

Association of Biomolecular Resource Facilities

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Skyline ABRF sPRG Data Processing Tutorial

This tutorial covers how to process your label-free quantitation data using the LC-MS instrument platform of your choice, upload peak integration results to Panorama, and export peak area ratios for submission to the ABRF sPRG.

In this tutorial you will work with data from a triplicate set of label-free LC-MS data.

This tutorial will cover the follow area critical to making effective use of Skyline for analysis of your ABRF sPRG study sample.

- Generating Skyline spectral libraries from discovery datasets
- Setting up a Skyline document for either MS 1 or data independent acquisition (DIA) filtering
- Importing raw data and using the retention time information for spectral libraries to direct peak picking during MS1 or DIA filtering
- Export results using a Skyline report template
- Uploading your Skyline results directly to Panorama
- Uploading your raw data and results to NIST server
- Filling out the ABRF sPRG 2013 Study Survey

Skyline is a freely-available, vendor-neutral platform for targeted proteomics investigation. It can import raw data for MS1 and DIA filtering from the instrument vendors AB Sciex, Agilent, Bruker, Thermo-Scientific and Waters. The ability to import data across various instrument platforms greatly facilitates cross-instrument comparisons and large multi-site studies.

Getting Started

To start this tutorial, download the following Zip file:

https://panoramaweb.org/labkey/files/ABRF%20sPRG/ABRF sPRG Skyline Tutorial Data.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\ABRF_Skyline

It will contain all the files necessary for this tutorial. Now start Skyline, and you will be presented with a new empty document.

Configuring a Skyline Document for a Data Dependent Dataset

Data was collected on the AB Sciex 5600 TripleTOF in three technical replicates with LC-MS label-free analysis (Figure 1).

Table 1. Filenames and Samples used for data analysis

File Name (wiff)	Sample Name	MASCOT results file (dat)
5600TT13-1070	Colangelo_ABRF HEK and JPT_042413_90minIDA_1_375_1_1	5600TT13-1070
5600TT13-1073	Colangelo_ABRF HEK and JPT_042413_90minIDA_1_375_1_2	5600TT13-1073
5600TT13-1076	Colangelo_ABRF HEK and JPT_042413_90minIDA_1_375_1_3	5600TT13-1076



Figure 1. Total Ion Chromatogram Overlay from 90min LC-MS label-free runs on ABRF sPRG 2013 Study Samples

After data collection, the resulting LC-MS label free runs, the wiff files were converted to mgfs using the AB Sciex Data Converter (beta 1.3), and searched by MASCOT (2.4.0) using the ABRFsPRG2013_v1.fasta database and the following parameters.

Search parameters

Type of search	: MS/MS Ion Search
Enzyme	: Trypsin
Fixed modifications	: ZCarbamidomethyl (C)
Variable modifications	: Deamidated (NQ), Label:13C(6)15N(2) (K), Label:13C(8)15N(2) (R), Condition (M)
Mass values	: Monoisotopic
Protein mass	: Unrestricted
Peptide mass tolerance	: ± 25 ppm (# ¹³ C = 1)
Fragment mass tolerance	: ± 0.2 Da
Max missed cleavages	: 1
Instrument type	: ESI-QUAD-TOF

The resulting Mascot .dat files are included in the ZIP file for this tutorial. Follow the steps below to build a spectral library in Skyline.

Setttings.....Peptide Settings....Library Tab....Click on Build Name the library "ABRF sPRG 2013 Yale"

tu	Build Library	×
	Name:	
	ABRF_sPRG_2013_Yale	
	Output Path:	
	C:\Users\cmc96\Documents\Skyline\ABRF_sPRG_20	Browse
	Action:	
	Create V Keep redundant library	
	Qut-off score: 0.9	
	Lab Authority (e.g. proteome.gs.washington.edu):	
	keck.med.yale.edu	
	Library [D: ABRF_sPRG_2013]	
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Use the Add Files button to add the .dat files, as shown below, from the ABRF_Skyline folder extracted from the ZIP file.

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Input Files: Imput Files: </td <td></td> <td>Add <u>Files</u> Add <u>Directory</u></td>		Add <u>Files</u> Add <u>Directory</u>
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Check the checkbox for the new Spectral Library.

Peptide Settings
Digestion Prediction Filter Library Modifications
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Pick peptides matching: Library Rank peptides by:
Limit peptides per protein Peptides
OK Cancel

From the **Modifications** tab, check "Carbamidomethyl (C)" as a structural modification and "Label:13C(6)15N(2) (C-term K)" and "Label: 13C(6)15N(4) (C-term R)" as isotope modifications and then click **OK**

SettingsTransitions settings	Full-	Scan	Tab

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MS/MS filtering
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Isolation scheme: Resolution:
Retention time filtering
Include all matching scans
Use only scans in retention time scheduling windows
Use only scans within by minutes of MS/MS IDs
OK Cancel

Settings...Transitions settings.....Instrument Tab

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Settings....Filter Tab...Then click OK.

Transition Settings
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Product ions
Always add: Nterminal to Proline Cterminal to Glu or Asp
Precursor m/z exclusion window:
V Auto-select all matching transitions
OK Cancel

File....Import....FASTA...and select "ABRFsPRG2013_v1.fasta".....click Open

A total of 975 peptide sequences were identified and imported as shown below.



File....Import.....Results

Save the Results to "Y	ale sPRG2013	ABRF Label	Free.sky	"click Save
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Hide Folders	Save	Cancel

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Add <u>multi-injection</u> replicates in directories	Cancel
<u>A</u> dd one new replicate <u>Name:</u>	
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 Add files to an existing replicate <u>N</u>ame: 	

Select the three label-free wiff samples and click Open



Next Skyline will ask you to remove the prefix and click Do not Remove



Save export "Yale_sPRG2013_ABRF_Label_Free.csv".

For your own personal data file please rename your data results file with a unique anonymous identifier composed of six alphanumeric characters (e.g. "12345A"). If you are submitting several data reports corresponding to alternative analytical strategies, keep the first six characters the same and concatenate sequential numbers (e.g., "12345A-01," "12345A-02," and "12345A-03"). Retain this identifier for future as this will be the only way for you to find your data in any presentation of our study results. (Your data will only be referred to by its unique identifier in any presentation).

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File...Share....choose complete dataset....Save Skyline Folder

Next you will also publish your data to Panorama......

Using Skyline go to Tools > Options and add the PanoramaWeb.org server to Skyline. This has to be done only once for each Panorama server that you would like to connect to from Skyline. Use "submit" for the password.

Panorama	
Servers:	
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	OK Cancel

Open the Skyline document that you want to publish to a Panorama server and click on **Publish to Panorama**... in the **File** menu. Select a folder on the server where you want to add the document. Folders for which you do not have access will be grayed-out.

The Publish Document
File:
BRF_Skyline\Yale_sPRG2013_ABRF_Label_Free_2013-07-31_15-18-53.zip Browse
Panorama Folders:
□
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OK Cancel

The file will be uploaded to the **server**.



Your data should now be seen on the ABRF sPRG 2013 Panorama Results Page under the Targeted MS Runs.

https://panoramaweb.org/labkey/project/ABRF%20sPRG/ABRF%20sPRG%202013%20Data%20Results/begin.v

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If you prefer to you can upload Skyline data to the Panorama server via a web-browser First go to Skyline and **File**.....**Share**

You will get a pop-up box and next click the **Complete** button



You will get a dialog box. Give your zip data results a name and click Save



Next open a web broswer and go to

https://panoramaweb.org/labkey/project/ABRF%20sPRG/ABRF%20sPRG%202013%20Data%20Results/begin.v iew?

At the top of the page, under Data Pipeline, click on the "PROCESS AND IMPORT DATA" link under Data Pipeline



Browse to the shared zip file that you want to import and upload it to the Panorama server.

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Select the "Yale_sPRG2013_ABRF_Label_Free2.zip" and click **Open** and then Click **Upload**

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📝 5600TT13-1073.dat	5/17/2013 12:25 PM	DAT File	37,527 KB		
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Select the zip file you uploaded and click on "IMPORT DATA"

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Support Help	Tale_sPRG2013_ABRF_Label_Free_2013-07-11_13-00-03.zip	2013-07-11 13:05:4
	<pre></pre>	► ta%20Results/%40files/Yal

You will see a pop-up box asking you to import Skyline Results and click Import.

ABRF sPRG 2013 Data Results	Targeted
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Import Skyline Results using 1 out of 1 file(s) 🕡	
IMPORT	CANCEL
	ABRF sPRG 2013 Data Results Import Data Import Skyline Results using 1 out of 1 file(s) • Import Skyline Results

Finally, navigate back to the ABRF sPRG 2013 Data Results Page

https://panoramaweb.org/labkey/project/ABRF%20sPRG/ABRF%20sPRG%202013%20Data%20Results/begin.view?

Your data results will be shown under the Targeted MS Runs

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VIEWS * CHARTS * EXPORT * PRINT PAGE SIZE * I	DELETE ADD TO RUN	GROUP	MOVE			
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Yale_sPRG2013_ABRF_Label_Free_2013- 07-11_13-00-03.zip	2013-07-11 10:06	1	975	2248	6744	

Upload RAW Data to NIST FTP Client

Use an FTP client to upload all your raw data and to ABRF sPRG 2013 ftp server

First create a folder using a unique anonymous identifier composed of six alphanumeric characters (e.g. "12345A"). Retain this identifier for future as this will be the only way for you to find your data in any presentation of our study results. (Your data will only be referred to by its unique identifier in any presentation).



Within the folder place your Raw data files and Excel reporting template



Then zip the folder with "12345A.zip

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Next login to ABRF sPRG 2013 FTP server using the following login information:

<u>sPRG 2013 Participants</u> Server: chemdata.nist.gov User: sprg2013 Passwd: 2013SPRG_100

Here we are using an FTP client like CoreFTP Fill in the **HOST/IP/URL** with**chemdata.nist.gov** Username: **sprg2013** Password: **2013SPRG_100** Then click **Connect** button

Site Manager	×
NIST_sPRG Keckmascot2 ABRF NIST_sPRG_participants	Site Name NIST_sPRG_participants Host / IP / URL chemdata.nist.gov Advanced
	Username sprg2013 Password Don't save password
	Port Timeout Retries 21 60 2 Retry On
	Connection
- Comments	- SSL Options
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New Site New Category	Connect Manager Close

Drag and Drop the "12345A.zip" file to the right On the bottom bar (highlighted in red box) you should see its progress as it is uploaded

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After upload, your file should now be listed on the NIST FTP site (right hand side)

Once your data has been uploaded all that is left to do is fill out the ABRF sPRG Survey Using your web browser navigate to <u>http://www.surveymonkey.com/s/XLMP2WV</u> and complete the survey.

