# Configuring Skyline Batch for Bruderer Data

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

## Getting Started

Install Skyline

Install Skyline Batch

Create a "Bruderer" folder in a drive on your computer that has at least 70GB of free space. This folder will be used to store all the data and associated files for the configurations.

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

https://panoramaweb.org/dia-bruderer15.url

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

| Panorama Dashboard | Raw Data | Files |
|---|---|---|

- 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)

*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)

- 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 Bruderer 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:
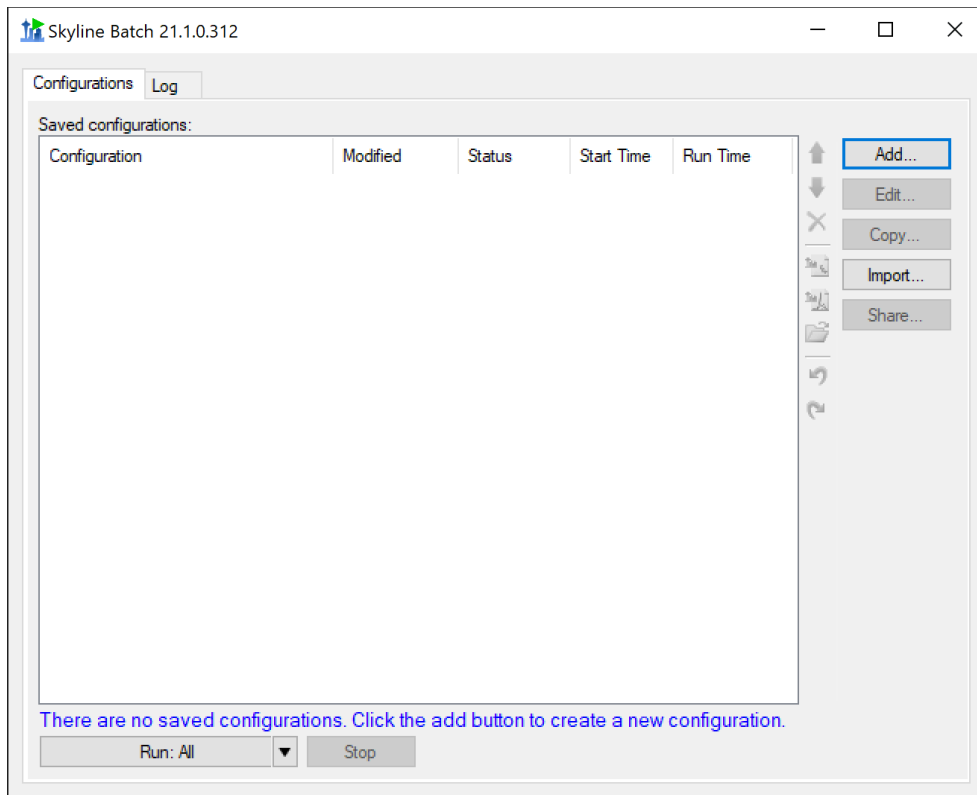


*Note: This tutorial requires a version of Skyline Batch after 21.1.0.312. If you have an earlier version number (seen in the top left corner of the main window) or no version number, you will need to install the newest version of Skyline Batch from Skyline Batch (washington.edu).

- Click the **Add** button.

This brings up the **Skyline Batch Configuration** dialog.
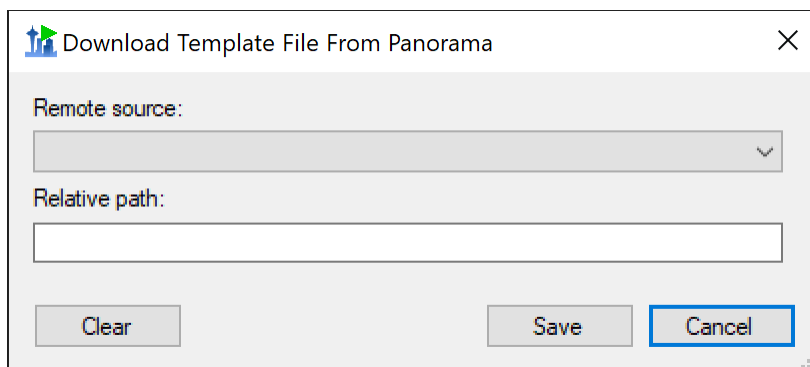
- Click on the **Files** tab.
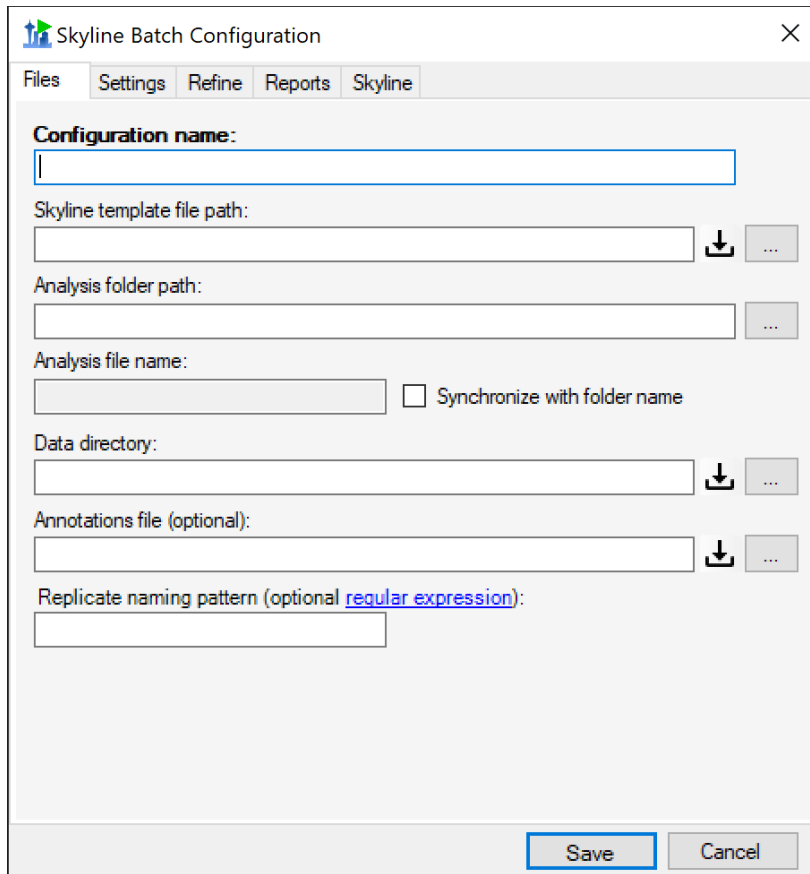
- Select the **Configuration name** textbox and type "Bruderer"

**Skyline template file path:**

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

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



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

This opens the **Remote source** dialog:

- Select the **Name** textbox and type "Bruderer folder"

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

https://panoramaweb.org/dia-bruderer15.url

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



This brings you to the list of files in the 2015-Bruderer folder.

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

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-Bruderer/@files/

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

Return to Skyline Batch.

- Paste (CTRL+V) into the **Folder URL** textbox in the remote source dialog.

The dialog should now look like this:
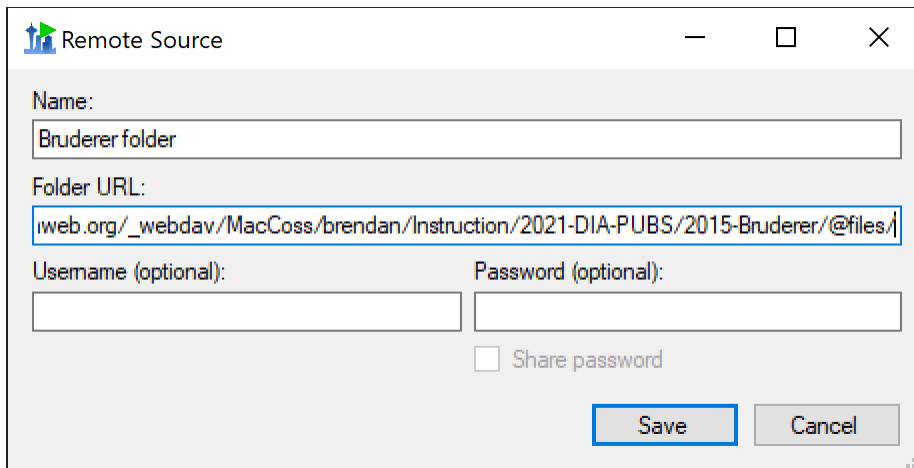


- Click **Save**.

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

- Select the **Relative path** textbox and type "Bruderer.sky.zip"

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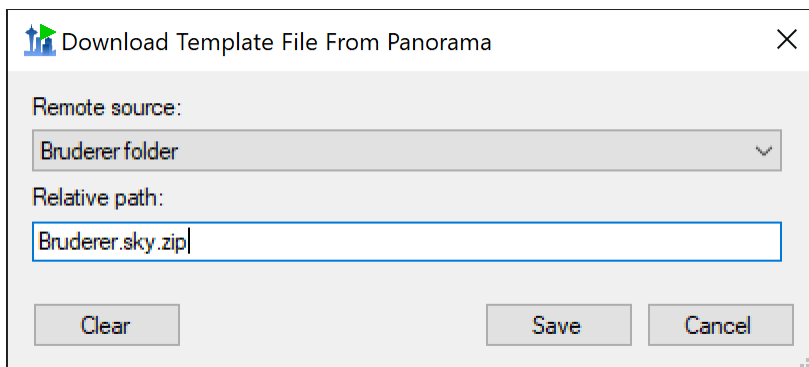


- Click **Save**.

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:

Skyline template file path:
\Bruderer.sky.zip

- Click the (**…**) button next to **Skyline template file path**.
- Select your "Bruderer" folder in the folder explorer and click **OK**.

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

Skyline template file path:
C:\Bruderer\Bruderer.sky.zip

**Analysis folder path:**

- Click the (**…**) button next to **Analysis folder path**.
- Select your "Bruderer" folder in the folder explorer and click **OK**.
- Add "\Bruderer_ppm15c_rt10" to the end of the **Analysis folder path**.

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

Analysis folder path:
C:\Bruderer\Bruderer_ppm15c_rt10
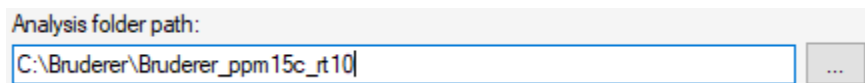
**Analysis file name:**

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

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

Analysis file name:
Bruderer.sky          ☑ Synchronize with folder name
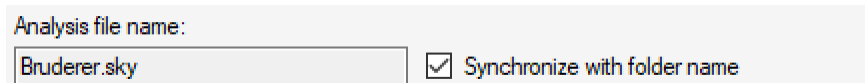
This will change the name of the generated Skyline file to Bruderer_ppm15c_rt10.sky, which matches the processed file name on Panorama.

**Data directory:**

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

This brings up the **Download Remote Data Files** dialog:
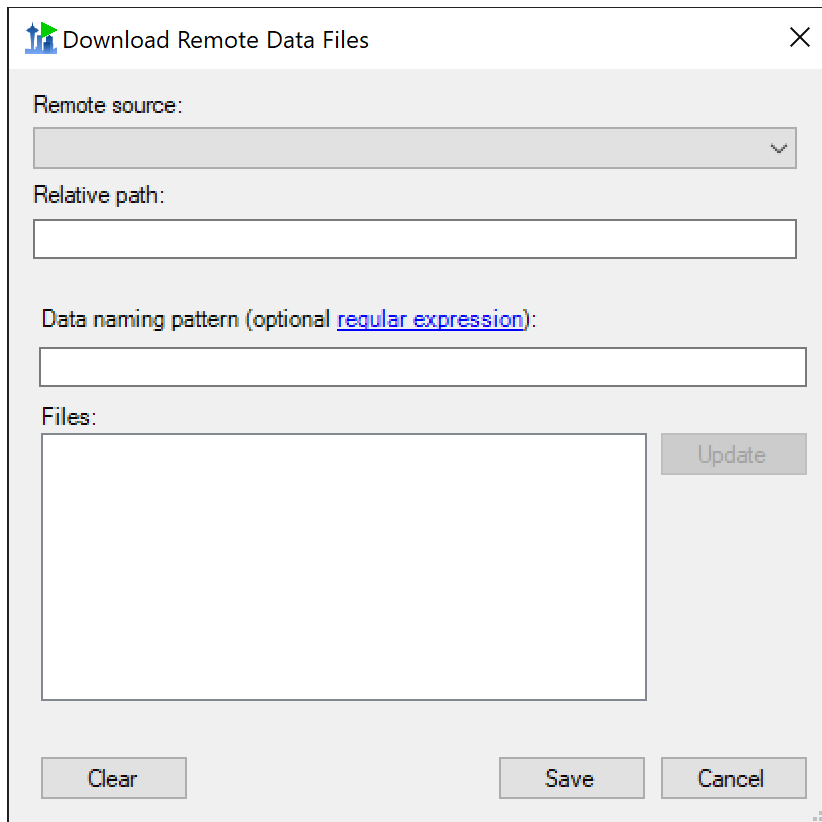
- Expand the **Remote source** drop-down list and select **Bruderer folder**
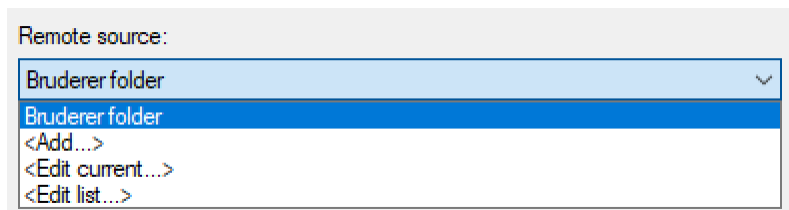


- Select the **Relative path** textbox and type "RawFiles\"
- Click **Update**.

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:
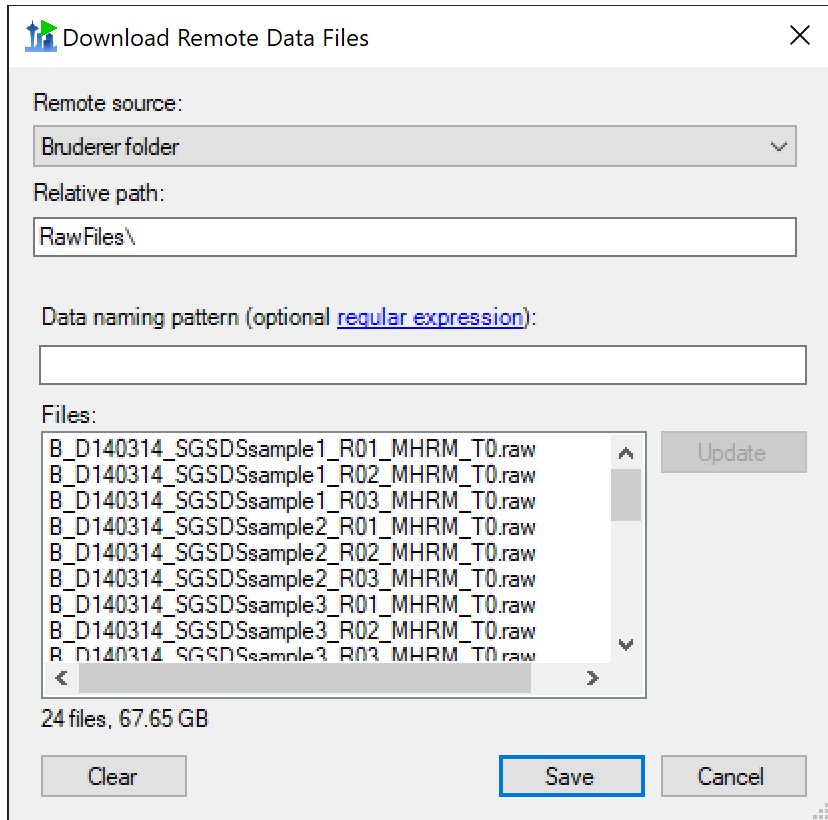
Double-check that Skyline Batch found 24 files with a combined size of 67.65 GB.

- Click **Save**.

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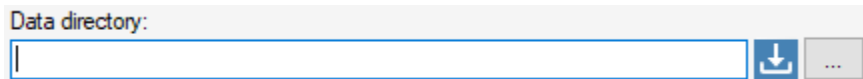


- Click the (**…**) button next to **Data directory**.
- Select your "Bruderer" folder in the folder explorer and click **OK**.
- Add "\raw" to the end of the **Data directory**.

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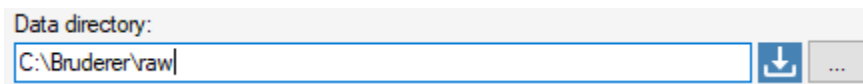


**Annotations file:**

- 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 **Bruderer folder**

- Select the **Relative path** textbox and type "reports\Bruderer-annotations.csv"

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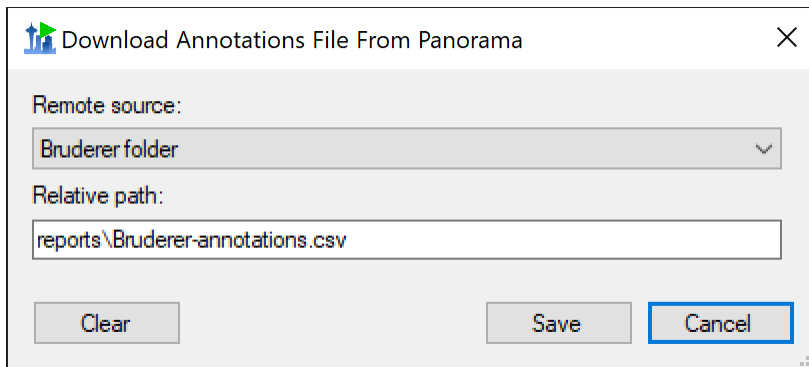


- Click **Save**.

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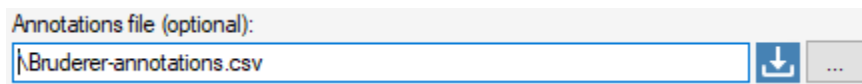


- Click the (**…**) button next to **Annotations file**.
- Select your "Bruderer" folder in the folder explorer and click **OK**.

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



**Replicate naming pattern:**

- In the **Replicate naming pattern** textbox, paste "(sample._R0.)"

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



The completed **Files** tab should look like this:
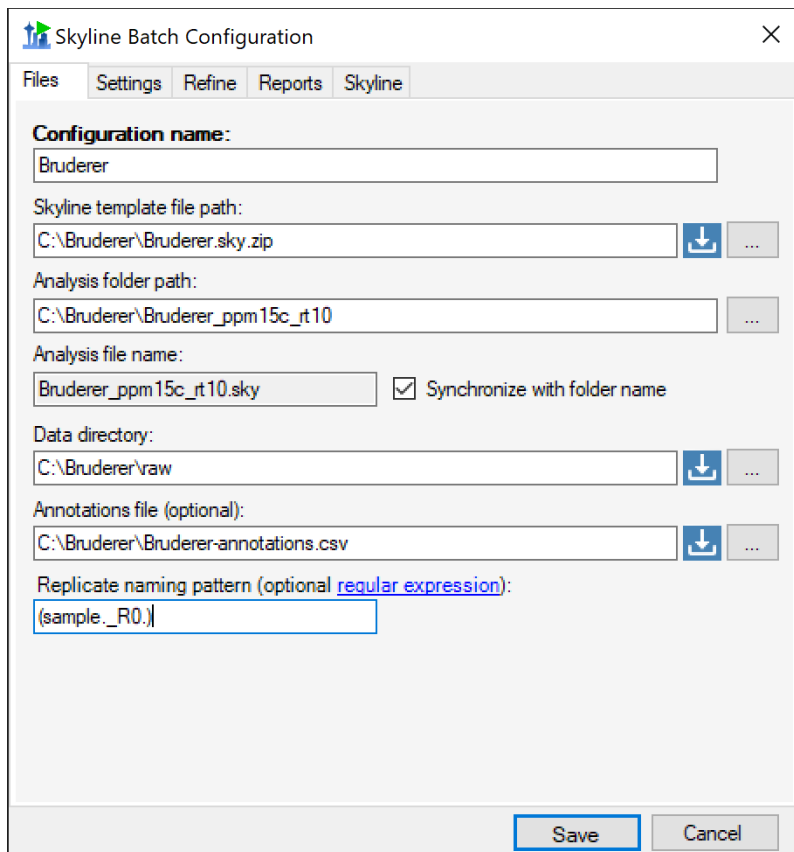
- Click on the **Settings** tab
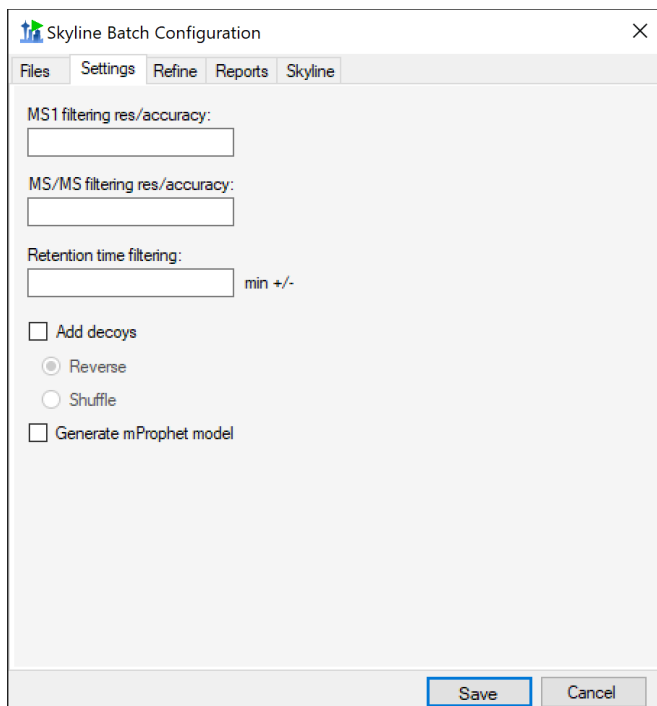


- Check the **Add decoys** checkbox.

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

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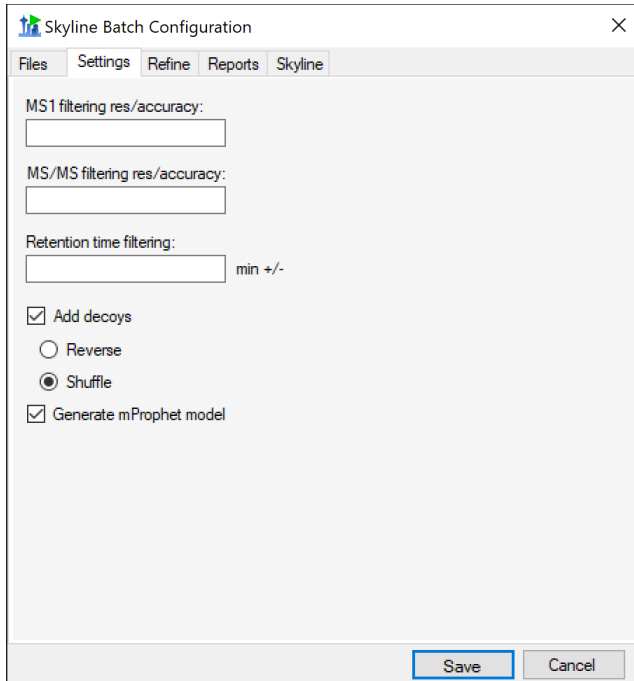


- Click on the **Reports** tab.
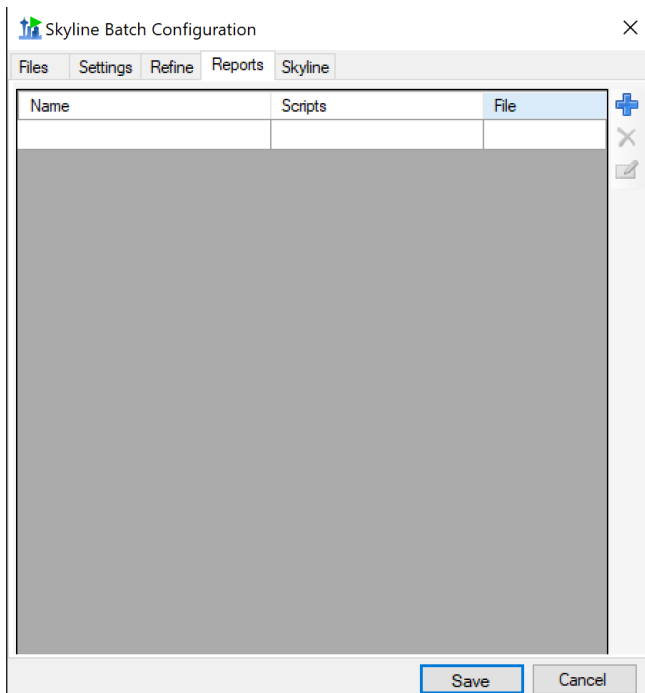


- Click the Add icon (✚) to add a new report.
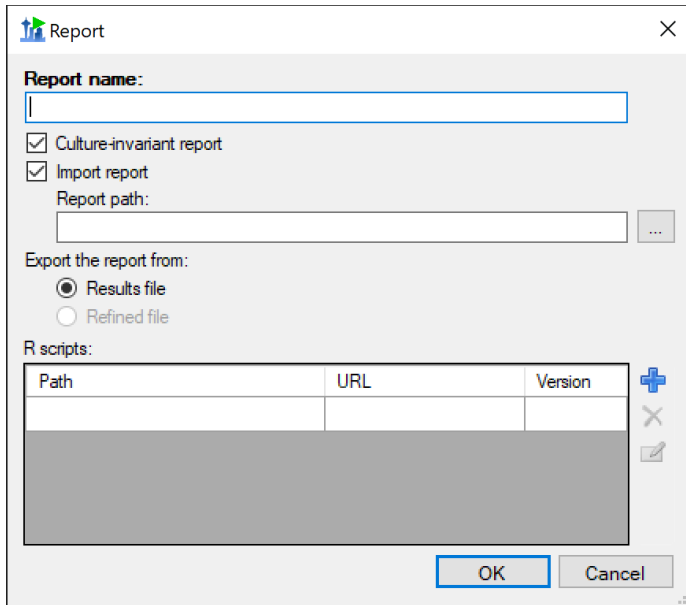
- In **Report name** put "MSstats Input-plus"
- Uncheck the **Import report** checkbox (this report already exists in our Skyline template document, so it does not need to be imported)

The Report dialog should now look like this:
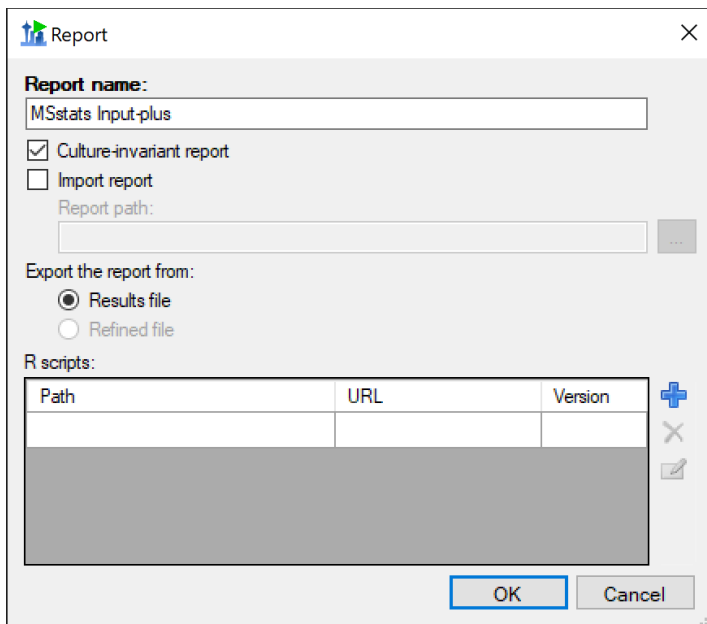


- Click **OK**.

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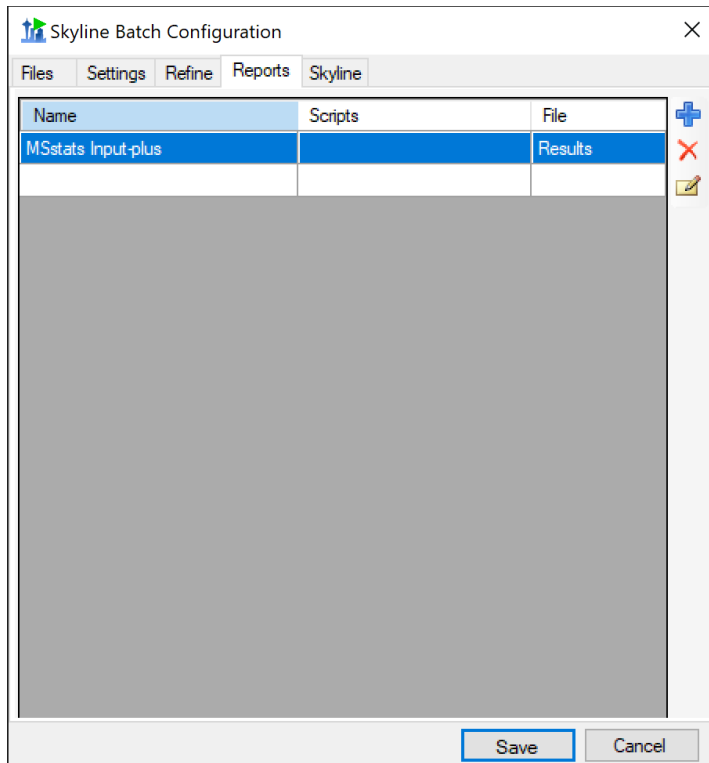
- Click the **Skyline** tab
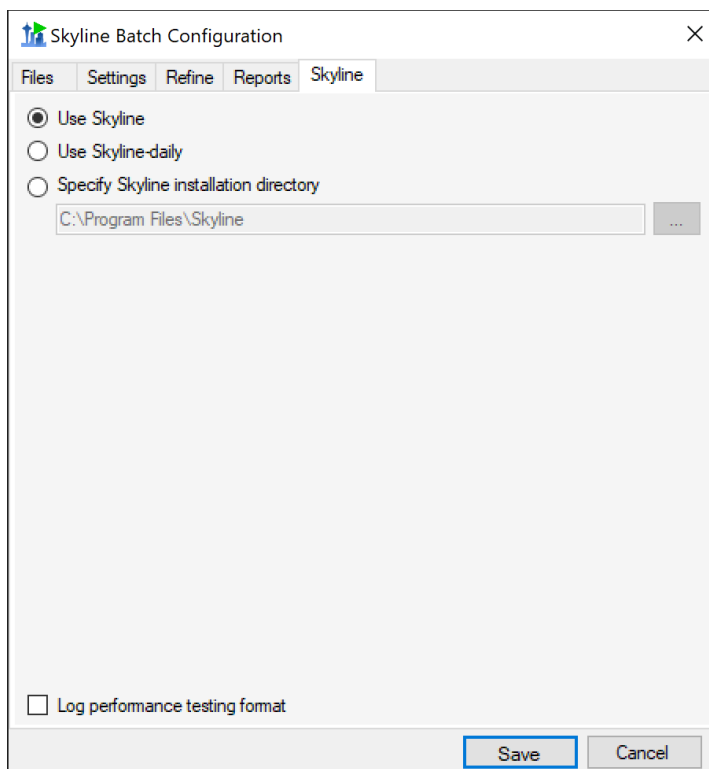


- Select the **Use Skyline** radio button if it is not already selected.
- Click **Save**.

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:
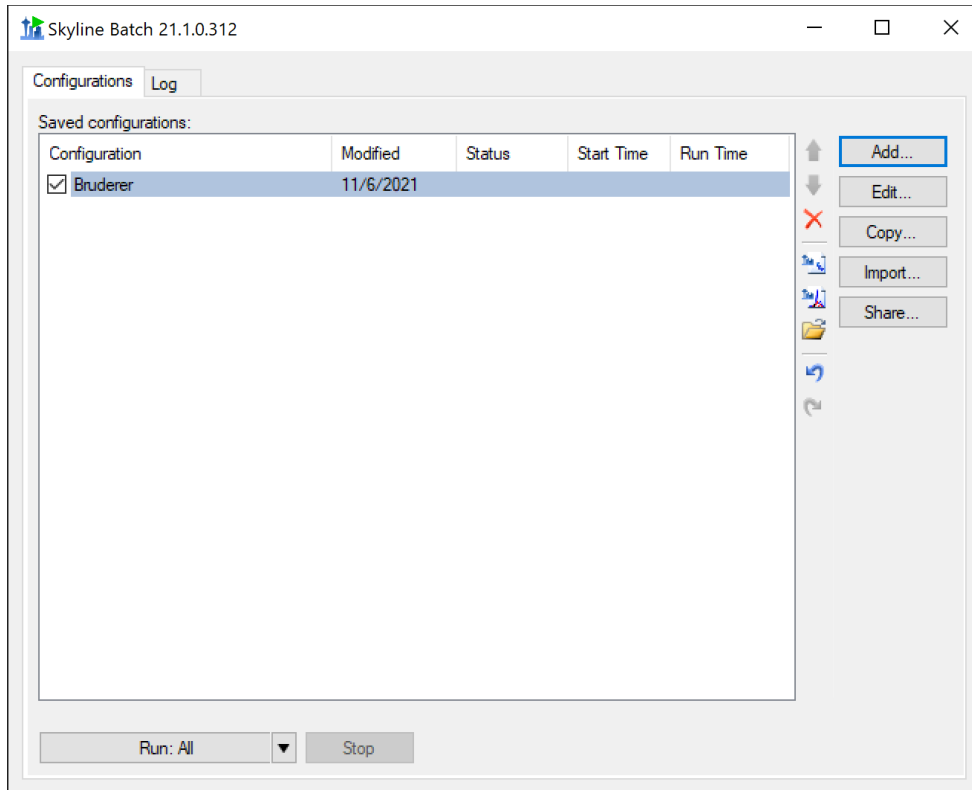


## Creating the Bruderer (MSstats) Configuration

- Click on "Bruderer" to select it.
- Click **Copy**.

This will bring up a copy of Bruderer, which we will use to create Bruderer (MSstats).

- Click the **Files** tab.
- In **Configuration name**, change "Bruderer" to "Bruderer (MSstats)"
- In **Analysis folder path** replace "Bruderer (MSstats)" with "Bruderer_ppm15c_rt10"

The files tab will now look like this:
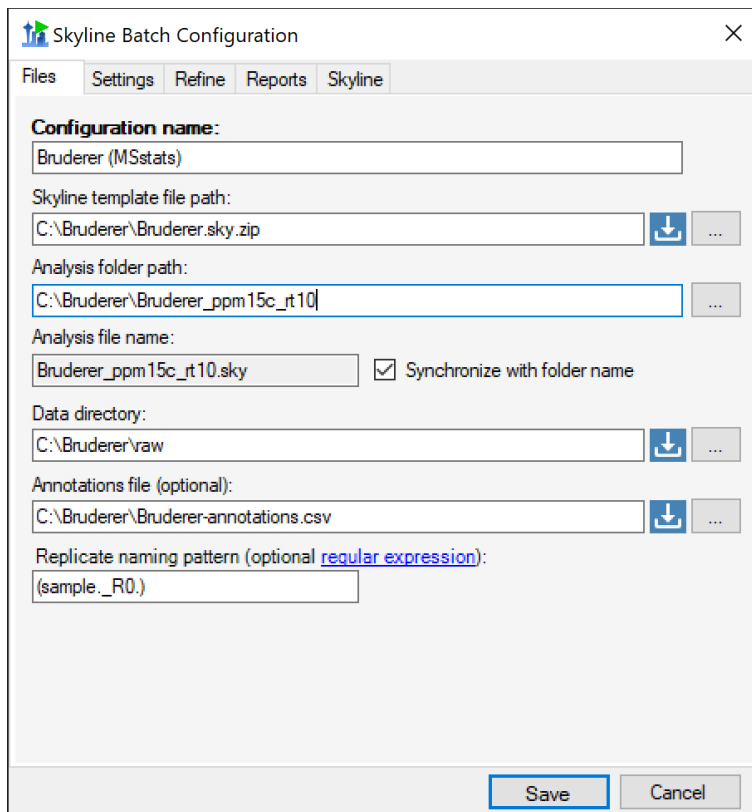
- Click on the **Reports** tab.
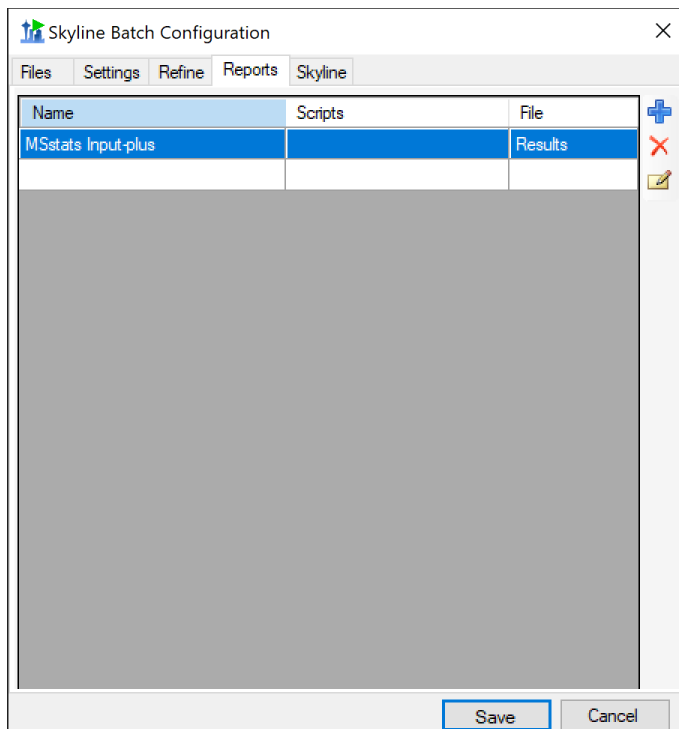


- Click on the MSstats Input-plus row to select it.

- Click the Edit ( ) icon.

You will see the **Report** form:



- Click 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 **Bruderer folder**
- Select the **Relative path** textbox and type "reports/MSstats_Bruderer.R"

The **Download R Script From Panorama** dialog should now look like this:
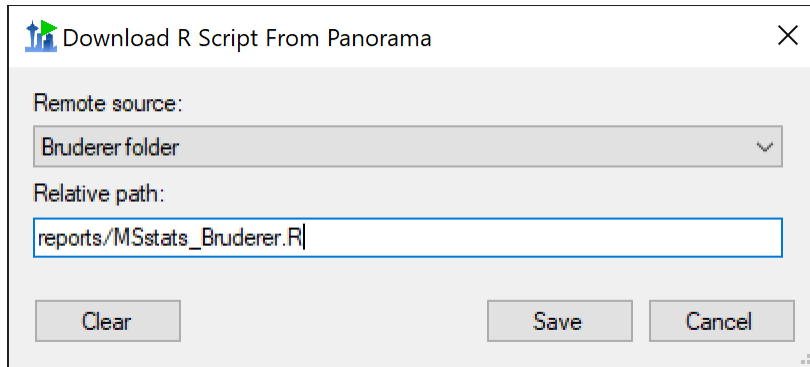
- Click **Save**.

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:
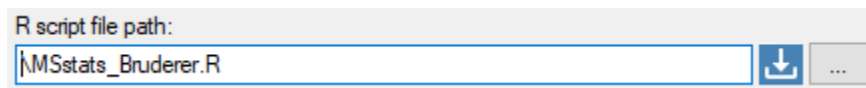


- Click the (**…**) button next to **R script file path**.
- Select your "Bruderer" folder in the folder explorer and click **OK**.

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:
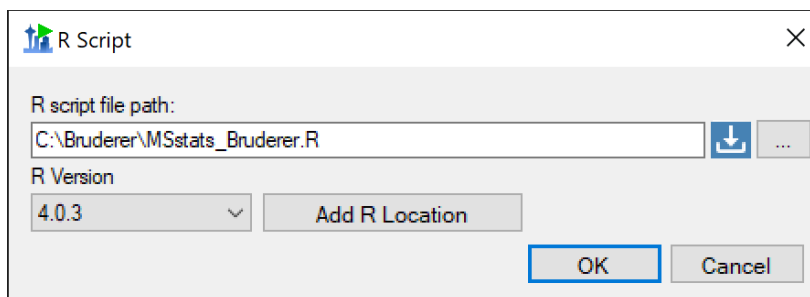


- Click **OK**.

The R script will appear in the **Report** dialog:
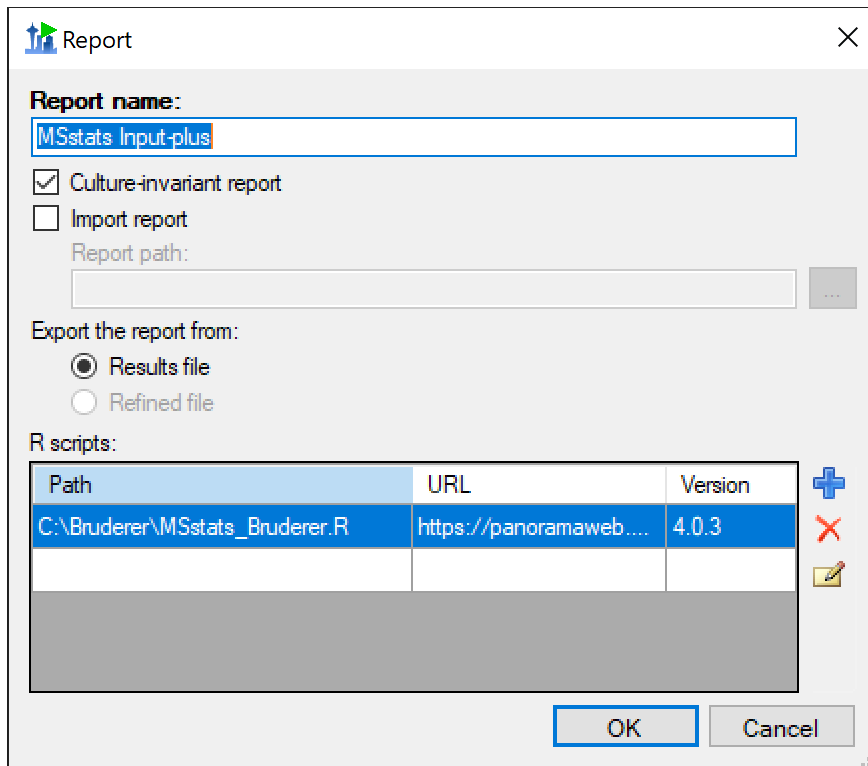
- Click **OK**.

The **Reports** tab will now look like this:
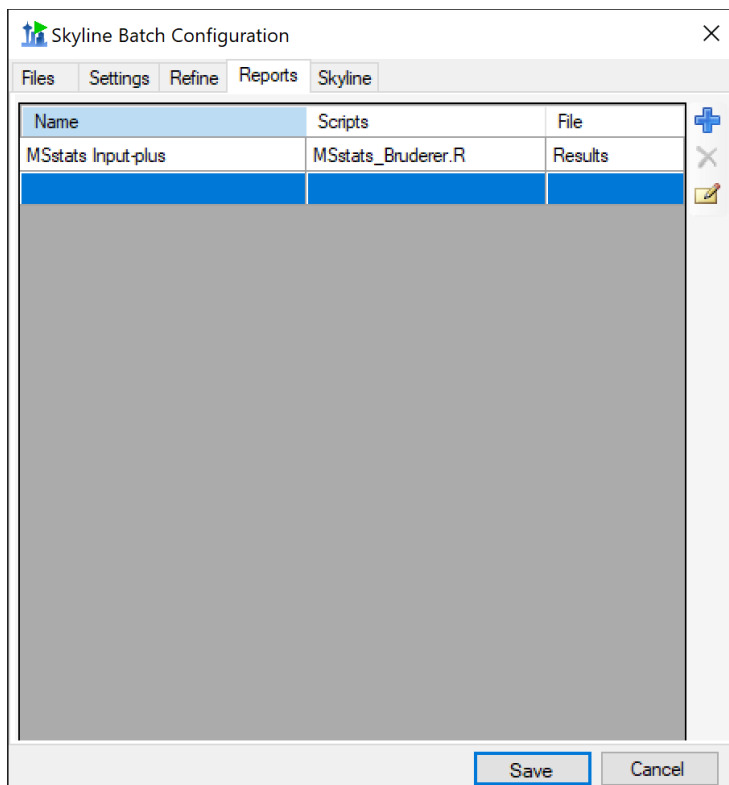
- Click **Save** to save the Bruderer (MSstats) configuration.
- Uncheck the Bruderer (MSstats) configuration

Checked configurations will be run when the **Run: All** button (or any of the other run options) are clicked. Since Bruderer (MSstats) is a copy of the Bruderer configuration with one added R script, it does not make sense to run them both as they will execute much of the same analysis. This is explained more in the **Running the configurations** section.

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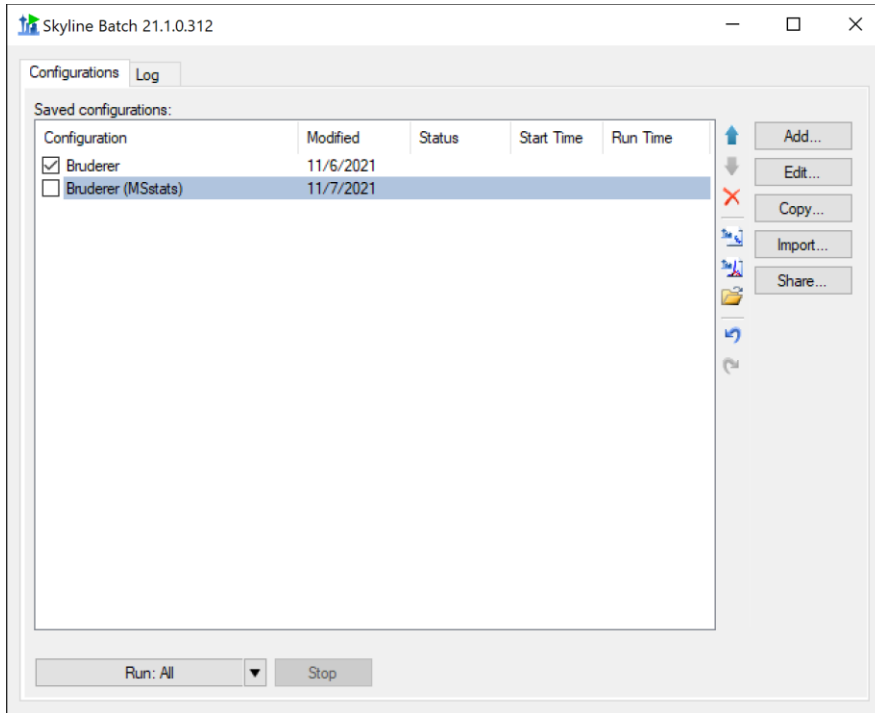


## Sharing the Configurations

- Click **Share…**

This brings up the **Share Configurations** dialog:
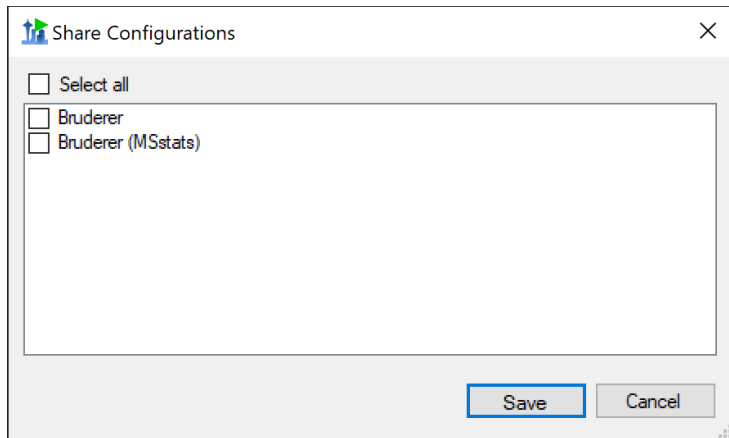
- Check the **Select all** checkbox.
- Click **Save**.
- Navigate to your "Bruderer" folder and save the file as "Bruderer.bcfg"

*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 opened and run from any computer with a current version of Skyline Batch.

## Running the Configurations

- Uncheck the Bruderer (MSstats) configuration
- 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 configuration has 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 configuration from the "R scripts only" step.

- Uncheck the Bruderer configuration.
- Check the Bruderer (MSstats) configurations.
- Click the downward arrow next to **Run: All** to expand the run options
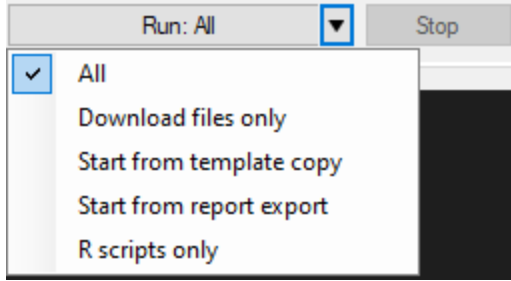
- Click **R scripts only**

This will run the MSstats script for Bruderer (MSstats). Wait until the configuration has finished running.

Navigate to your "Bruderer" folder, which will contain the following files and folders:



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