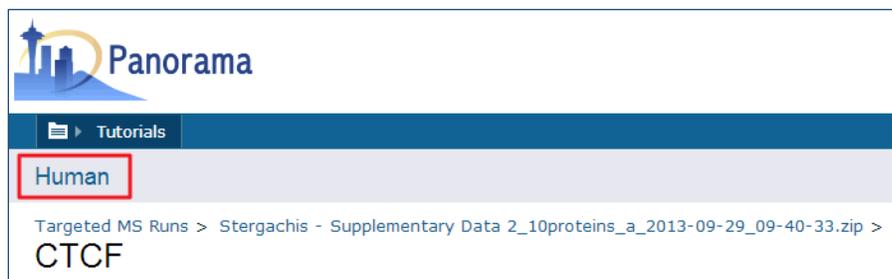
	Peptide	Peptide Neutral Mass	Missed Cleavages	Avg. Measured RT	Predicted RT	RT Score
DETAILS	MEGDAVEAIVEESETFIK	1995.9347	0	24.9907		
DETAILS	DPDYQPPAK	1029.4767	0	25.5342		
DETAILS	VVGNMKPPKPTK	1294.7432	0	24.8102		
DETAILS	TFQCELCSYTCPR	1720.7008	0	37.8053		
DETAILS	SHTDERPHK	1105.5265	0	24.3577		
DETAILS	NHLNTHGTGRPHK	1511.7705	0	46.2283		
DETAILS	CPDCDMAFVTSGELVR	1855.7903	0	42.3704		
DETAILS	HTHEKPFK	1022.5298	0	24.7994		
DETAILS	CSMCDYASVEVSK	1534.6102	0	34.4654		
DETAILS	SHTGERPFQCSLCSYASR	2141.937	0	24.7531		
DETAILS	THSGEKPYECYICHAR	2006.8727	0	45.1026		
DETAILS	FTQSGTMK	898.4219	0	24.8384		
DETAILS	HTENVAK	797.40314	0	24.7963		
DETAILS	FHCPHCDTVIAR	1511.6761	0	26.5643		
DETAILS	SDLGVHLR	895.48755	0	28.8680		
DETAILS	QHSYIEQ GK	1088.525	0	24.7637		
DETAILS	YCDAVFHER	1195.508	0	28.5533		
DETAILS	YALIQHQK	999.5502	0	25.4094		
DETAILS	CDQCDYACR	1246.4165	0	25.0130		
DETAILS	THTGEKPYACSHCDK	1789.7512	0	24.6605		
DETAILS	QLLDMHFK	1030.527	0	36.4861		
DETAILS	YHDPNFVPAAFVCSK	1750.8137	0	38.9469		
DETAILS	HADNCAGPDGVEGENGGETK	2012.813	0	24.8724		

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Below the **Peptides** table is a bar graph that displays the peak areas of the peptides, ordered from the highest to the lowest measured peak area



Go back to the home page of your library folder either by clicking your browser's back button or by clicking the folder name ('Human') above 'CTCF' as shown in the image below.



Updating chromatogram libraries

As you develop assays for more proteins or peptides, you can publish Skyline documents containing those assays to the same library folder. Panorama will build a new version of the library each time a Skyline document is published to the folder. Any proteins that were not already included in the library will be added.

Open the file "Stergachis-SupplementaryData_2_b.sky" in Skyline. This file contains another subset of transcription factor assays from *Stergachis et al.* 2011¹. This file also contains measurements for the transcription factor MAX that was already included in the first document that you published to the library folder. Two additional peptide measurements are included for this protein in this document.

Publish this document to the 'Human' chromatogram library folder on Panorama by doing the following:

- Click the **Publish to Panorama** button in the Skyline toolbar.
- Select the 'Human' folder in the **Panorama Folders** tree of the **Publish Document** form.
- Click the **OK** button.
- Wait for the file import to complete.
- Go to your browser and refresh the library folder page.

You will see that a new version of the library is now available in Panorama, as indicated by the higher revision number (2) in the **Chromatogram Library Download** box. This library includes the new proteins, peptides and transitions from the Skyline file that you just uploaded, reflected in the updated library statistics. You should also see a warning message below the library statistics about a conflicting protein. This message is displayed because the document you just uploaded contains a protein (transcription factor MAX) which already existed in the library.

Chromatogram Library Download

Human Library

Download

Human_rev2.clib (1 MB)
Revision 2

ARCHIVED REVISIONS >

Library Statistics:

The library contains 19 proteins with 231 ranked peptides.
The 231 ranked peptides contain 1,668 ranked transitions.

There are 1 conflicting proteins in this folder. [Resolve conflicts](#)

In case of a conflict, Panorama marks the incoming version of the protein as conflicted, and continues to include the older version of the protein in the library, till the conflict is resolved. The library built after uploading "Stergachis-SupplementaryData_2_b.sky" still contains peptides, transitions and chromatograms for protein MAX from the first document that was uploaded to the library folder. To tell Panorama to use the newer version of the protein:

- Click the **Resolve conflicts** link.

You will now see a table containing a list of conflicting proteins displayed side by side. The left column, titled **Conflicting Proteins in Document**, displays the version of the protein contained in the document

that you just uploaded. The right column, titled **Current Library Proteins** has the version contained in the current library.

Library Protein Conflicts

Check the proteins that you would like to include in the library.

Resolve conflicts for Stergachis-SupplementaryData_2_b_2013-10-02_09-26-21.zip.

Conflicting Proteins in Document		Current Library Proteins	
Protein	Document	Protein	Document
<input checked="" type="checkbox"/> ⊕_MAX	Stergachis-SupplementaryData_2_b_2013-10-02_09-26-21.zip	<input type="checkbox"/> ⊕_MAX	Stergachis-SupplementaryData_2_a_2013-10-02_09-21-07.zip
Select All		Select All	

[APPLY CHANGES](#) [CANCEL](#)

Click on the [+] icon next to the protein name to view the list of peptides measured for this protein in the two versions. The new document contains measurements for two additional peptides that are marked in the image below.

Library Protein Conflicts

Check the proteins that you would like to include in the library.

Resolve conflicts for Stergachis-SupplementaryData_2_b_2013-10-02_09-26-21.zip.

Conflicting Proteins in Document		Current Library Proteins	
Protein	Document	Protein	Document
<input checked="" type="checkbox"/> ⊕_MAX	Stergachis-SupplementaryData_2_b_2013-10-02_09-26-21.zip	<input type="checkbox"/> ⊕_MAX	Stergachis-SupplementaryData_2_a_2013-10-02_09-21-07.zip
Peptide	Rank	Peptide	Rank
ATEYIQYMR	1	ATEYIQYMR	1
DSVPSLQGEK	2	DSVPSLQGEK	2
DSFHSLR	3	-	-
NHTHQQDIDDLK	4	-	-
AHHNALER	5	AHHNALER	3
QNALLEQQVR	6	QNALLEQQVR	4
MSDNDIEVESDADK	7	MSDNDIEVESDADK	5
SSAQLQTNYPSSDNSLYTNAK	8	SSAQLQTNYPSSDNSLYTNAK	6
Select All		Select All	

[APPLY CHANGES](#) [CANCEL](#)

To resolve this conflict by choosing the new version of the protein, do the following:

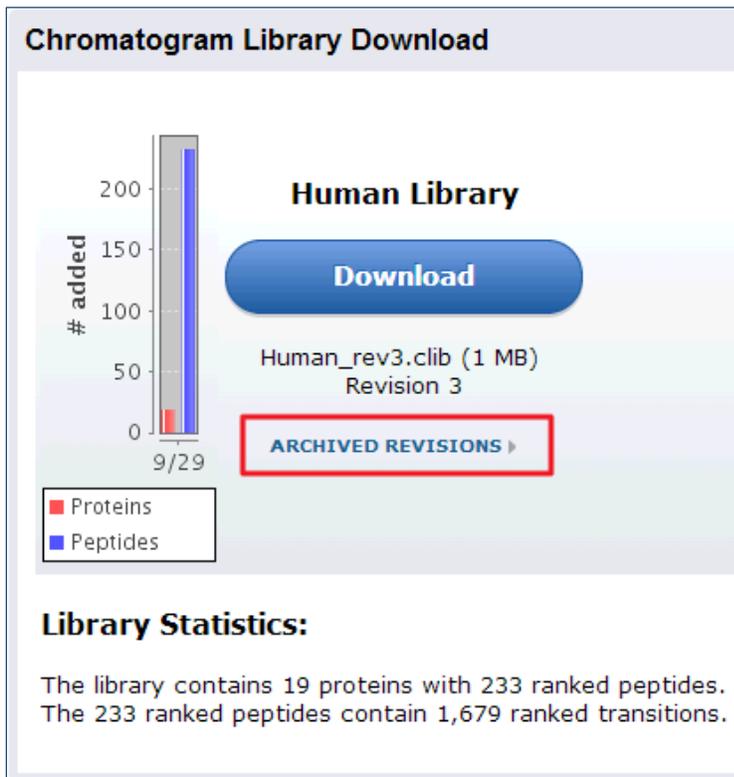
- Under **Conflicting Proteins in Document**, click the check box next to the protein name.
- Click the **Apply Changes** button.

Panorama will now rebuild the library and include the new version of the protein with all its peptides and transitions. Any peptides or transitions that existed only in the older version of the protein will be removed from the library. Once the new library has been built you will be taken to the home page of the library folder where you will see that a new revision (3) of the library is available. The conflict message should no longer be displayed.

- Click on the **Download** button and save the chromatogram library file (Human_rev3.clib) to your computer.

You will use this library in the next section of the tutorial.

You can access older revisions of the library by clicking on the **Archived Revisions** link below the library file name, shown in the image below.



A table with all the library revisions will be displayed. You can download older revisions by clicking on a file name in the **Archived Revisions** table.

Download Chromatogram Library

Archived Revisions

Revision #	File name	Size	Date created
1	HUMAN_REV1.CLIB >	607 KB	2013-09-29 09:40
2	HUMAN_REV2.CLIB >	1 MB	2013-09-29 14:04
3	HUMAN_REV3.CLIB >	1 MB	2013-09-29 14:51

Using chromatogram libraries in Skyline

You will now prepare a new Skyline document to measure the proteins CTCF and MAX in another sample. The library you just downloaded from Panorama contains chromatograms for peptides from these proteins. You will use the library to filter the set of peptides and transitions in your Skyline document to the best responding ones contained in the library. You will also see how you can compare fragment ion or transition chromatograms obtained in a new experiment with chromatograms contained in the library to confirm peptide identifications.

Before you begin, make sure that the peptide and transition settings in Skyline are configured correctly, by creating a new file and adjusting its settings as necessary:

- On the **File** menu, click **New** (Ctrl-N).

Configure the peptide settings as follows:

- On the **Settings** menu, click **Peptide Settings**.
- Click the **Digestion** tab.
 - In the **Enzyme** dropdown list, select “Trypsin [KR | P]”.
 - In the **Max Missed Cleavages** dropdown list, select 0.
 - In the **Background proteome** dropdown list, select “None”.
- Click the **Filter** tab.
 - In the **Min length** field, enter ‘7’.
 - In the **Max length** field, enter ‘23’.
 - In the **Exclude N-terminal AAs** field, enter ‘0’.
 - Uncheck **Exclude potential ragged ends**.
 - Uncheck any selected options in the **Exclude peptides containing** list.
 - Check **Auto-select all matching peptides**.
- Click the **Library** tab.
 - Uncheck any selected libraries.
- Click on the **Modifications** tab.
 - Check the “Carbamidomethyl (C)” modification in the **Structural modifications** list.
 - Uncheck any other selected modifications in the **Structural modifications** list.
 - Uncheck any selected modifications in the **Isotope modifications** list.
- Click the **OK** button in the **Peptide Settings** form.

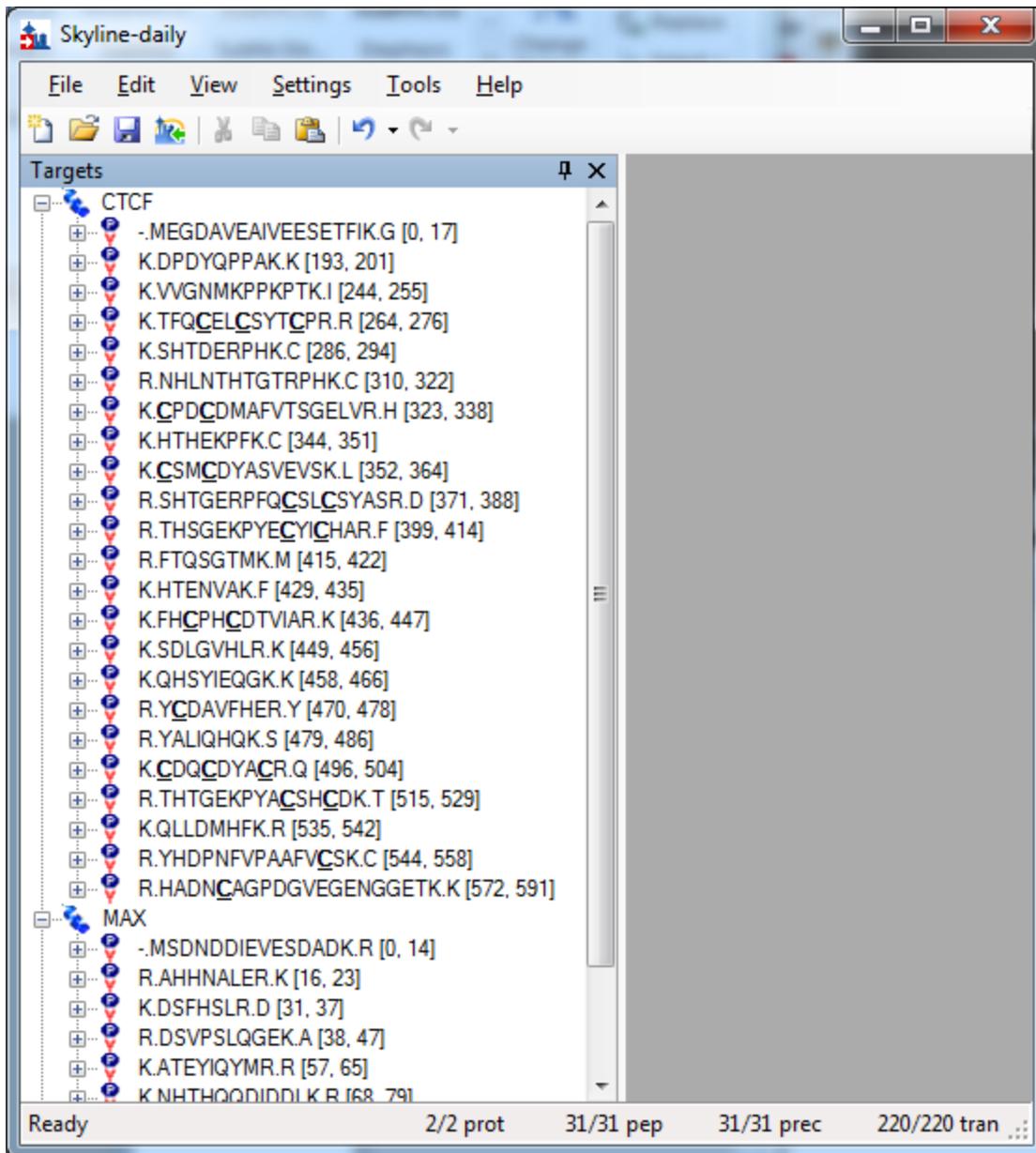
Configure the transition settings as follows:

- On the **Settings** menu, click **Transition Settings**.
- Click the **Filter** tab.
 - In the **Precursor charges** field, enter '2'.
 - In the **Ion charges** field, enter '1'.
 - In the **Ion types** field, enter 'y'.
 - In the Product ions **From** dropdown list, select "ion 3".
 - In the Product ions **To** dropdown list, select "last ion – 1".
 - In the **Always add** list, uncheck any selected options.
 - Make sure that the **Precursor m/z exclusion widow** field is blank.
 - Check **Auto-select all matching transitions**.
- Click on the **Library** tab.
 - Uncheck **If a library spectrum is available, pick its most intense ions**
- Click on the **Instrument** tab.
 - Enter 50 in the **Min m/z** textbox.
 - Enter 1500 in the **Max m/z** textbox.
 - Uncheck **Dynamic min product m/z**.
- Click the **OK** button in the **Transition Settings** form.

The files you downloaded for this tutorial include the file 'CTCF_MAX.txt'. This file contains sequences for the proteins CTCF and MAX. To add these proteins to the new Skyline document do the following:

- On the **File** menu, choose **Import** and click **FASTA**.
- Browse to and select 'CTCF_MAX.txt' in the file browser.
- Click the **Open** button.

The Skyline window should now look like this:



The settings you configured in Skyline have resulted in 31 peptides for the two proteins. If you export a transition list for this document without applying any filtering you would be measuring 220 transitions. However, you can take advantage of the information contained in the chromatogram library to limit your measurements to the best responding precursors and product ions for the two proteins. Follow these steps to filter the list of peptides and transitions:

- On the **Settings** menu, click **Peptide Settings**.
- Click the **Library** tab.
- Click the **Edit List** button.
- Click on the **Add** button in the **Edit Libraries** form.

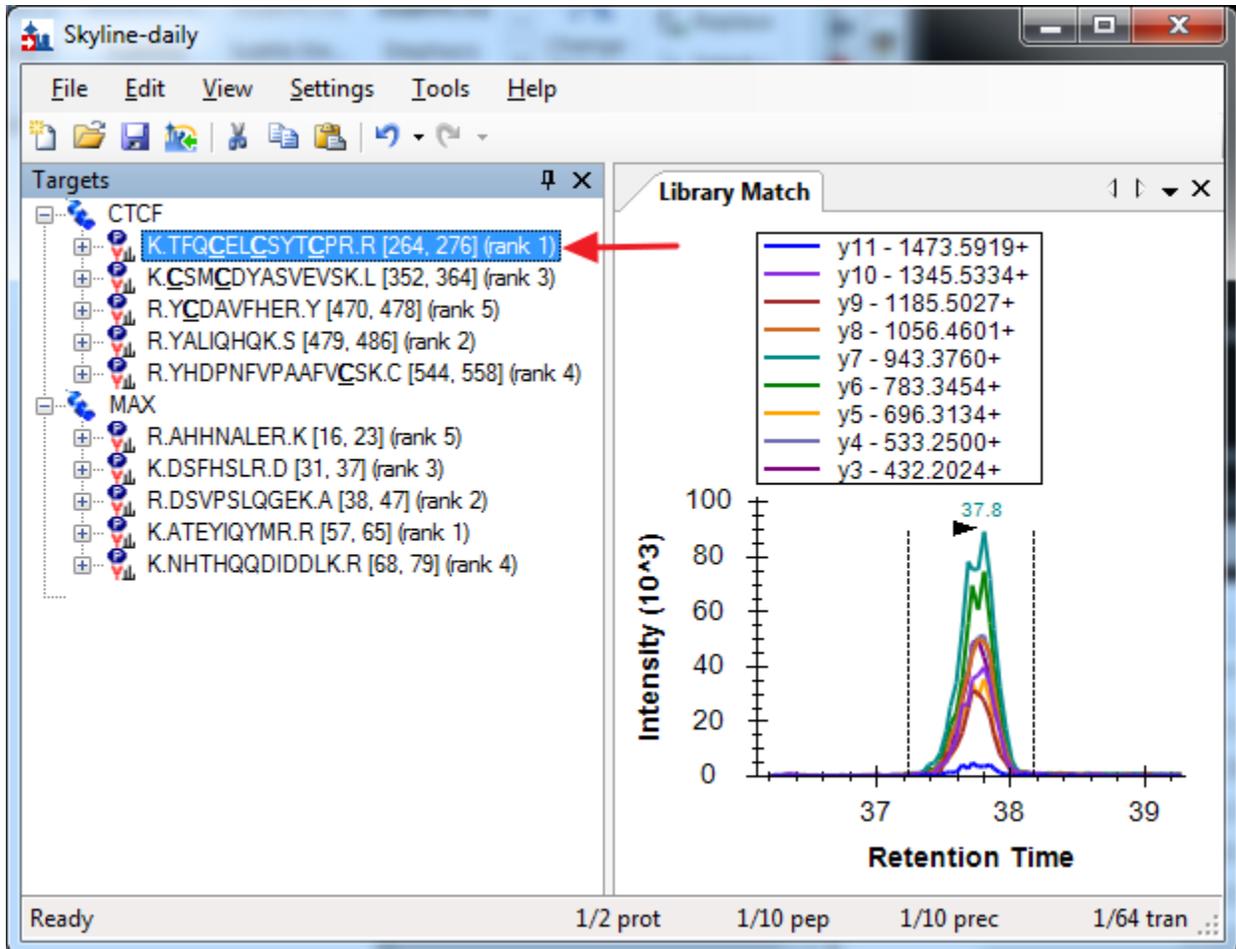
- In the **Name** field of the **Edit Library** form, enter 'Panorama Human Library'.
- Click on the **Browse** button.
- Select the 'Human_rev3.clib' chromatogram library file that you downloaded earlier.
- Click the **OK** button in the **Edit Library** form.
- Click the **OK** button in the **Edit Libraries** form.
- Check the box next to 'Panorama Human Library', in the **Libraries** list.
- In the **Pick peptides matching** dropdown list, select **Library**.
- In the **Rank peptides by** dropdown list, select **Peak Area**.
- Click the **Limit peptides per protein** check box
- In the **Peptides** field, enter '5'.

The **Peptide Settings** form should now look like this:

The screenshot shows the 'Peptide Settings' dialog box with the 'Library' tab selected. The 'Libraries' list contains 'Panorama Human Library' with a checked checkbox. To the right of the list are buttons for 'Edit list...', 'Build...', and 'Explore...'. Below the list, the 'Pick peptides matching:' dropdown is set to 'Library', and the 'Rank peptides by:' dropdown is set to 'Peak Area'. The 'Limit peptides per protein' checkbox is checked, and the 'Peptides' field contains the number '5'. At the bottom of the dialog are 'OK' and 'Cancel' buttons.

- Click the **OK** button.

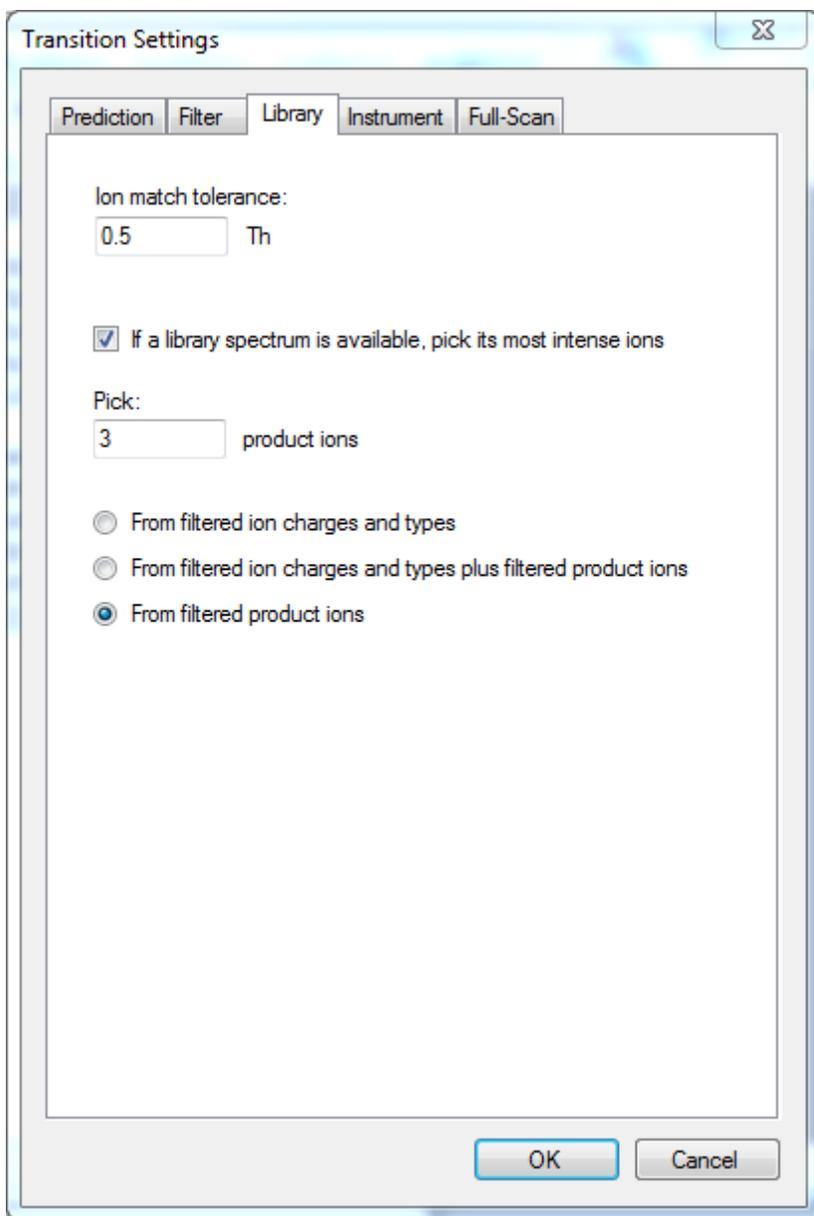
The number of transitions in the document should have decreased from 220 to 64. Skyline also displays a rank for each peptide as indicated in the image below. This rank is based on the total peak area, summing all transition peak areas, for the peptide in the library.



This number can be further reduced by limiting the transitions to the top ranking transitions in the library.

- On the **Settings** menu, click **Transition Settings**.
- Click the **Library** tab.
- Check the check box **If a library spectrum is available, pick its most intense ions**.
- In the **Pick product ions** field, Enter '3'.
- Select the **From filtered product ions** option.

The **Transition Settings** form should look like this:



- Click the **OK** button.

The number of transitions in this document should now be 30.

You can now export a transition list or a native instrument method for this document, and acquire data. Skyline offers the ability to export transition lists and SRM methods for instruments from several different instrument manufacturers.

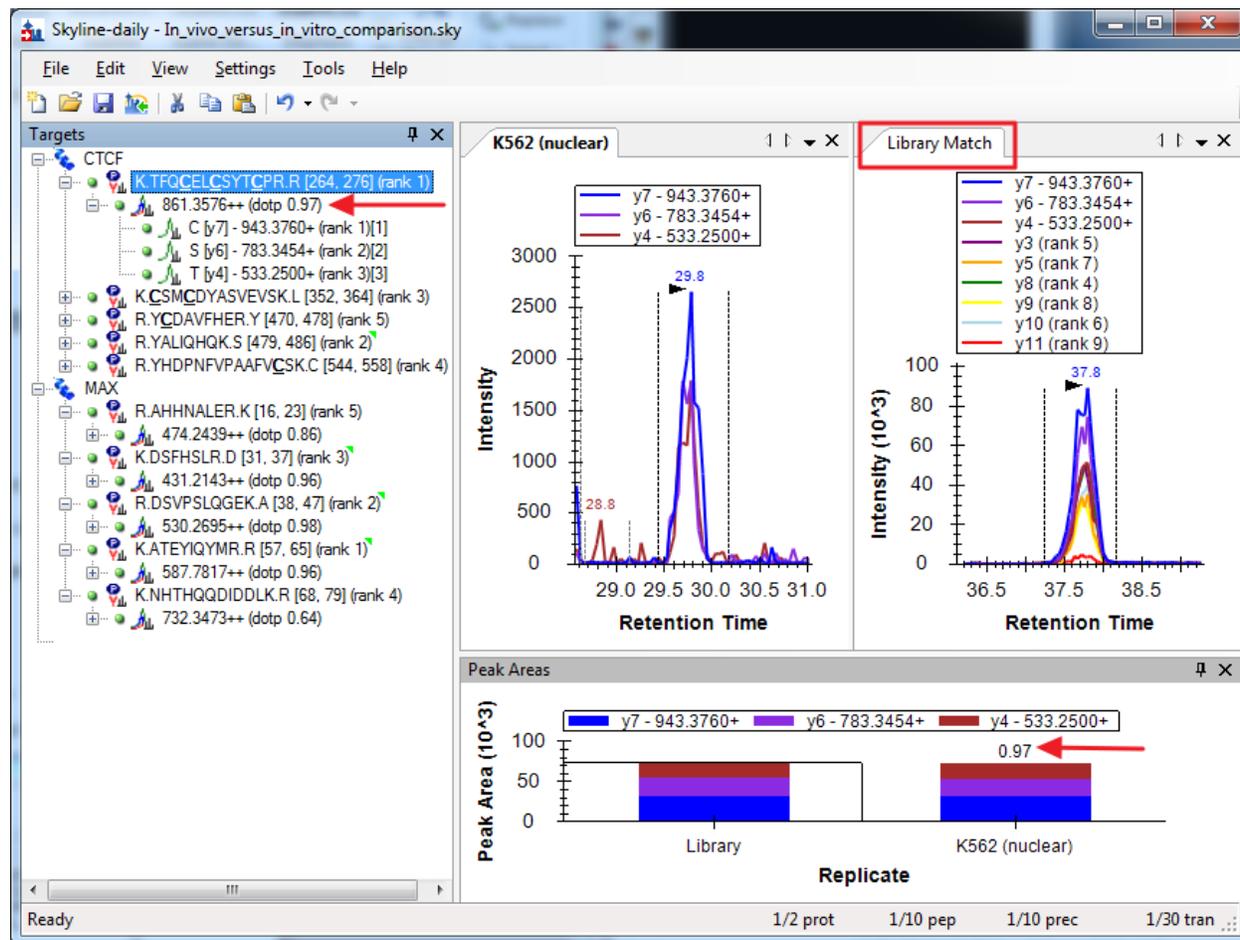
- On the **File** menu, choose **Export** and click either **Transition List** or **Method**.
- To view the list of supported instrument types in the **Instrument type** dropdown menu.

Please see the [Targeted Method Editing](#) and [Targeted Method Refinement](#) Skyline tutorials for more information on exporting transition lists and SRM Methods.

The files you downloaded for this tutorial already contain a document with data acquired on a triple quadrupole mass spectrometer. To begin exploring how acquired data and your new chromatogram library can be used together:

- Open the file “In_vivo_versus_in_vitro_comparison.sky” in Skyline.
- In the **Targets** view, select on the peptide **TFQCELCSYTCPR**, under the **CTCF** protein.

The Skyline window should look like this:



You should see the chromatograms for this peptide from the newly acquired data side-by-side with the chromatograms from the Human_rev3.clb library. The tab titled **K562 (nuclear)** has the chromatograms from the new data, and the tab titled **Library Match** displays the library chromatograms.

If you do not see the **Library Match** tab you can display it by as follows:

- On the **View** menu, click **Library Match**.

Skyline also calculates a dot product as a measure of similarity between the peak areas in the new data and the library peak areas. This is displayed next to the precursor, as marked in the image above. For the peptide **TFQCELC₂SYTCPR** the similarity is high, with a dot product of 0.97, and that gives a measure of confidence in the peptide identification.

Conclusion

In this tutorial, you have gone through the steps of creating a library folder in Panorama for curated, targeted results. You have built a chromatogram library in Panorama, and used it in Skyline to select the peptides and product ions to measure in a new experimental setting. You have also compared chromatograms acquired in a new experiment with data contained in the library to validate peptide identifications. By using these techniques with your own experimental data, you can build an extensive knowledge base of the targeted assays you have investigated and refined in your laboratory, and avoid duplicating this effort in new experiments. You can share these libraries with collaborators or the general public, as others are already doing, helping others to reproduce your results, or use them for new applications in targeted proteomics.

Reference List

1. Stergachis, A., MacLean, B., Lee, K., Stamatoyannopoulos, J. A., & MacCoss, M. J., Rapid empirical discovery of optimal peptides for targeted proteomics *Nature Methods* **8**,1041–1043 (2011)