

Installation of necessary software:

To run the presented data analysis pipeline using the R-script as well as Skyline Batch (SB), you will need to install Skyline (version 20.2 or later), SB as well as R.

You can get a Skyline installation of your choice here:

https://skyline.ms/wiki/home/software/Skyline/page.view?name=install-64-disconnected_23_1

You can install SB from here:

<https://skyline.ms/wiki/home/software/Skyline/page.view?name=skyline-batch>

A current version of R can be obtained here:

<https://cran.r-project.org/>

Furthermore, we recommend to install RStudio after you have installed R, as it allows for an easy check whether all packages required by the provided R-script are also installed on your system (just open the R-script in RStudio before using it with Skyline Batch and RStudio will guide you through the installation process of necessary missing packages, if required):

<https://posit.co/download/rstudio-desktop/>

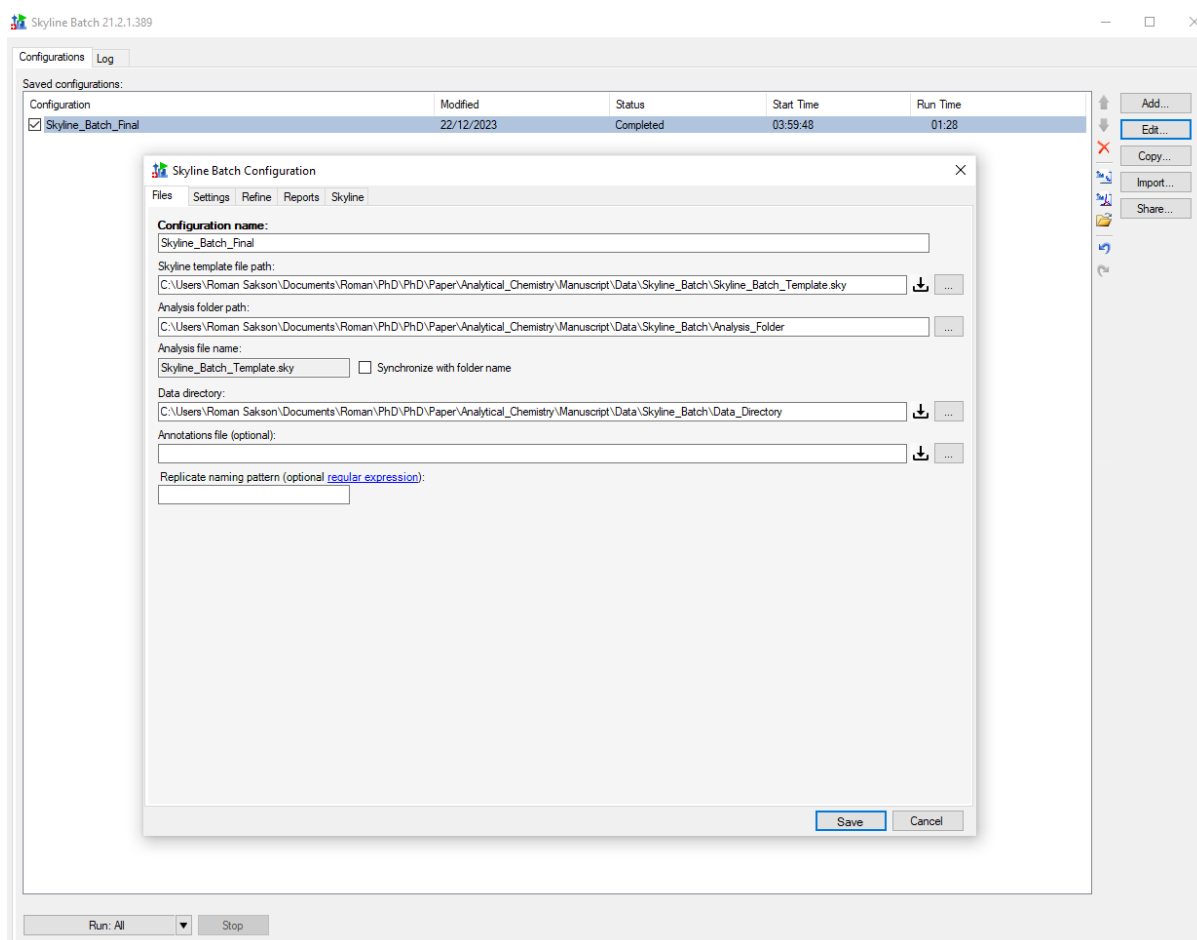
Getting started:

Please, consult the general SB documentation first:

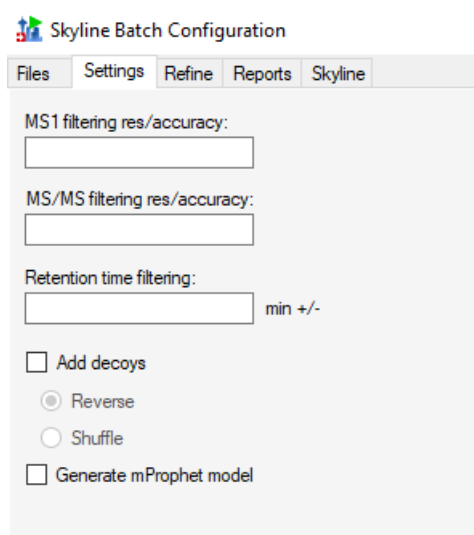
<https://skyline.gs.washington.edu/software/SkylineBatch/SkylineBatchDocumentation.pdf>

All necessary files, such as the bcfg file, the "Skyline_Batch_Template" Skyline file and the skyr report for transition area export as well as the R-script have been deposited on Panorama Public (see main manuscript for accession details).

Below, screenshots of our SB configuration are provided to facilitate the setup of the pipeline:



Settings and Refine options were not used:



Skyline Batch Configuration

×

Files

Settings

Refine

Reports

Skyline

Save refined file as:

...

☐ Remove data

☐ Remove decoys

▼ Refining the targets list

Minimum peptides

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

Prefer larger products

Missing results

Minimum time correlation

Minimum dot-product

Minimum idotp

Use best result

CV remove above cutoff

CV global normalize

CV reference normalize

CV transitions

CV transitions count

CV ms level

Q value cutoff

Minimum detections

Group comparison p value cutoff

Group comparison fold change cutoff

MS level

Group comparison name

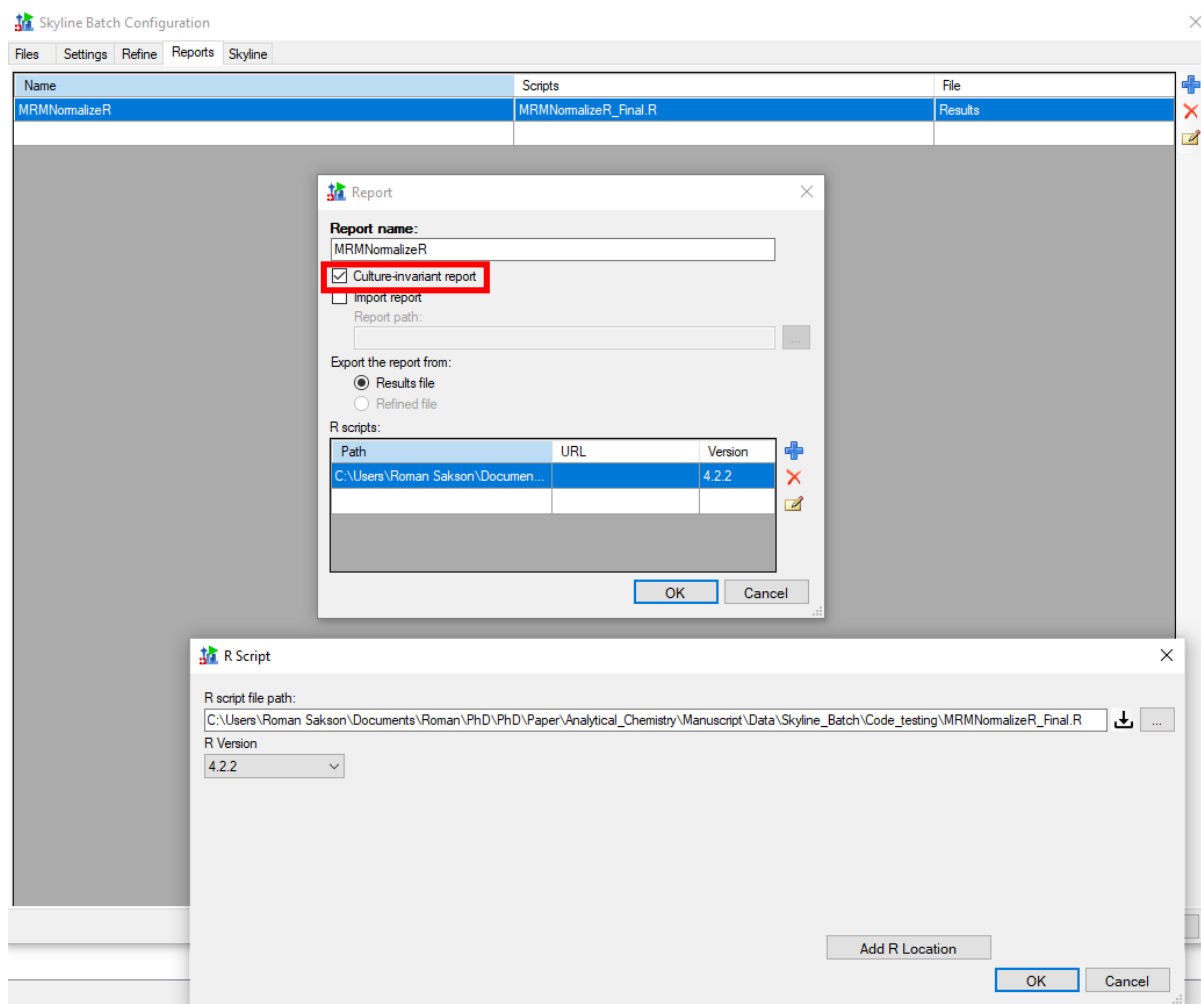
Minimum peptides

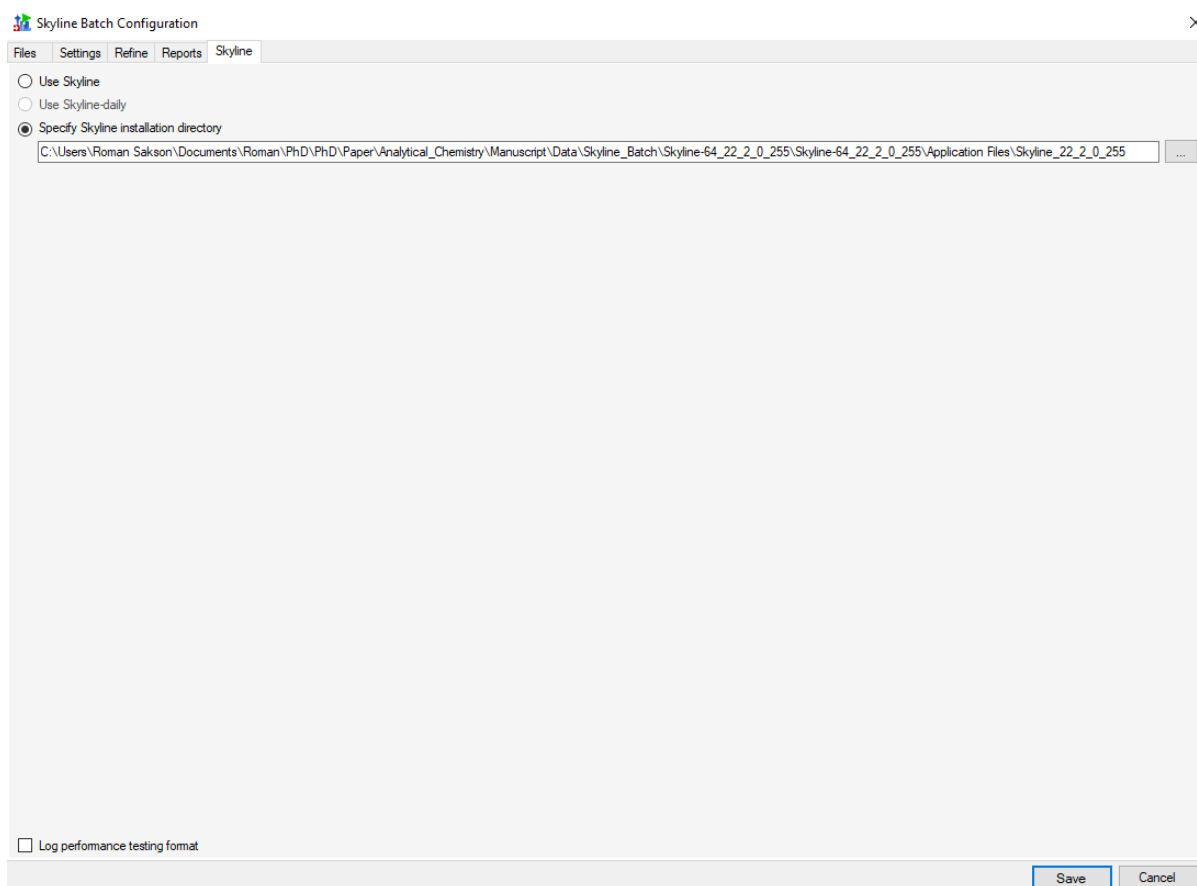
Proteins with fewer than this number of peptides will be removed from the document.

Save

Cancel

Make sure to check the “culture-invariant report” box to avoid spaces in the column header names of the Skyline custom export report, otherwise the R-script will not run through:





Here an example of a successful log record (note that there will be a pop-up fenster to set the working directory where your R-script is saved):

