

Configuring Skyline Batch for Selevsek Data

This tutorial covers how to use Skyline Batch to create and run five sharable configurations with the Selevsek 2015 data.

Getting Started

Install Skyline Batch from:

[Skyline Batch \(washington.edu\)](http://www.washington.edu/skylinebatch)

Create a “Selevsek” folder in a drive on your computer that has at least 134GB of free space. This folder will be used to store all the data and associated files for the configurations.

To download the R script to install the necessary R packages, go to:

<https://panoramaweb.org/dia-selevsek15.url>

- Click on the **Files** tab in the upper right-hand corner



- Double-click on the **reports** folder to open it.
- Check the checkbox next to **install_R_packages.R**.
- Click the download icon to download the file into your downloads folder.

Setting Up R

Check if you have a “C:\Program Files\R\ R-4.0.3” folder. If you do not, go to:

[Index of /bin/windows/base/old/4.0.3 \(r-project.org\)](http://www.r-project.org/bin/windows/base/old/4.0.3/)

*Note: You can use an R-4.0.3 installation located somewhere else on your computer. However, you will have to add your R directory to Skyline Batch.

Install R-4.0.3 from the R-4.0.3-win.exe link. When it is done downloading, the setup installer will open.

- Click the **Next** button until you see a green Installing progress bar.
- Click the **Finish** button.

Go to the Rtools website:

[Building R for Windows \(r-project.org\)](http://www.r-project.org/tools/)

- Click on “Rtools35.exe” to begin downloading the correct version of Rtools.
- Click the **Next** button until you reach **Install** to accept the default settings.
- Click the **Install** button.

- Click the **Finish** button.

If you do not have Rstudio, download it here:

[Download the RStudio IDE – Rstudio](#)

- Install it, accepting the default settings.
- Open RStudio.
- Go to **Tools** and click **Global Options**.
- Select the **General** tab on the left-hand side.
- Check if **R version** is showing R-4.0.3
 - If it is:
 - Click **OK**
 - If it is not:
 - Click **Change**
 - Select **R-4.0.3**
 - Click **OK** twice
 - Restart RStudio

You are now ready to start downloading packages in R-4.0.3

- In RStudio, click **File > Open File**
- Navigate to your downloads folder and double-click on “install_R_packages.R” to open it.
- Select line 2, then click the **Run** toolbar button. (Ctrl-Enter)

You should see this output if the download was successful:

```
package 'gplots' successfully unpacked and MD5 sums checked
package 'dplyr' successfully unpacked and MD5 sums checked
```

- Select lines 4-5, then click the **Run** toolbar button. (Ctrl-Enter)

You should see this output if the download was successful:

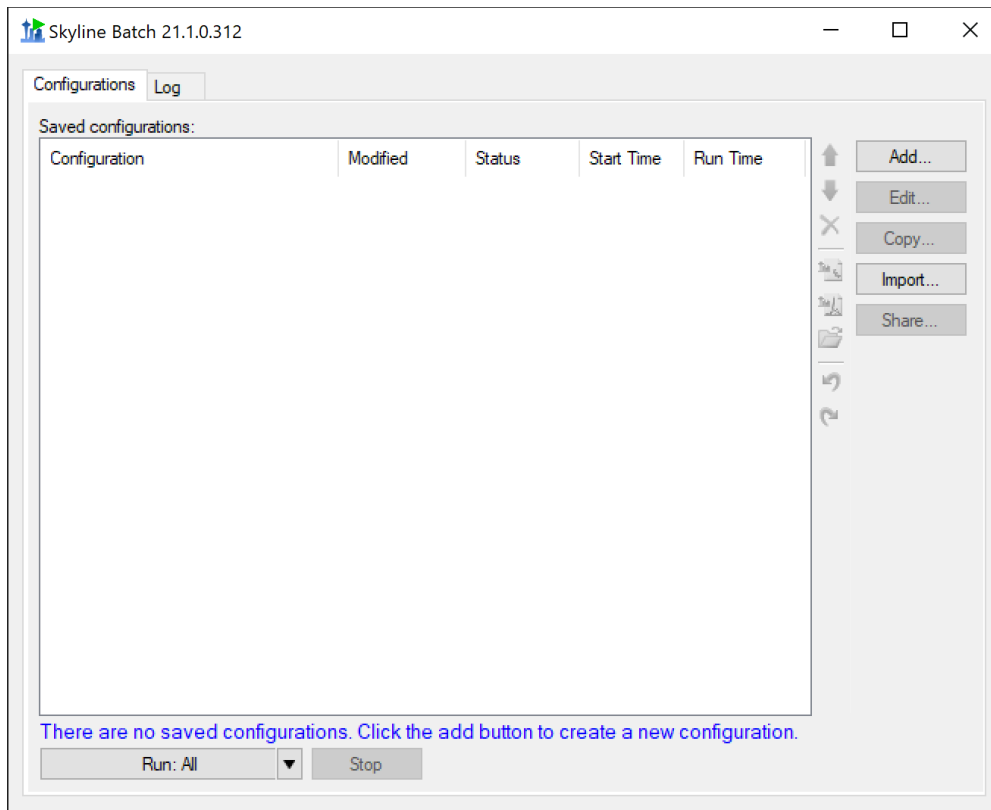
```
package 'BiocManager' successfully unpacked and MD5 sums checked
package 'MSstats' successfully unpacked and MD5 sums checked
```

Creating the Selevsek-all (MSstats) Configuration

- Open Skyline Batch.

Skyline Batch looks for Skyline when you open it the first time. If it does not find it, you will see a form to specify the path to the Skyline directory. Do that.

You will see the main window, which looks like this:

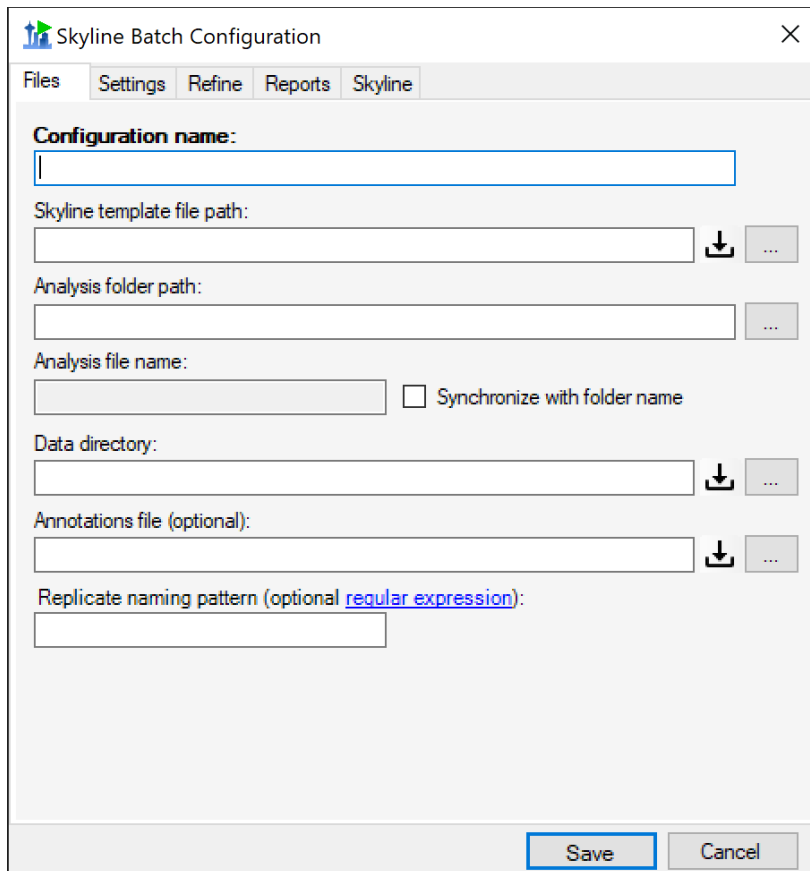


*Note: This tutorial requires a version of Skyline Batch after 21.1.0.312. If you have an earlier version number (seen in the top left corner of the main window) or no version number, you will need to install the newest version of Skyline Batch from [Skyline Batch \(washington.edu\)](http://www.washington.edu).

- Click the **Add** button.

This brings up the **Skyline Batch Configuration** dialog.

- Click on the **Files** tab.

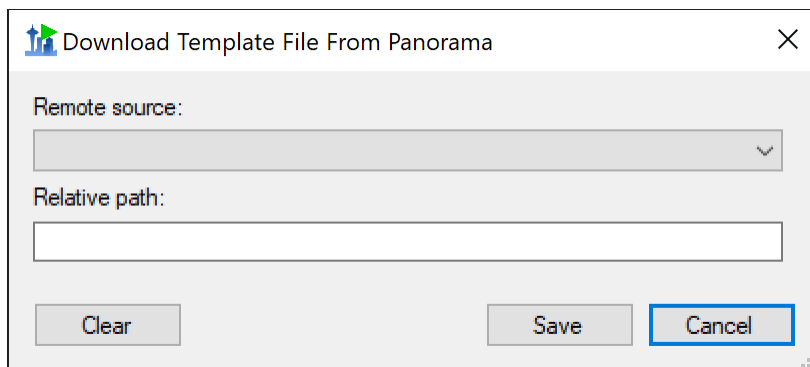


- Select the **Configuration name** textbox and type “Selevsek-all (MSstats)”

Skyline template file path:

- Click the download button (↓) next to **Skyline template file path**.

This opens the **Download Template File From Panorama** dialog:



- Expand the **Remote source** drop-down list
- Click <Add...>

This opens the **Remote source** dialog:

Remote Source

Name:

Folder URL:

Username (optional): Password (optional):

Share password

Save Cancel

- Select the **Name** textbox and type “Selevsek folder”

In a web browser, navigate to the 2015-Selevsek folder on Panorama:

<https://panoramaweb.org/dia-selevsek15.url>

- Click on the **Files** tab in the upper right-hand corner



This brings you to the folder view of the data.

- Click on the **Files** label underneath MacCoss-2015-Selevsek (shown in the red square below).

MacCoss - 2015-Selevsek MacCoss - 2015-Selevsek

Files

Import Skyline Results

	Name	Last Modified
<input type="checkbox"/>	assaydata	
<input type="checkbox"/>	docs	
<input type="checkbox"/>	RawFiles	
<input type="checkbox"/>	reports	
<input type="checkbox"/>	Selevsek	
<input type="checkbox"/>	Selevsek-all	

This displays the WebDav URL at the bottom of the page:

Name: fileset

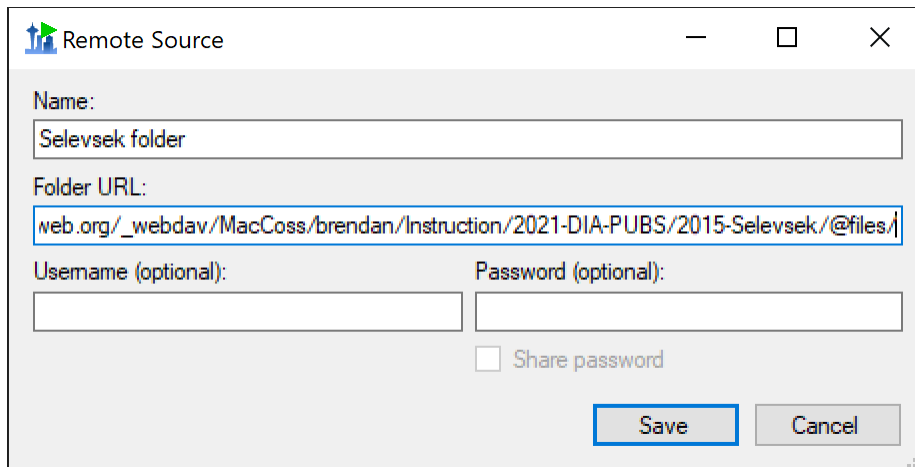
WebDav URL: https://panoramaweb.org/_webdav/MacCoss/brendan/Instruction/2021-DIA-PUBS/2015-Selevsek/@files/

- Right-click on the blue **WebDav URL** link.
- Click **Copy link**.

Return to Skyline Batch.

- Paste (CTRL+V) into the **Folder URL** of the **Download Source** dialog.

The **Remote Source** dialog should now look like this:



Remote Source dialog box showing:

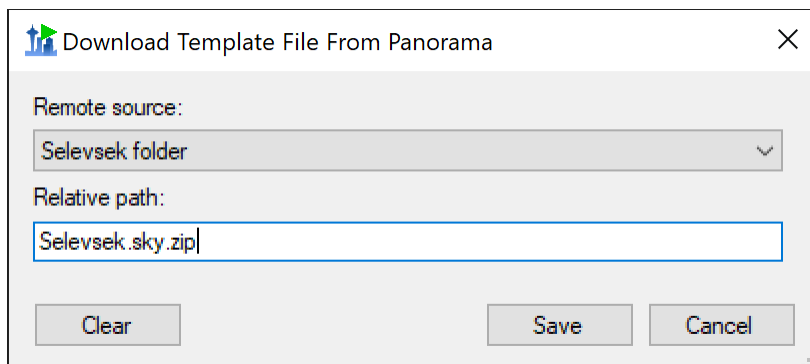
- Name: Selevsek folder
- Folder URL: web.org/_webdav/MacCoss/brendan/Instruction/2021-DIA-PUBS/2015-Selevsek/@files/
- Username (optional):
- Password (optional):
- Share password
- Buttons: Save, Cancel

- Click **Save**.

This has created a remote source for the 2015-Selevsek folder on Panorama that can be reused for other downloaded files.

- Select the **Relative path** textbox and type “Selevsek.sky.zip”

The **Download Template File From Panorama** dialog should now look like this:

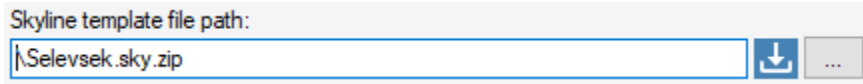


Download Template File From Panorama dialog box showing:

- Remote source: Selevsek folder
- Relative path: Selevsek.sky.zip
- Buttons: Clear, Save, Cancel

- Click **Save**.

The file name appears and the download icon next to **Skyline template file path** turns blue and white, indicating that the file will be downloaded:



- Click the (...) button next to **Skyline template file path**.
- Select your “Selevsek” folder in the folder explorer and click **OK**.

The **Skyline template file path** should now look like this:

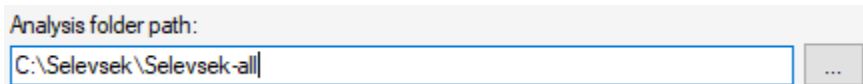


Analysis folder path:

- Click the (...) button next to **Analysis folder path**.
- Select your “Selevsek” folder in the folder explorer and click **OK**.
- Add “\Selevsek-all” to the end of the **Analysis folder path**.

This will create a “Selevsek-all” folder to hold the results of analysis when the configuration is run.

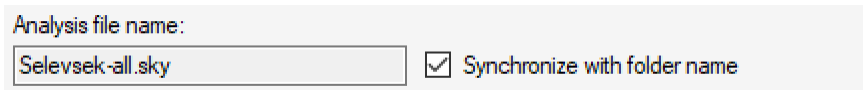
The **Analysis folder path** should now look like this:



Analysis file name:

- Check the **Synchronize with folder name** checkbox

The **Analysis file name** should now look like this:

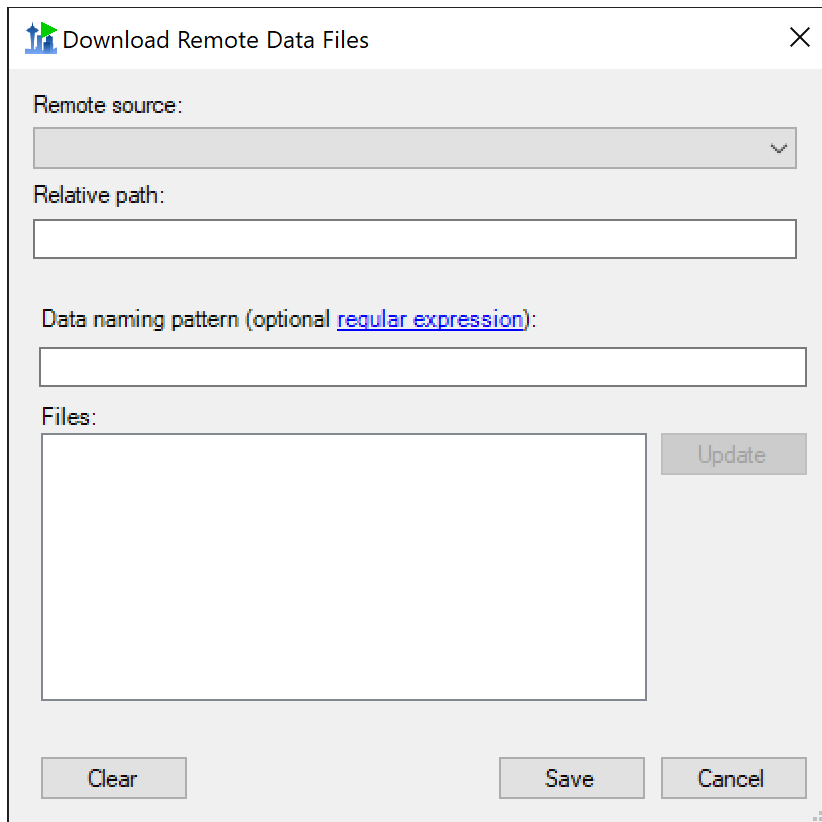


This will change the name of the generated Skyline file to Selevsek-all.sky, which matches the processed file name on Panorama.

Data directory:

- Click the download button (↓) next to **Data directory**.

This brings up the **Download Remote Data Files** dialog:

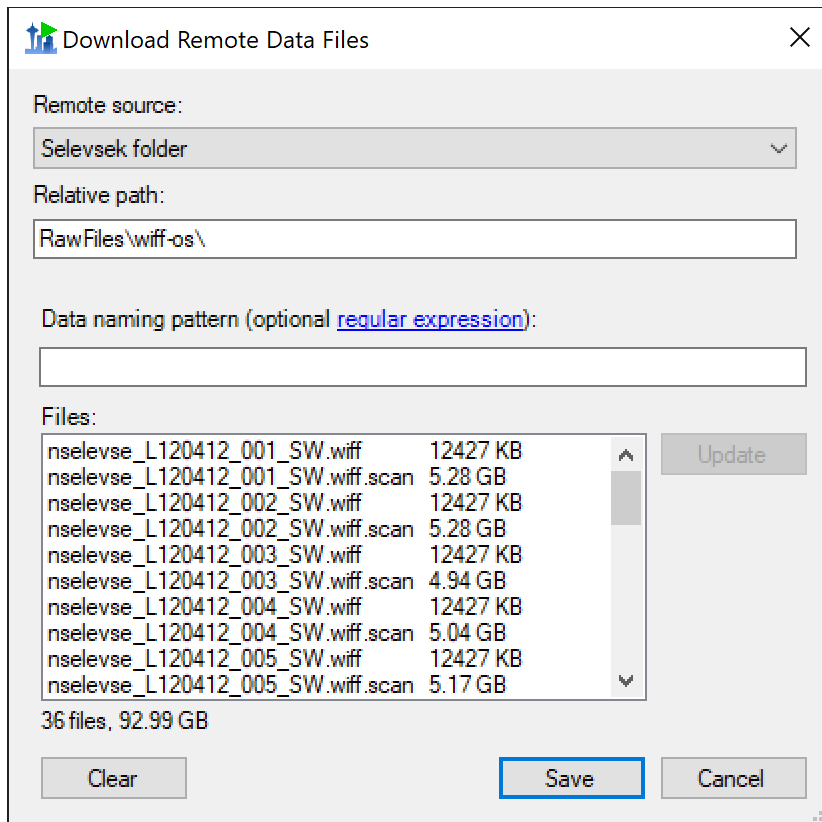


- Expand the **Remote source** drop-down list and select **Selevsek folder**



- Select the **Relative path** textbox and type “RawFiles\wiff-os\”
- Click **Update**.

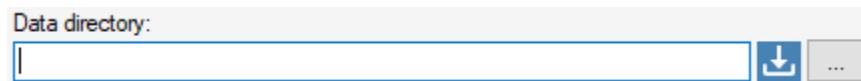
This retrieves all the file names and sizes from the URL. When it has finished loading, the **Download Remote Data Files** dialog should look like this:



Double-check that Skyline Batch found 36 files with a combined size of 92.99 GB.

- Click **Save**.

The download icon next to **Data directory** turns blue and white, indicating that the data files will be downloaded:



- Click the (...) button next to **Data directory**.
- Select your “Selevsek” folder in the folder explorer and click **OK**.
- Add “\OS” to the end of the **Data directory**.

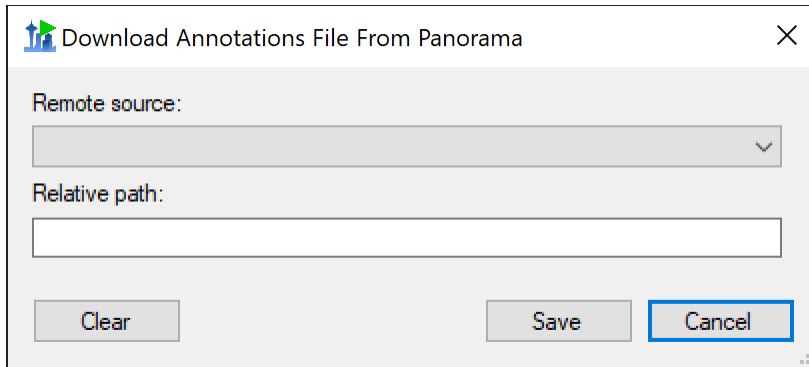
The **Data directory** should now look like this:



Annotations file:

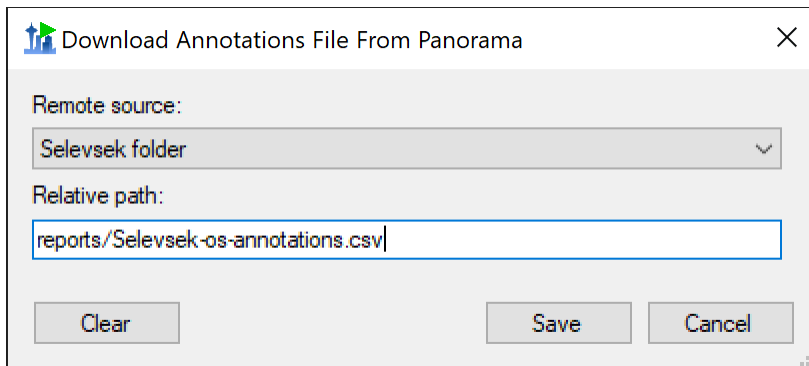
- Click the download button (↓) next to **Annotations file**.

This opens the **Download Annotations File From Panorama** dialog:



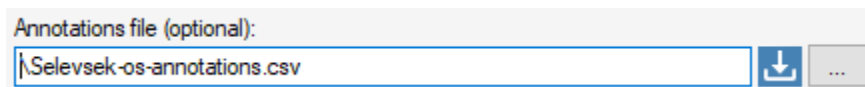
- Expand the **Remote source** drop-down list and select **Selevsek folder**
- Select the **Relative path** textbox and type “reports/Selevsek-os-annotations.csv”

The **Download Annotations File From Panorama** dialog should now look like this:



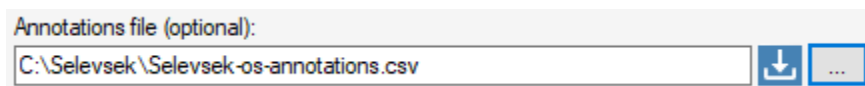
- Click **Save**.

The file name appears and the download icon next to **Annotations file** turns blue and white, indicating that the file will be downloaded:



- Click the (...) button next to **Annotations file**.
- Select your “Selevsek” folder in the folder explorer and click **OK**.

The **Annotations file** should now look like this:



Replicate naming pattern:

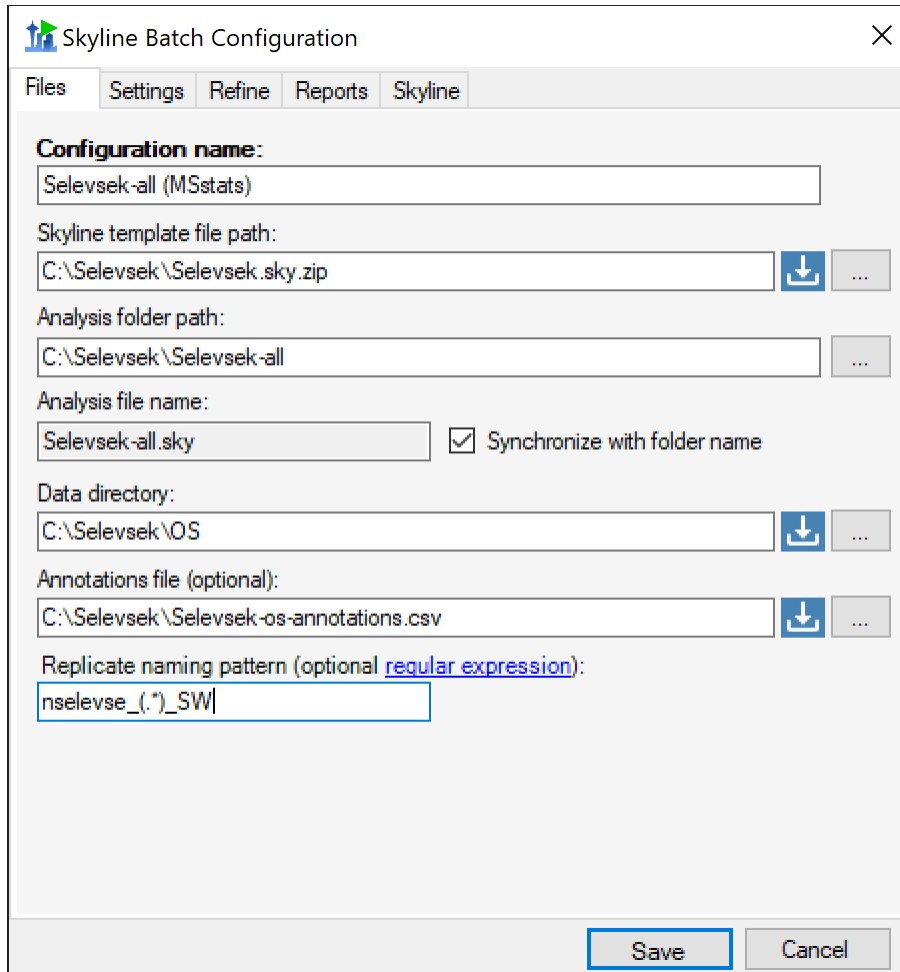
- In the **Replicate naming pattern** textbox, type “nselevse_(.*)_SW”

The **Replicate naming pattern** should look like this:

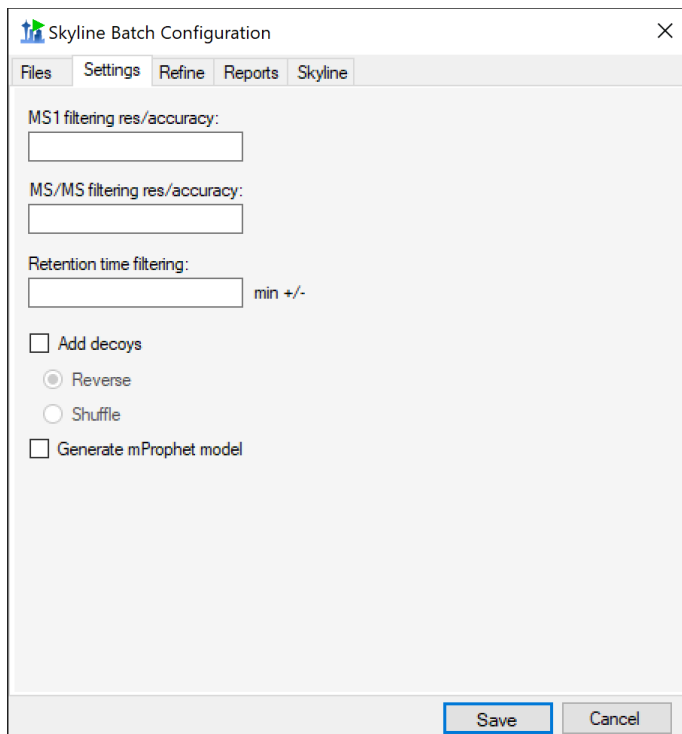
Replicate naming pattern (optional [regular expression](#)):

nselevse_(.*)_SW

The completed **Files** tab should look like this:

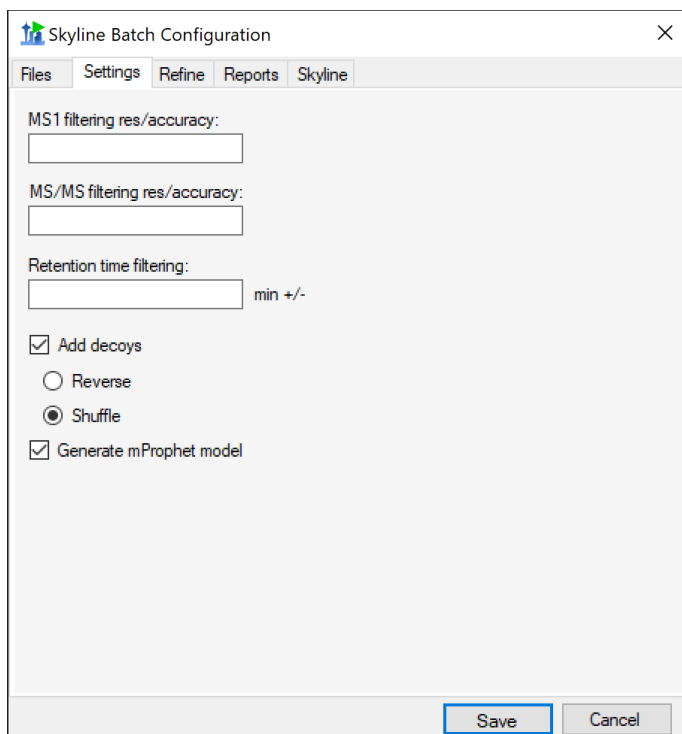


- Click on the **Settings** tab

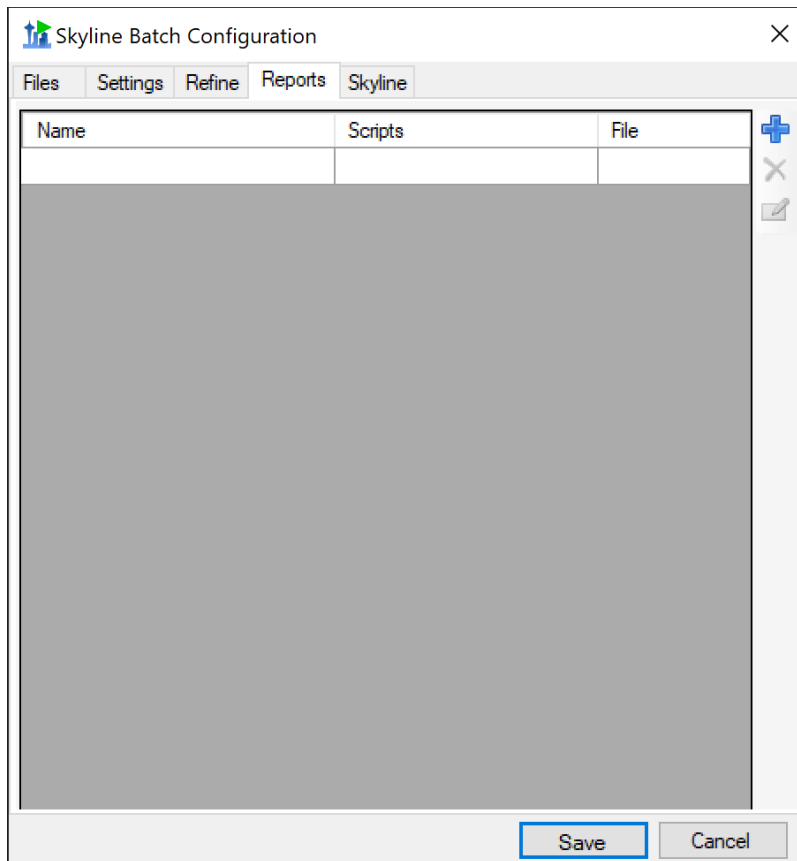


- Check the **Add decoys** checkbox.
- Select the **Shuffle** radio button.
- Check the **Generate mProphet model** checkbox.

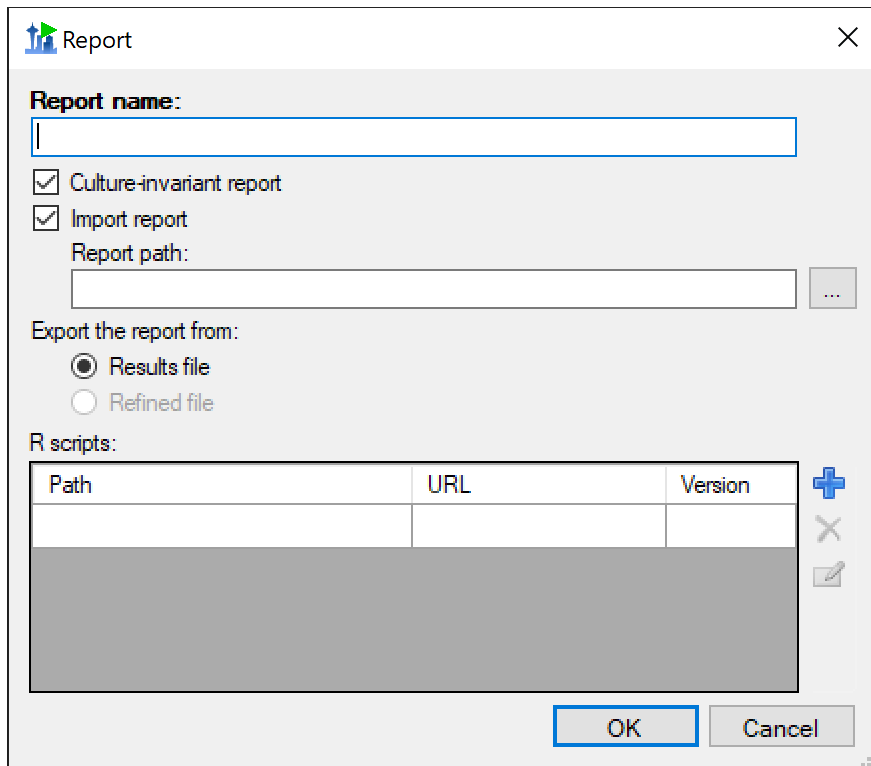
The **Settings** tab should now look like this:



- Click on the **Reports** tab.

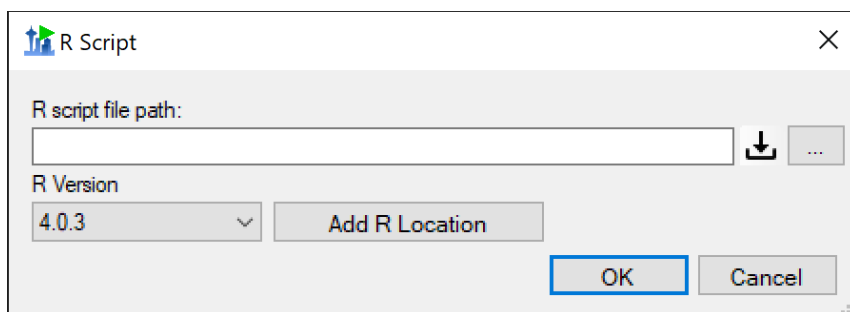


- Click the Add icon (+) to add a new report.



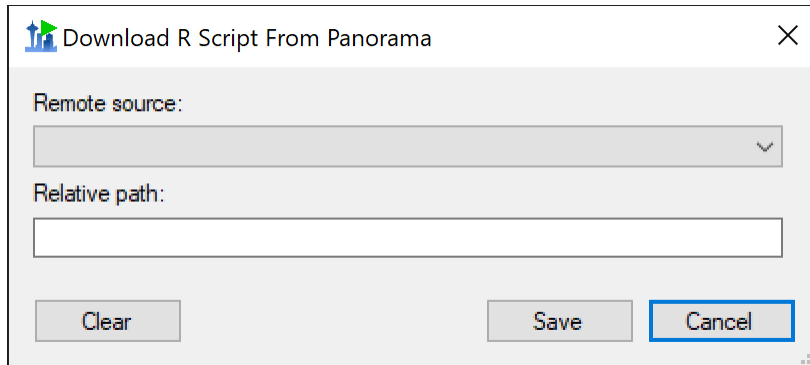
- In **Report name** put “MSstats Input-plus”
- Uncheck the **Import report** checkbox (this report already exists in our Skyline template document, so it doesn’t need to be imported)
- Click the Add icon (+).

This brings up the **R Script** dialog:



- Click the download button (↓) next to **R script file path**.

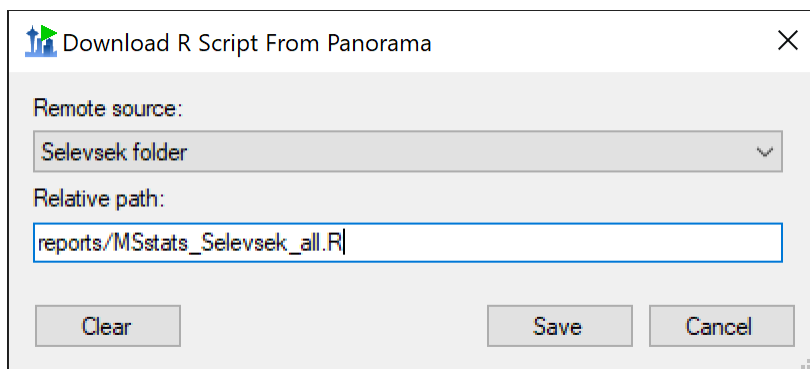
This opens the **Download R Script From Panorama** dialog:



This opens the **Download R Script From Panorama** dialog:

- Expand the **Remote source** drop-down list and select **Selevsek folder**
- Select the **Relative path** textbox and type “reports/MSstats_Selevsek_all.R”

The **Download R Script From Panorama** dialog should now look like this:



- Click **Save**.

The file name appears and the download icon next to **R script file path** turns blue and white, indicating that the file will be downloaded:



- Click the (...) button next to **R script file path**.
- Select your “Selevsek” folder in the folder explorer and click **OK**.

The **R script file path** should now look like this:

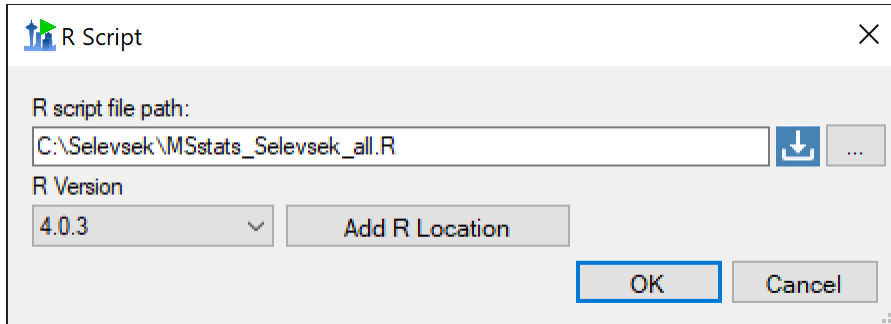


- Expand the **R version** drop-down list.

- Select **R-4.0.3**.

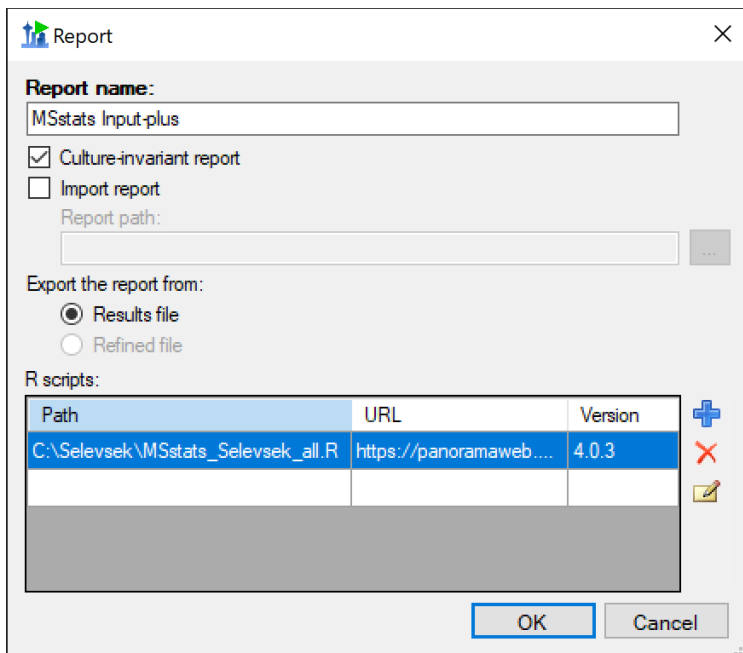
*Note: If R-4.0.3 does not appear in the list, you will need to add the location of R-4.0.3 by clicking on the **Add R Location** button.

Your **R Script** dialog should now look like this:



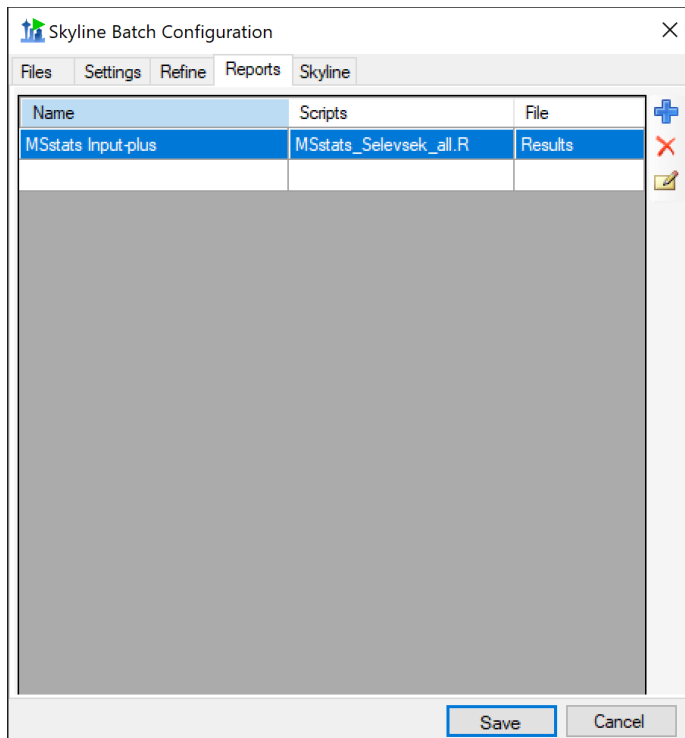
- Click **OK**.

The **Report** dialog should now look like this:

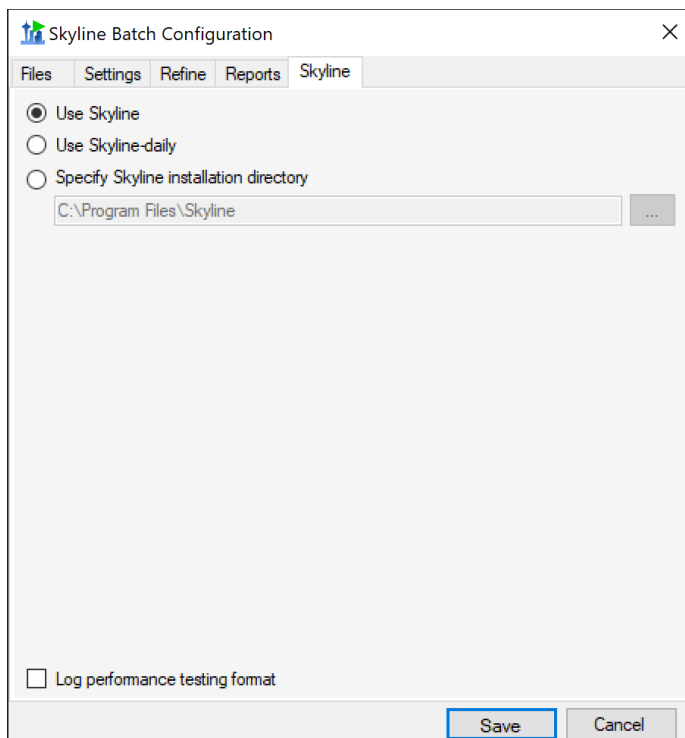


- Click **OK**.

The report will now appear in the list on the **Reports** tab:



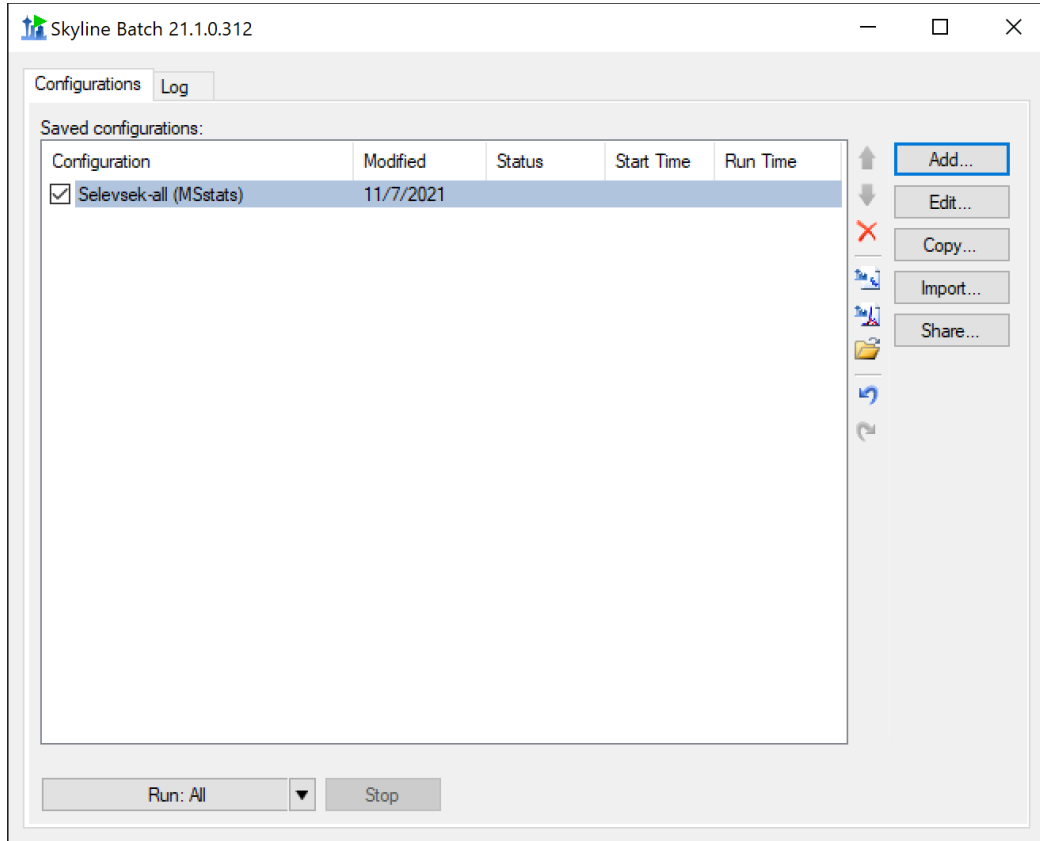
- Click the **Skyline** tab



- Select the **Use Skyline** radio button if it is not already selected.
- Click **Save** to save the configuration.

If there are any errors in the configuration, a warning will appear that will prevent you from saving. Fix the information causing the error and try to save again.

The **Skyline Batch Configuration** dialog will close, and you will see the new configuration in the main window:



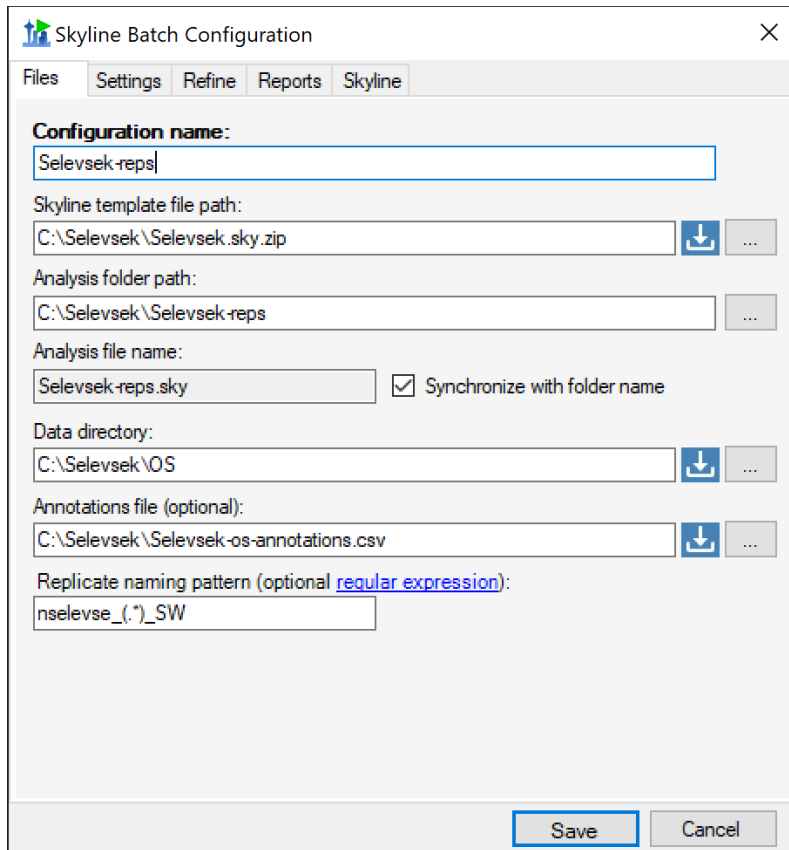
Creating the Selevsek-reps Configuration

- Click on “Selevsek-all (MSstats)” to select it.
- Click **Copy**.

This will bring up a copy of Selevsek-all (MSstats), which we will use to create Selevsek-reps.

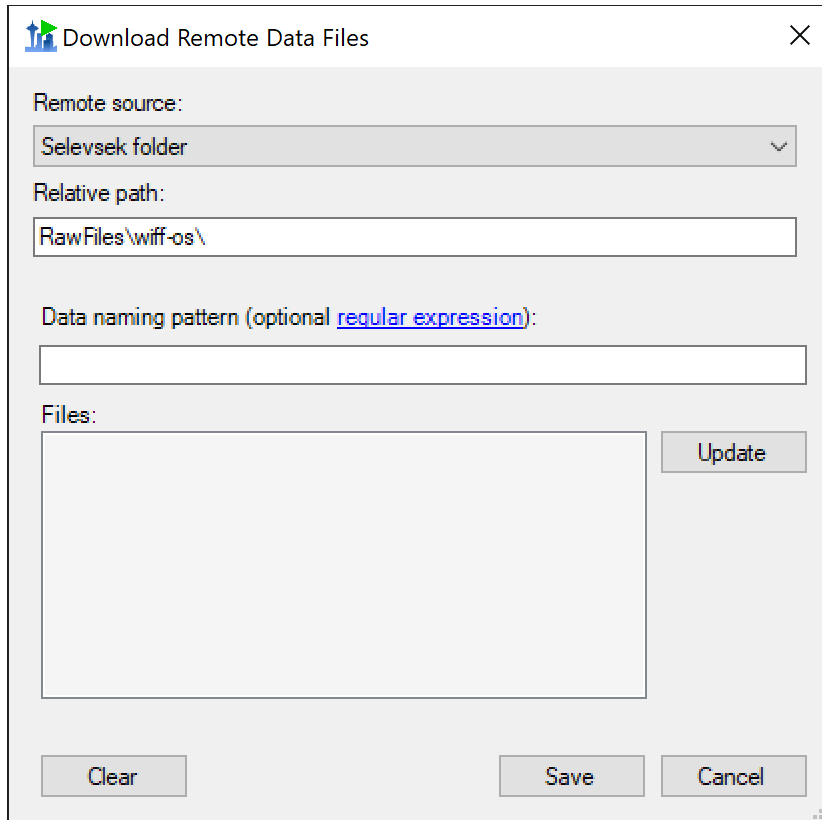
- Click the **Files** tab.
- In **Configuration name**, change “Selevsek-all (MSstats)” to “Selevsek-reps”

The files tab will now look like this:



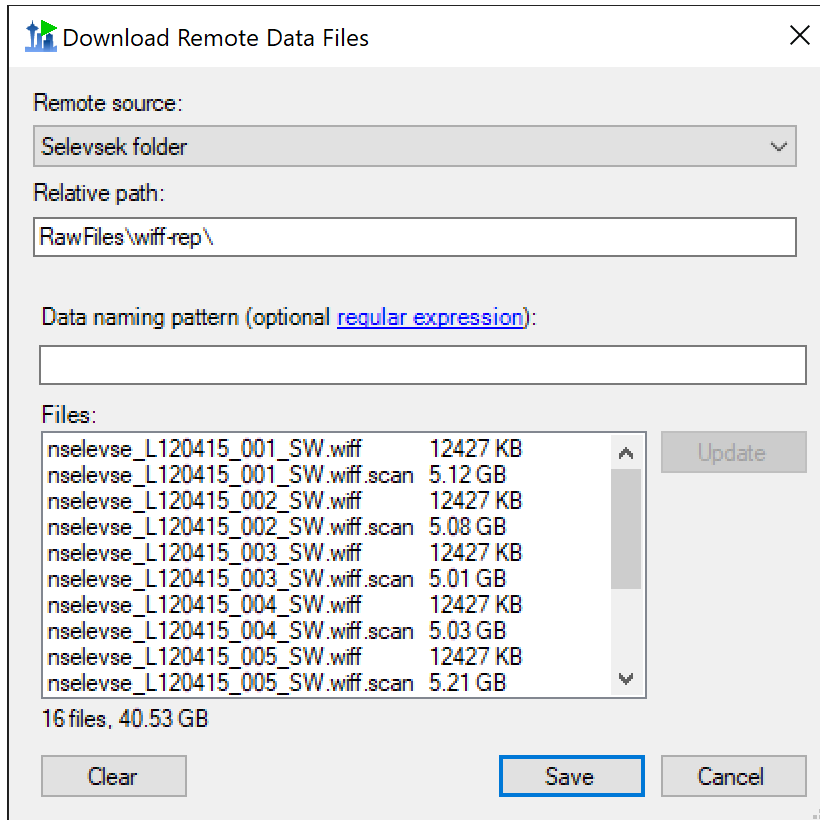
- Click the download button (📄) next to **Data directory**.

This brings up the **Download Remote Data Files** dialog:



- Select the **Relative path** textbox and change “wiff-os” to “wiff-rep”
- Click **Update**.

This retrieves all the file names and sizes from the URL. When it has finished loading, the **Download Remote Data Files** dialog should now look like this:



Double-check that Skyline Batch found 16 files with a combined size of 40.53 GB.

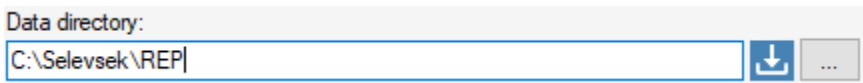
- Click **Save**.

The **Data directory** should look like this:



- In **Data directory**, replace “OS” with “REP”

The **Data directory** should now look like this:

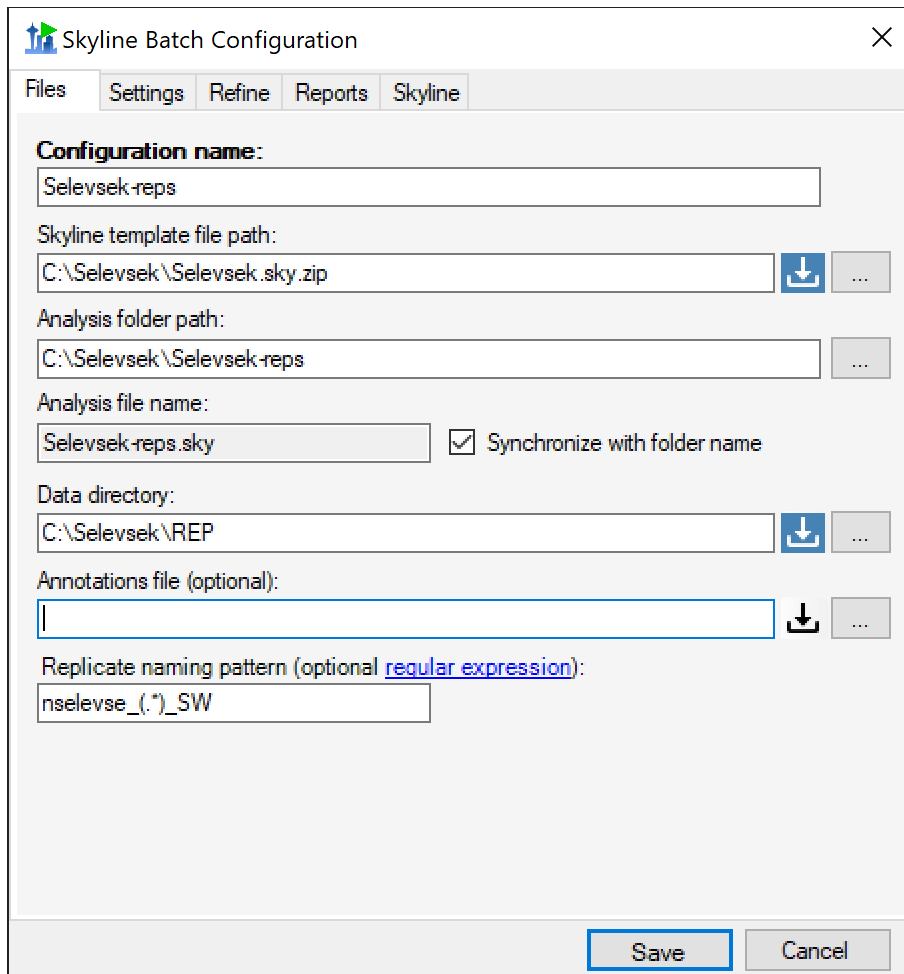


- In **Annotations file path**, delete the path

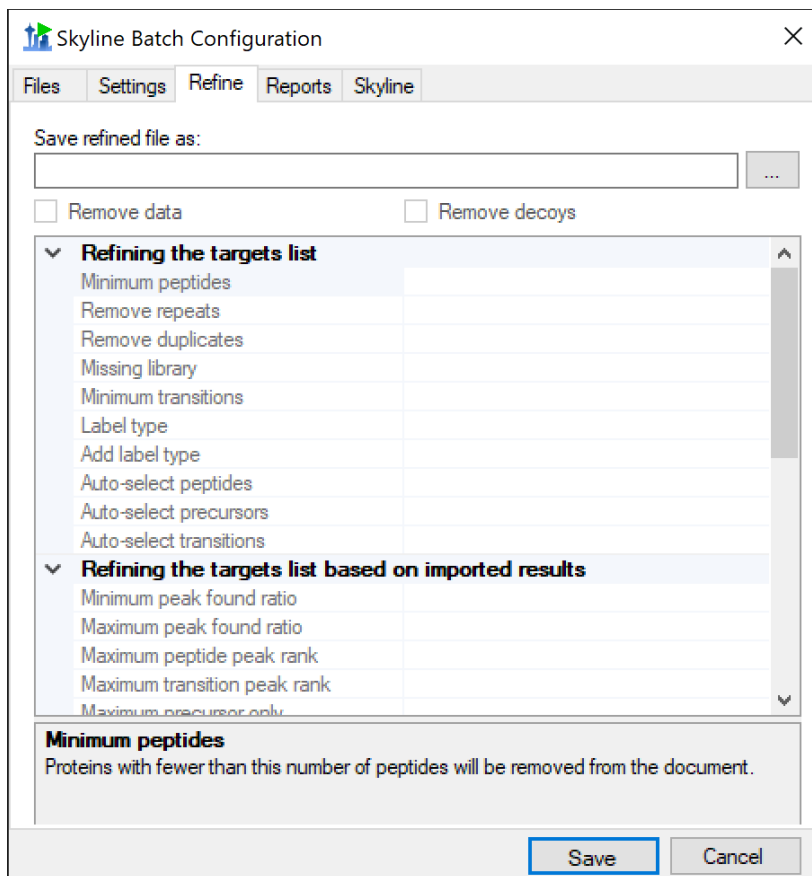
A dialog will appear warning you that if you change the annotations file it will not be downloaded from the server.

- Click **OK**.

The completed **Files** tab should look like this:

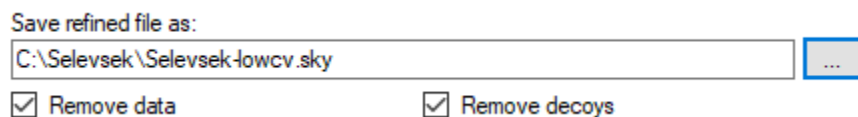


- Click on the **Refine** tab.



- Click the “...” button next to **Save refined file as**.
- Navigate to your “Selevsek” folder and name the file “Selevsek-lowcv.sky”

The top of the **Refine** tab should now look like this:



- Enter the following information in the data grid:

Minimum peptides: 1
Maximum precursor only: True
CV remove above cutoff: 20
CV global normalize: equalize_medians
Q value cutoff: 0.01
Minimum detections: 4

The **Refine** tab should now look like this (expanded to show complete list):

Skyline Batch Configuration

Files Settings **Refine** Reports Skyline

Save refined file as:
 ...

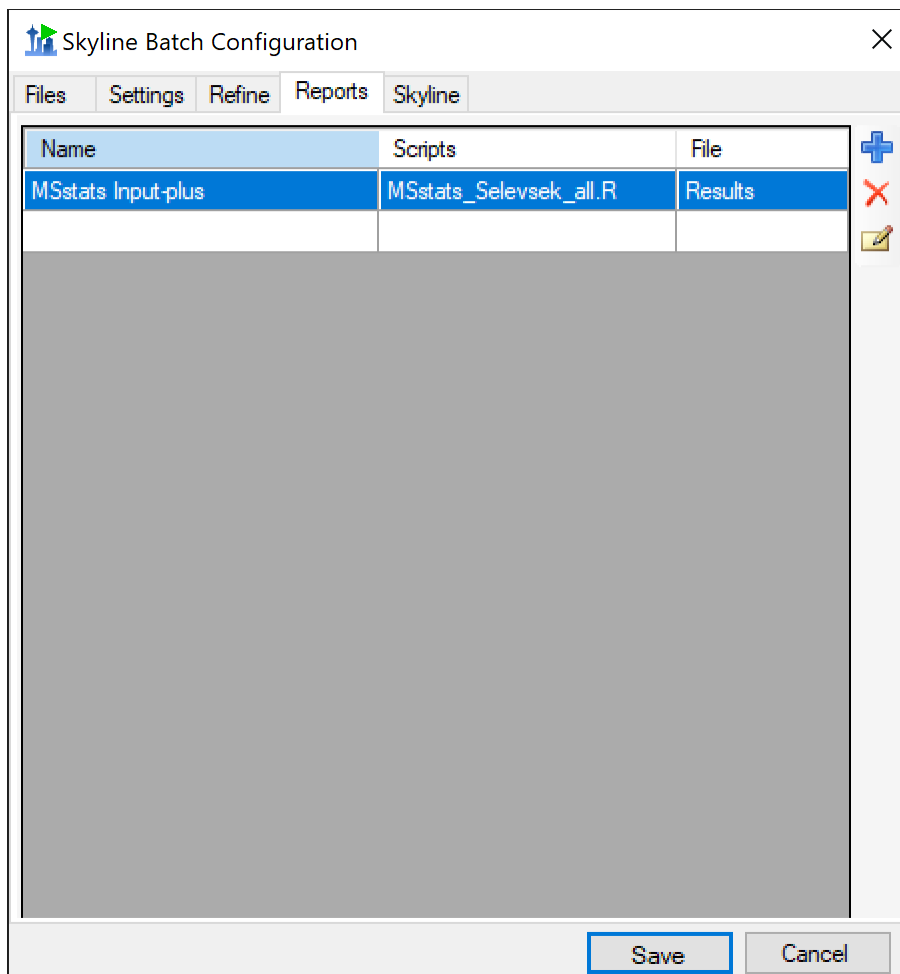
Remove data Remove decoys

Refining the targets list	
Minimum peptides	1
Remove repeats	
Remove duplicates	
Missing library	
Minimum transitions	
Label type	
Add label type	
Auto-select peptides	
Auto-select precursors	
Auto-select transitions	
Refining the targets list based on imported results	
Minimum peak found ratio	
Maximum peak found ratio	
Maximum peptide peak rank	
Maximum transition peak rank	
Maximum precursor only	True
Prefer larger products	
Missing results	
Minimum time correlation	
Minimum dot-product	
Minimum idotp	
Use best result	
CV remove above cutoff	20
CV global normalize	equalize_medians
CV reference normalize	
CV transitions	
CV transitions count	
CV ms level	
Q value cutoff	0.01
Minimum detections	4
Group comparison p value cutoff	
Group comparison fold change cutoff	
MS level	
Group comparison name	

Minimum detections
 All target precursors with less than the specified number of replicates with detection q value below the -refine-qvalue-cutoff are removed.

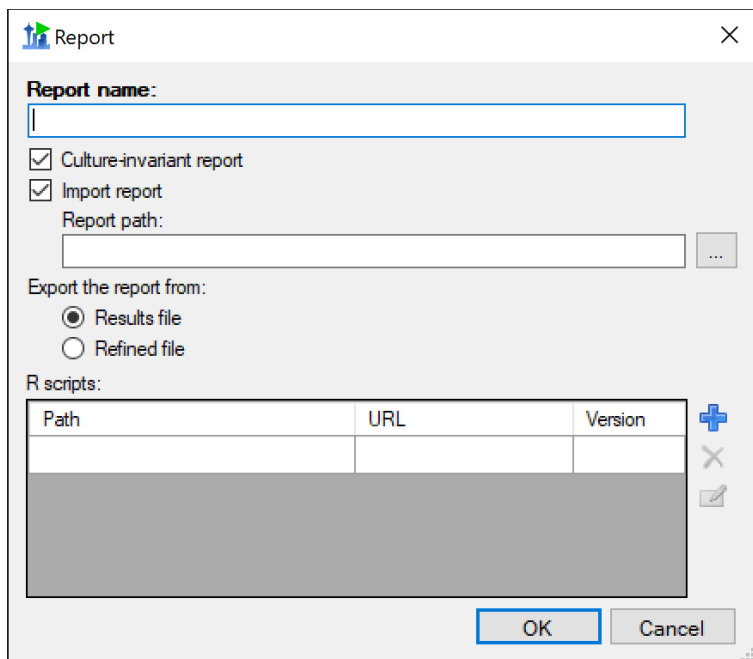
Save Cancel

- Click on the **Reports** tab.



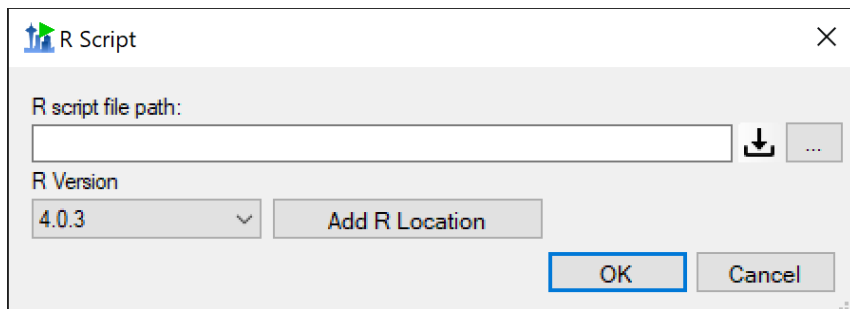
- Click on the MSstats Input-plus row to select it.
- Click the Delete (X) icon.
- Click the Add icon (+) to add a new report.

You will see the empty **Report** form:



- In **Report name** put “Precursor Peak Areas Scored”
- Uncheck the **Import report** checkbox
- Click the Add icon (+).

This brings up the **R Script** dialog:

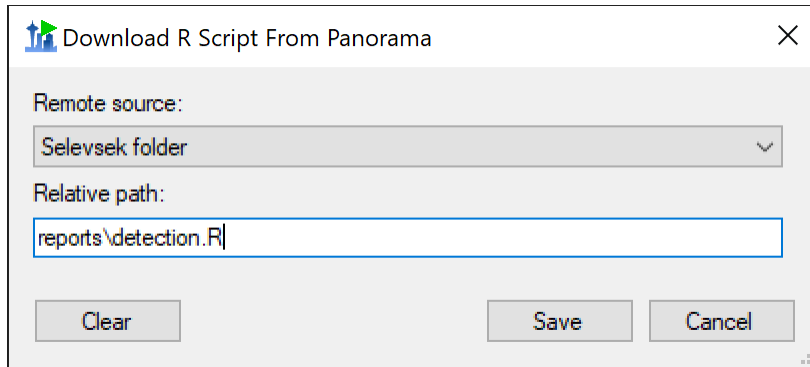


- Click the download button (↓) next to **R script file path**.

This opens the **Download R Script From Panorama** dialog:

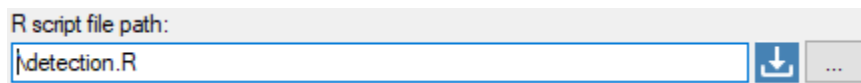
- Expand the **Remote source** drop-down list and select **Selevsek folder**
- Select the **Relative path** textbox and type “reports/detection.R”

The **Download R Script From Panorama** dialog should now look like this:



- Click **Save**.

The file name appears and the download icon next to **R script file path** turns blue and white, indicating that the file will be downloaded:



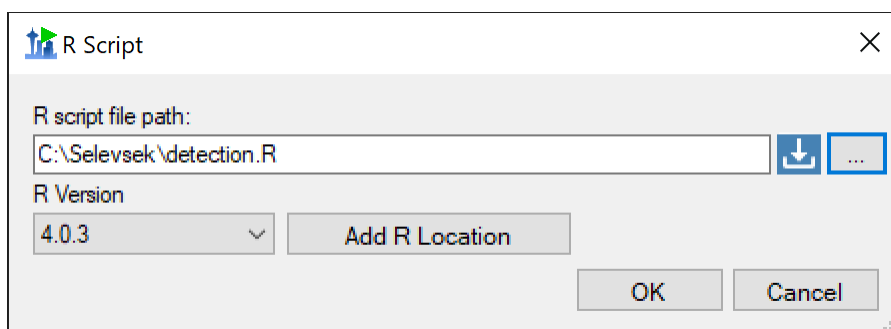
- Click the (...) button next to **R script file path**.
- Select your “Selevsek” folder in the folder explorer and click **OK**.

The **R script file path** should now look like this:



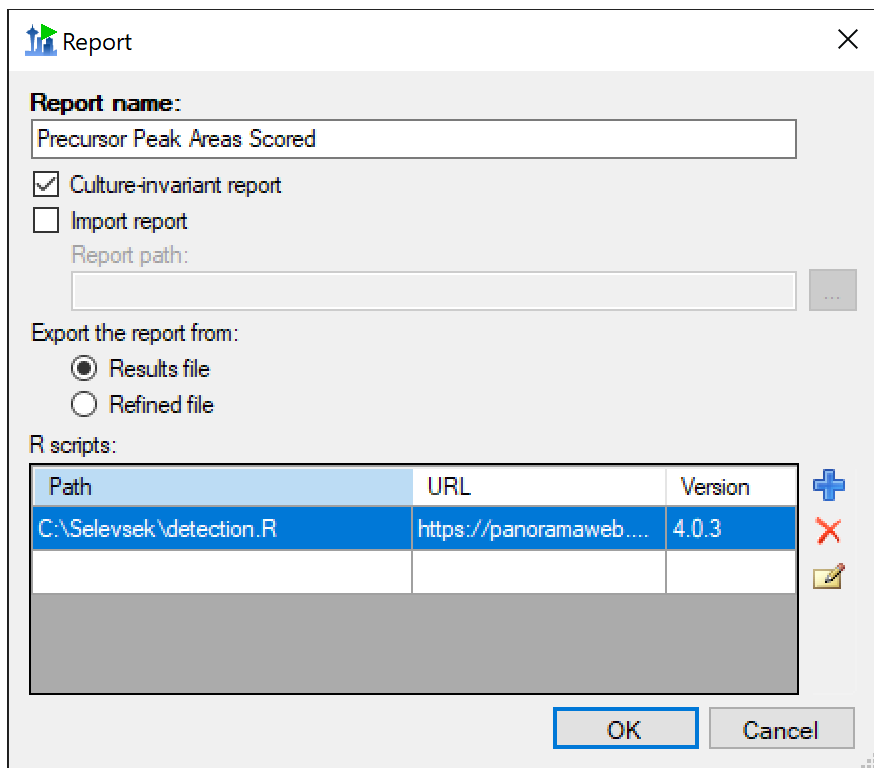
- Expand the **R version** drop-down list.
- Select **R-4.0.3**.

Your **R Script** dialog should now look like this:



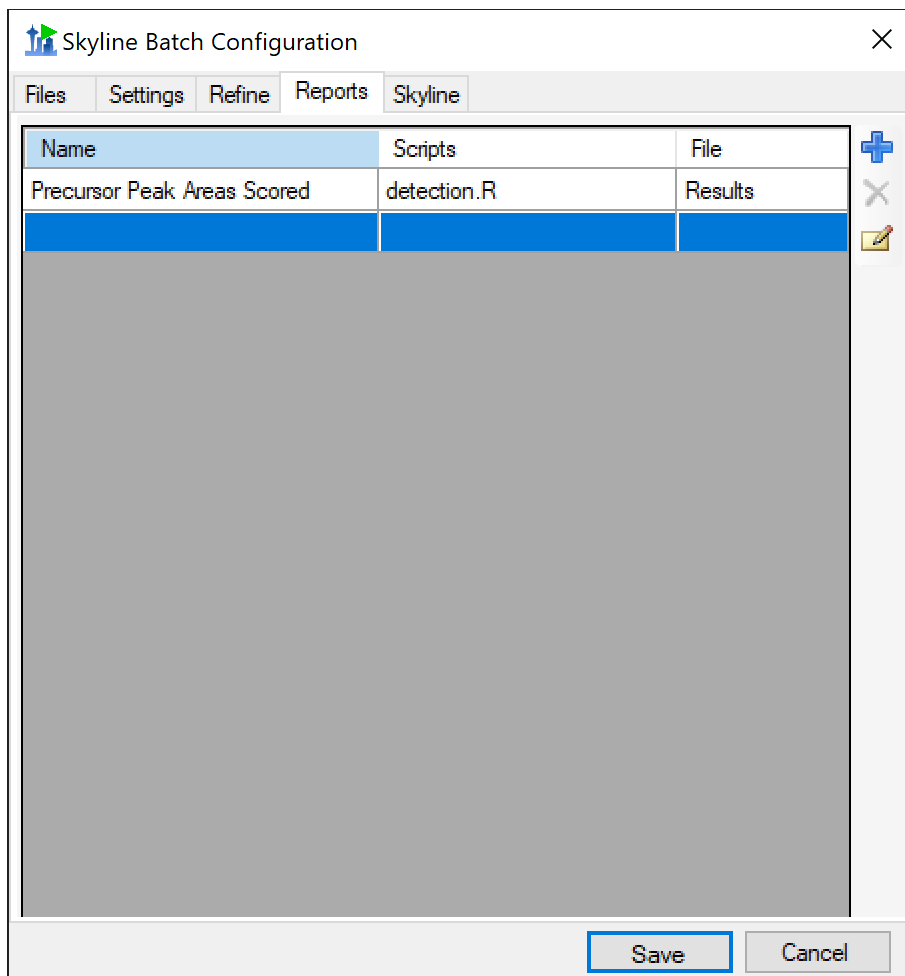
- Click **OK**.

The **Report** dialog should now look like this:



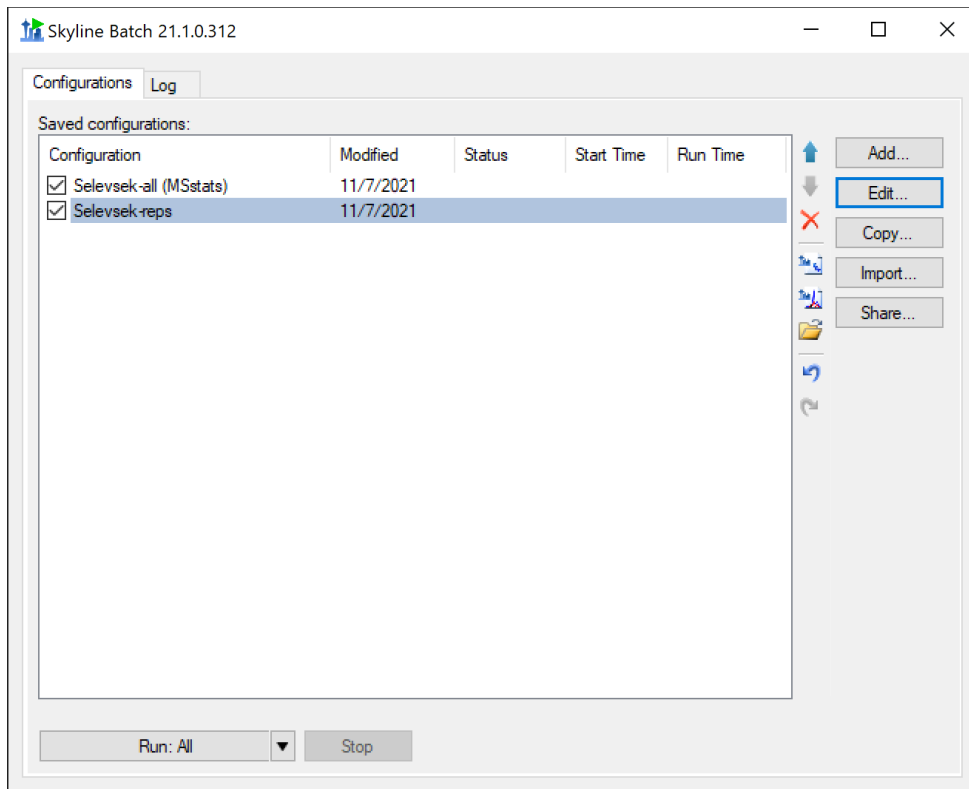
- Click **OK**.

The report will now appear in the list on the **Reports** tab:



- Click **Save** to save the Selevsek-reps configuration.

The main window now displays the two configurations we have created:

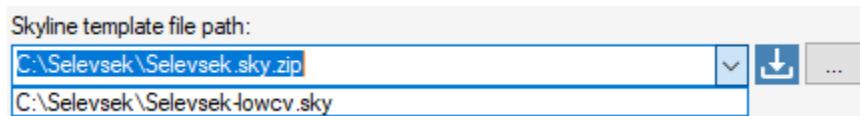


Creating the Selevsek-lowcv (MSstats) Configuration

- Click on “Selevsek-all (MSstats)” to select it.
- Click **Copy**.

This will bring up a copy of Selevsek-all (MSstats), which we will use to create Selevsek-lowcv (MSstats).

- Click the **Files** tab.
- In **Configuration name**, change “Selevsek-all (MSstats)” to “Selevsek-lowcv (MSstats)”
- In **Skyline template file path**, click the drop-down arrow



- Click on the “Selevsek-lowcv.sky” file path to select it.

A dialog will appear warning you that changing the template file path will prevent the file from being downloaded from Panorama.

- Click **OK**.

The **Skyline template file path** should now look like this:

Skyline template file path:
C:\Selevsek\Selevsek-lowcv.sky

- In the **Analysis folder path**, delete “ (MSstats)” from the end.

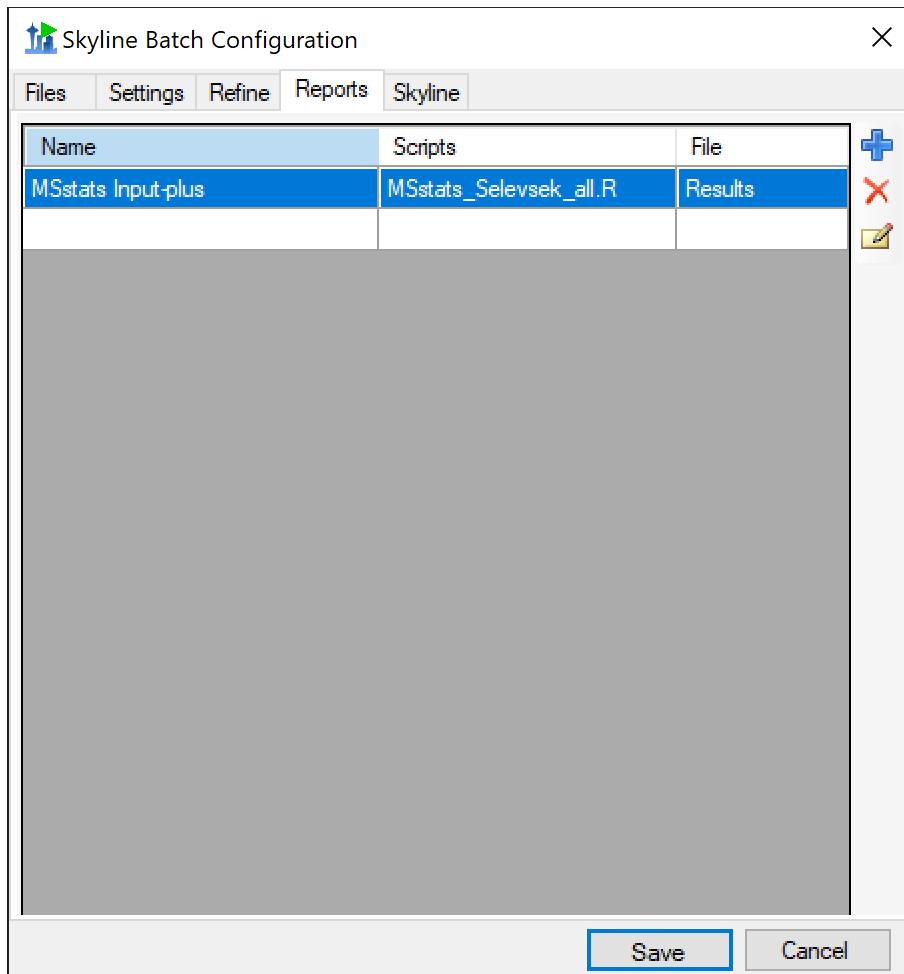
The completed **Files** tab for Selevsek-lowcv (MSstats) now looks like this:


The screenshot shows the 'Skyline Batch Configuration' dialog box with the 'Files' tab selected. The configuration details are as follows:

- Configuration name:** Selevsek-lowcv (MSstats)
- Skyline template file path:** C:\Selevsek\Selevsek-lowcv.sky
- Analysis folder path:** C:\Selevsek\Selevsek-lowcv|
- Analysis file name:** Selevsek-lowcv.sky Synchronize with folder name
- Data directory:** C:\Selevsek\OS
- Annotations file (optional):** C:\Selevsek\Selevsek-os-annotations.csv
- Replicate naming pattern (optional regular expression):** nselevse_(.*)_SW

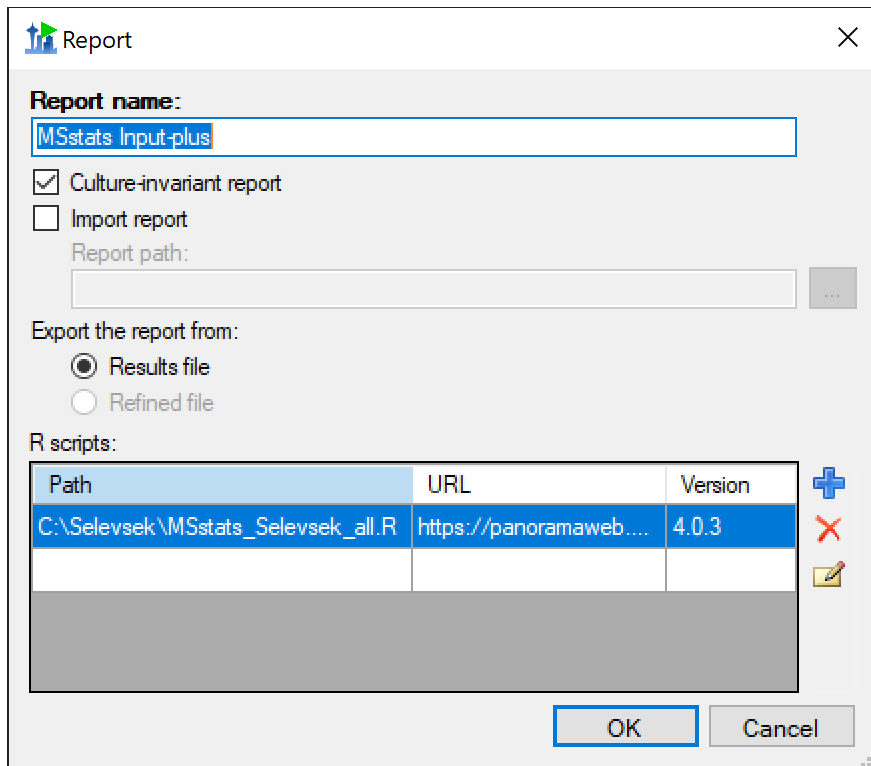
Buttons at the bottom: Save, Cancel

- Click on the **Reports** tab.



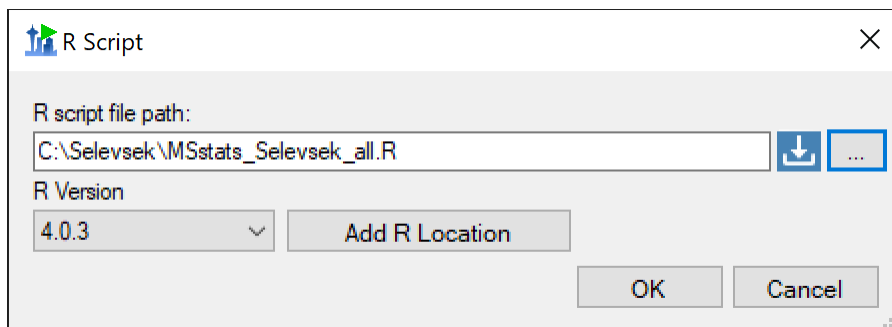
- Click on the MSstats Input-plus row to select it.
- Click the Edit () icon.

You will see the **Report** form:



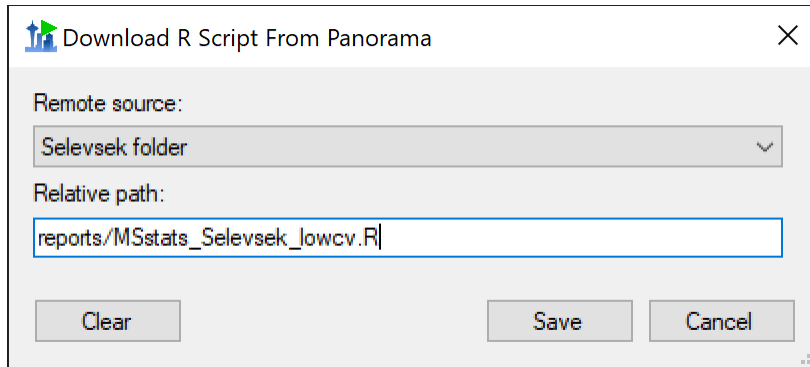
- Double-click on the MSstats_selevsek_all.R file path to edit it.

This brings up the **R Script** dialog:



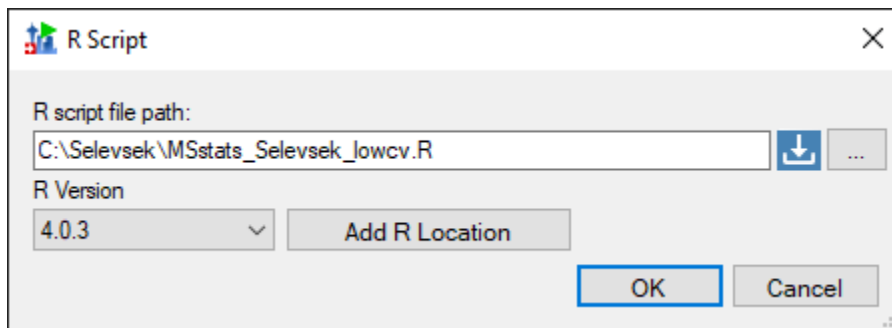
- Click the download button (📄) next to **R script file path**.
- In the **Relative path** replace “MSstats_Selevsek_all.R” with “MSstats_Selevsek_lowcv.R”

The **Download R Script From Panorama** dialog should look like this:



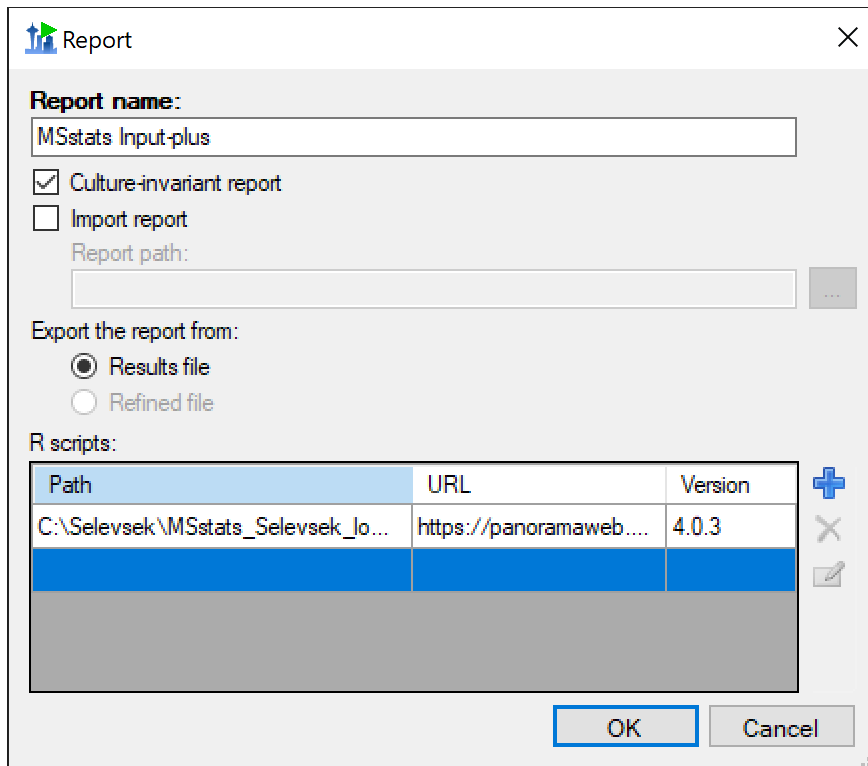
- Click **Save**.

The **R script file path** updates with the new file:



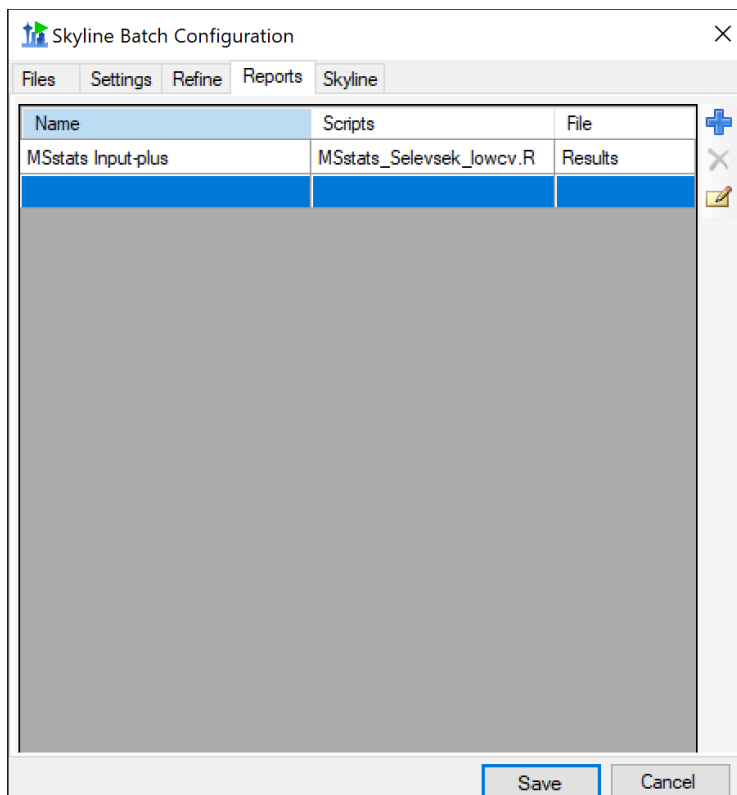
- Click **OK**.

Your **Report** form should now look like this:



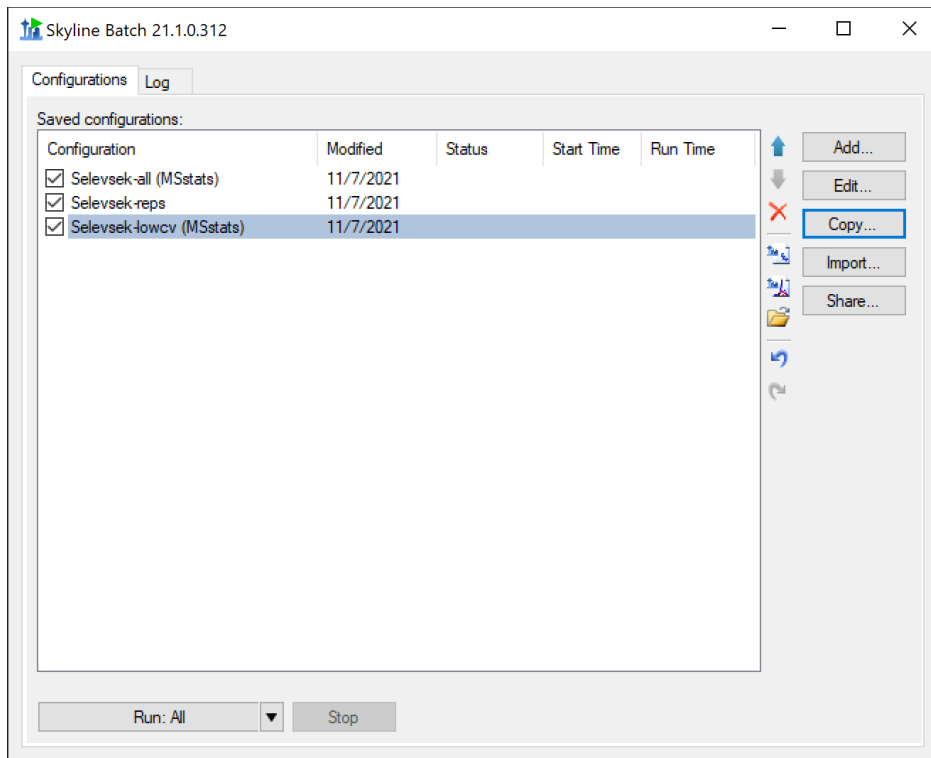
- Click **OK**.

The MSstats Input-plus report with the lowcv R script now appears in the list:



- Click **Save**.

There are now three configurations in the main window:



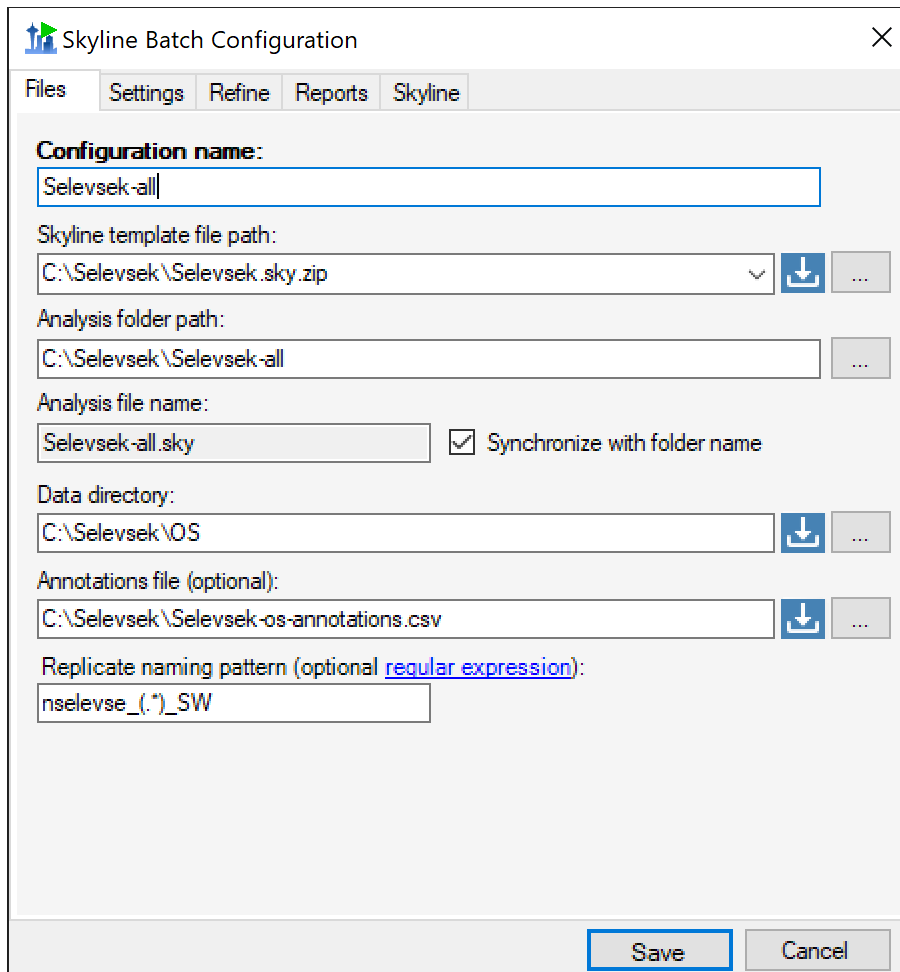
Creating the Selevsek-all and Selevsek-lowcv Configurations

Since MSstats can take a long time to run on this data, we want to create copies of the existing configurations without the MSstats R script. When these configurations are run, Skyline Batch will stop after the reports have been exported.

This section explains how to copy the “Selevsek-all (MSstats)” configuration and remove the R script to create “Selevsek-all”

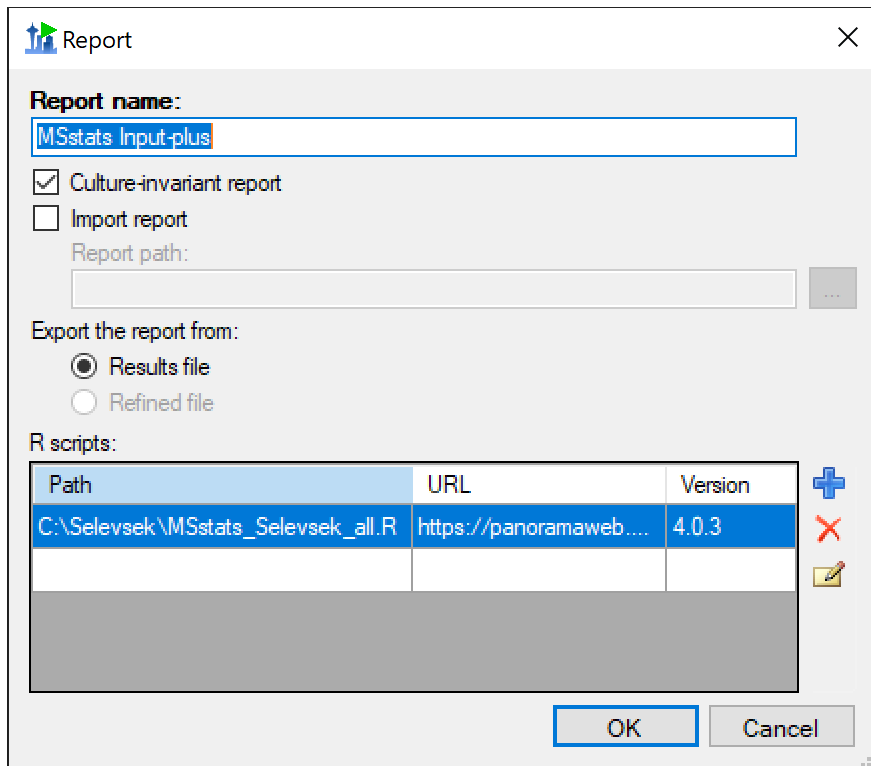
- In the main window, click on “Selevsek-all (MSstats)” to select it.
- Click **Copy** to bring up the **Skyline Batch Configuration** form.
- Click on the **Files** tab.
- In **Configuration name** delete “ (MSstats)”

The **Files** tab should now look like this:



- Click on the **Reports** tab.
- Click on the MSstats-Input plus row to select it.
- Click the edit (✎) icon.

You will see the **Report** form:



- Click on the MSstats_Selevsek_all.R file path.
- Click the delete (X) icon.
- Click **OK**.

You have now removed the MSstats R script from this configuration.

- Click **Save** to add Selevsek-all to the configuration list in the main window.

This section explains how to copy the “Selevsek-lowcv (MSstats)” configuration and remove the R script to create “Selevsek-lowcv”

- Click on “Selevsek-lowcv (MSstats)” to select it.
- Click **Copy** to bring up the **Skyline Batch Configuration** form.
- Click on the **Files** tab.
- In **Configuration name** delete “ (MSstats)”

The **Files** tab should now look like this:

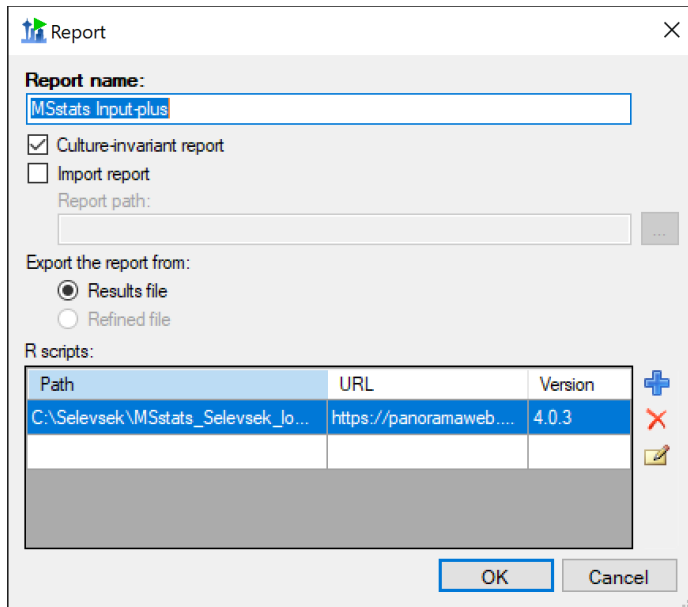
The screenshot shows the 'Skyline Batch Configuration' dialog box with the 'Files' tab selected. The configuration fields are as follows:

- Configuration name:** Selevsek-lowcv
- Skyline template file path:** C:\Selevsek\Selevsek-lowcv.sky
- Analysis folder path:** C:\Selevsek\Selevsek-lowcv
- Analysis file name:** Selevsek-lowcv.sky, with a checked box for 'Synchronize with folder name'.
- Data directory:** C:\Selevsek\OS
- Annotations file (optional):** C:\Selevsek\Selevsek-os-annotations.csv
- Replicate naming pattern (optional regular expression):** nselevse_(.*)_SW

At the bottom of the dialog, there are 'Save' and 'Cancel' buttons.

- Click on the **Reports** tab.
- Click on the MSstats-Input plus row to select it.
- Click the edit (✎) icon.

You will see the **Report** form:

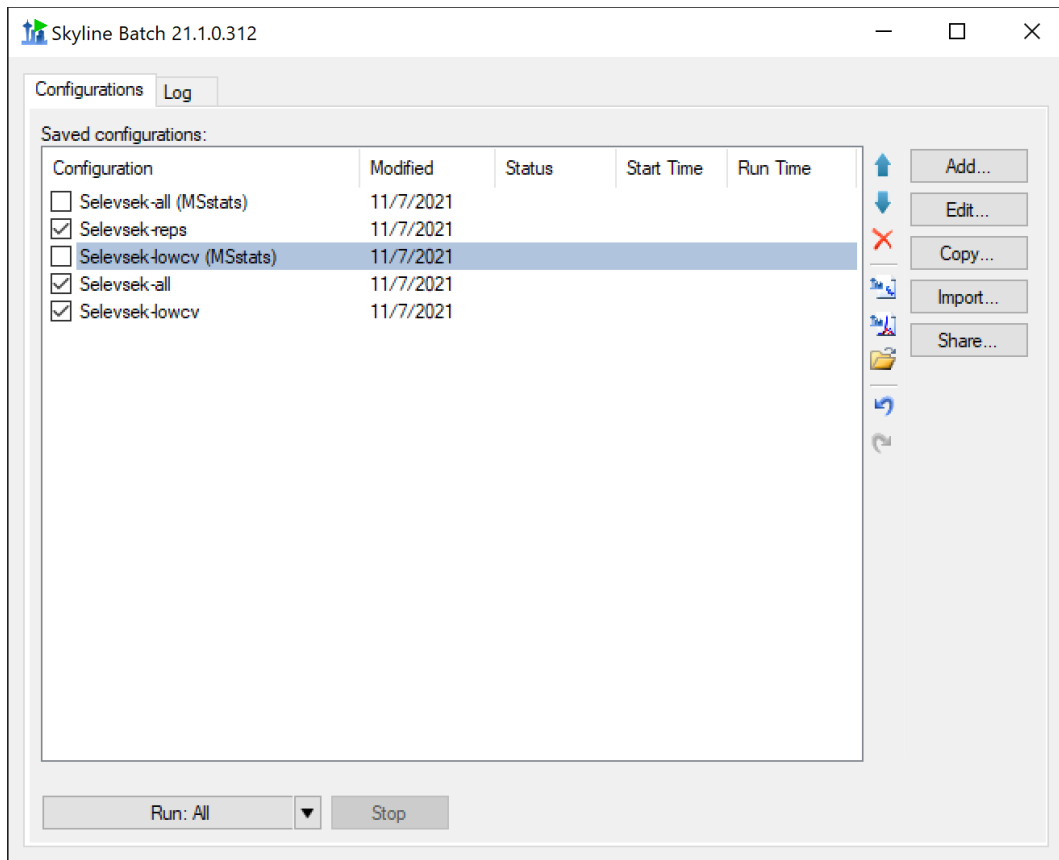


- Click on the MSstats_Selevsek_lowcv.R file path.
- Click the delete (X) icon.
- Click **OK**.

You have now removed the MSstats R script from this configuration.

- Click **Save** to add Selevsek-lowcv to the configuration list in the main window.
- Uncheck the MSstats configurations (Selevsek-all (MSstats) and Selevsek-lowcv (MSstats))

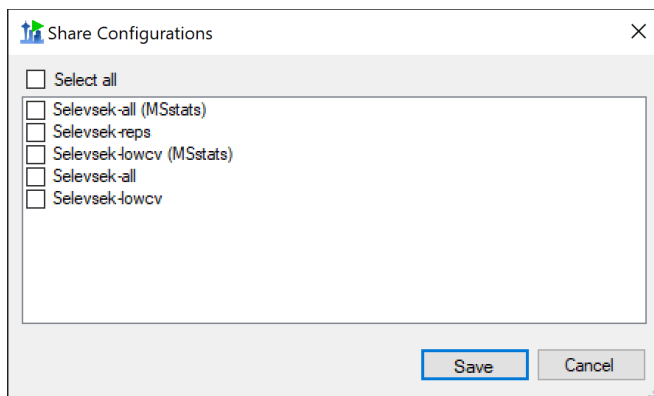
The complete list of configurations should look like this:



Sharing the Configurations

- Click **Share...**

This brings up the **Share Configurations** dialog:



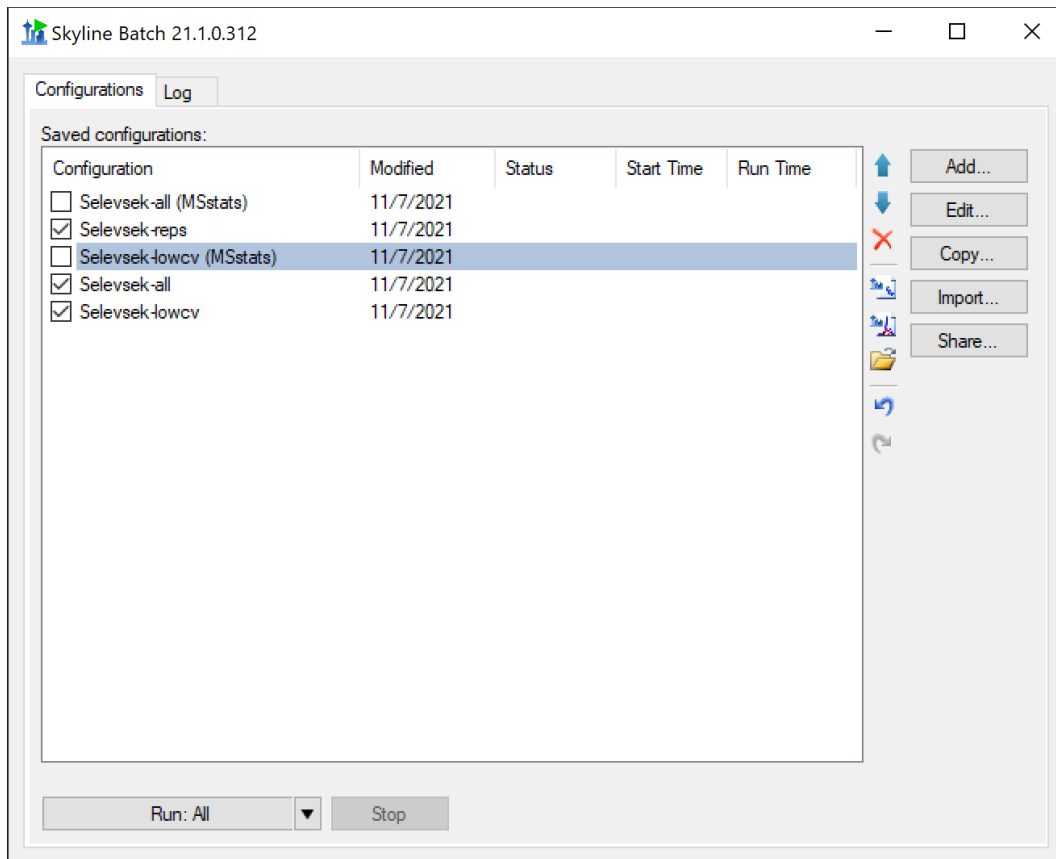
- Check the **Select all** checkbox.
- Click **Save**.

- Navigate to your “Selevsek” folder and save the file as “Selevsek.bcfg”

*Note: When sharing configurations, it is important to save the Skyline Batch configuration file close to where the files are stored. This allows Skyline Batch to do file path replacement when the configuration file is opened on a new computer.

This configuration file is now ready to be shared. Since the files are downloaded from Panorama, these configurations can be open and run from any computer with a current version of Skyline Batch.

Running the Configurations



- Click the **Run: All** button.

Skyline Batch brings you to the **Log** tab to show the output of running the configurations. It will download data, import it, and export reports for all three configurations.

When the configurations have finished running, it will display a **Completed** status in the main window. Wait until the Skyline Batch run has completed.

To run the MSstats processing, we can run the MSstats configurations from the “R scripts only” step.

- Uncheck all the configurations.

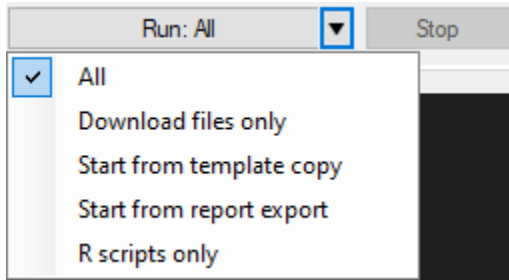
- Check the (MSstats) configurations.

The configurations should now look like this:

Configuration

- Selevsek-all (MSstats)
- Selevsek-reps
- Selevsek-lowcv (MSstats)
- Selevsek-all
- Selevsek-lowcv



















- Click the downward arrow next to **Run: All** to expand the run options






- Click **R scripts only**

This will run the MSstats scripts for Selevsek-all (MSstats) and Selevsek-lowcv (MSstats). Wait until the configurations have finished running.

Navigate to your “Selevsek” folder, which will contain the following files and folders:

 OS	Data for Selevsek-all and Selevsek-lowcv
 REP	Data for Selevsek-reps
 Selevsek-all	Analysis folders
 Selevsek-lowcv	
 Selevsek-reps	
 detection.R	
 MSstats_Selevsek_all.R	R script for Selevsek-all
 MSstats_Selevsek_lowcv.R	R script for Selevsek-lowcv
 Selevsek.bcfg	Skyline Batch configuration file containing five sharable configurations
 Selevsek.sky	Template document and associated files
 Selevsek.sky.view	
 Selevsek.sky.zip	
 Selevsek.skyl	
 Selevsek90.blib	
 Selevsek90.irtdb	Refined template document outputted from Selevsek-reps
 Selevsek-lowcv.sky	
 Selevsek-lowcv.skyl	Annotations file for Selevsek-all and Selevsek-lowcv
 Selevsek-os-annotations.csv	

You can view files in Skyline Batch by clicking on a configuration to select it, and using the following buttons:

-  Opens the Skyline template document
-  Opens the Skyline results document
-  Opens the analysis folder

If you want to learn more about Skyline Batch daily, visit the documentation at:

[SkylineBatchDocumentation.pdf](#)