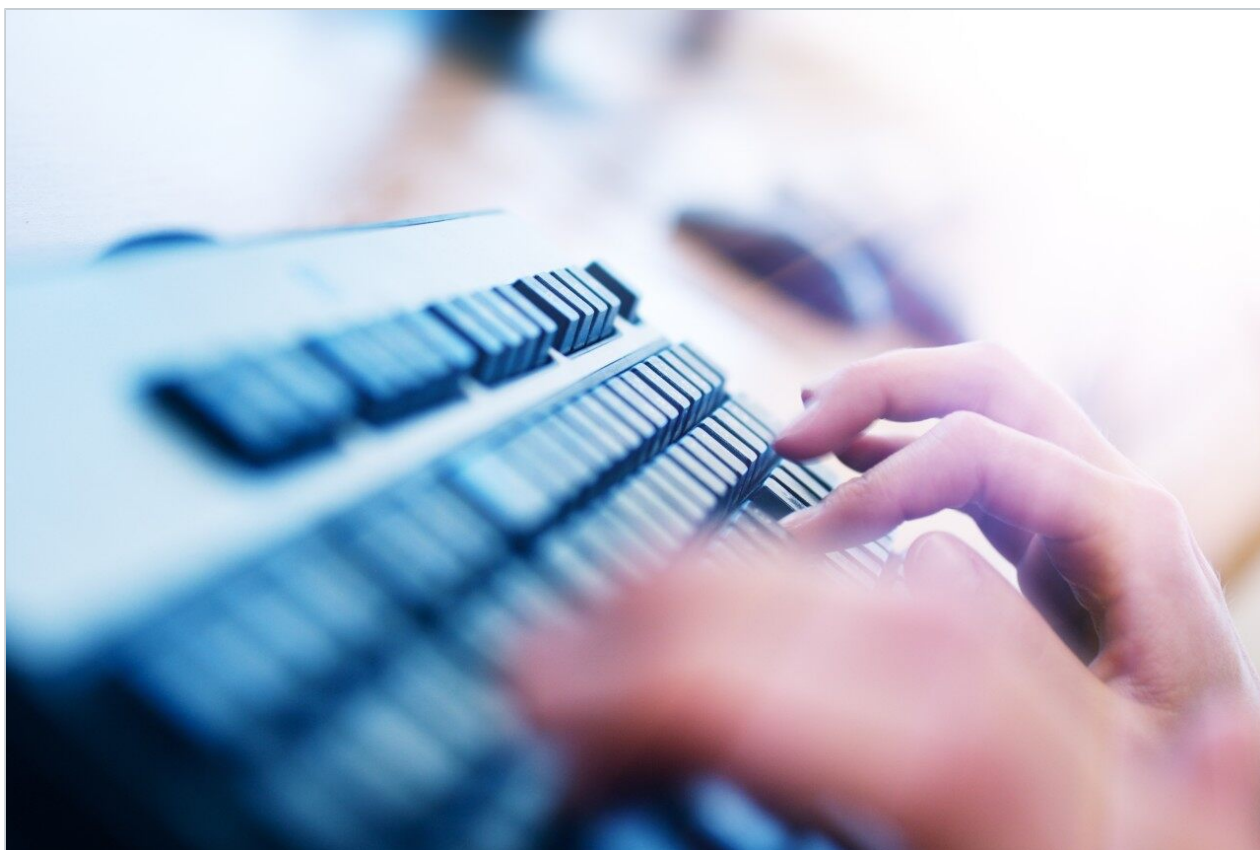


Automating MetaboQuan-R and LipidQuan Data Processing with Symphony

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For research use only. Not for use in diagnostic procedures.

This is an Application Brief and does not contain a detailed

Experimental section.

Abstract

This application brief demonstrates the workflow to automate data processing in TargetLynx and Skyline for MetaboQuan-R and LipidQuan targeted assays. The pipelines are configured to automatically generate a results report, which is directly compatible with MetaboAnalyst.

Benefits

Increase throughput of MetaboQuan-R and LipidQuan assays with automation of the data processing workflow in TargetLynx and Skyline using Symphony.

Introduction

MetaboQuan-R and LipidQuan are two targeted assays designed to compare samples from large cohorts and highlight potential markers of interest in a rapid, high-throughput manner. All LC and MS methods for both assays are provided in a Quanpedia database and do not require any further development. More specifically, MetaboQuan-R allows for the rapid screening and semi-quantitative analysis of different compound classes (acylcarnitines, bile acids, amino acids, triglycerides, and peptides) using the same LC conditions. LipidQuan is a comprehensive and high-throughput HILIC-based methodology for the separation and quantification of polar and non-polar lipid classes. Here, we show how the use of the Symphony Software improves the efficiency of data processing and thereby increases the throughput of these assays.

Results and Discussion

The Symphony data pipeline is specifically designed to form custom chains of data processing steps to improve the efficiency in complex LC-MS analyses. The MetaboQuan-R and LipidQuan generated data can be processed using TargetLynx and/or Skyline (MacCoss Lab, University of Washington). The outputs from these processed datasets can be visualized and statistically analyzed with the

freely available MetaboAnalyst software to determine potential markers of interest. In this technology brief, we demonstrate how Symphony can be applied to streamline the processing and automatically generate a report compatible with MetaboAnalyst from TargetLynx and/or Skyline.

Symphony pipeline – TargetLynx processing

The pipeline created in Symphony to generate a report from TargetLynx is described in Figure 1. Data are acquired using MassLynx prior to being loaded into TargetLynx. The Symphony pipeline triggers the transfer of the raw data to a processing computer and/or server for storage. The processing is then automated to generate an .xml output from TargetLynx and to convert the file, via an Excel macro, to a .csv results file. The resulting .csv file is compatible for import into MetaboAnalyst, allowing additional interrogation and statistical analysis.

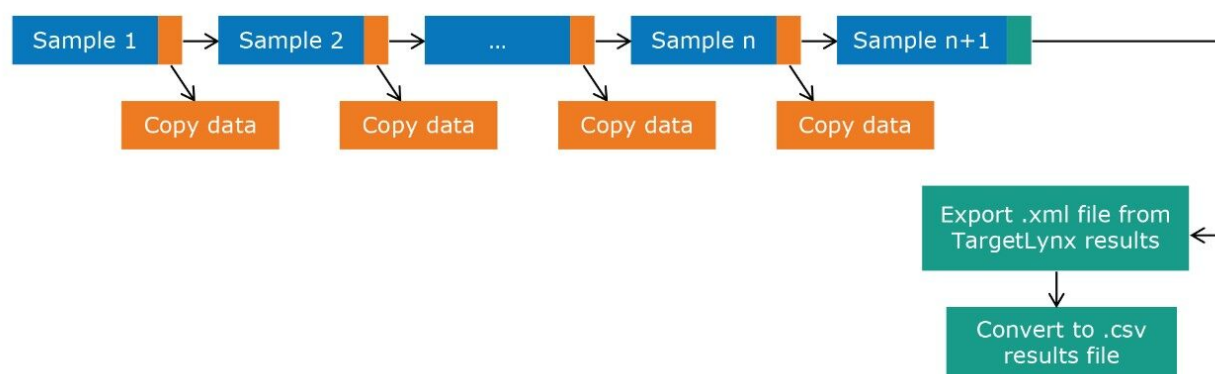


Figure 1. Symphony pipeline to automatically trigger the processing of data from MetaboQuan-R and LipidQuan assays using TargetLynx. A result report compatible with MetaboAnalyst is generated directly after acquisition. All data are copied to a server following acquisition. At the end of the MassLynx batch, a mock sample (sample n+1) is used to trigger the remaining tasks of the pipeline: generate an .xml file from TargetLynx and convert this to a .csv file via an Excel macro for compatibility with MetaboAnalyst.

Symphony pipeline – Skyline processing

Figure 2 outlines a pipeline created using Symphony for data processing and report generation through Skyline. The pipeline consists of three tasks: Data transfer to a storage server and/or processing PC, data import and processing using Skyline, and finally data export as a .csv result file. On completion of the MassLynx analysis batch, two files are obtained: A Skyline file containing all the raw data and a .csv file consisting of 1) peak area for each sample (raw, normalized against an internal standard or light/heavy ratios) and 2) sample conditions/groups. Key user parameters for

each pipeline are shown in Figures 3 and 4. An example amino acid highlighted using the MetaboQuan-R assay and processed via the Skyline/MetaboAnalyst pipeline for a lung and bladder cancer sample set² is provided in Figure 5.

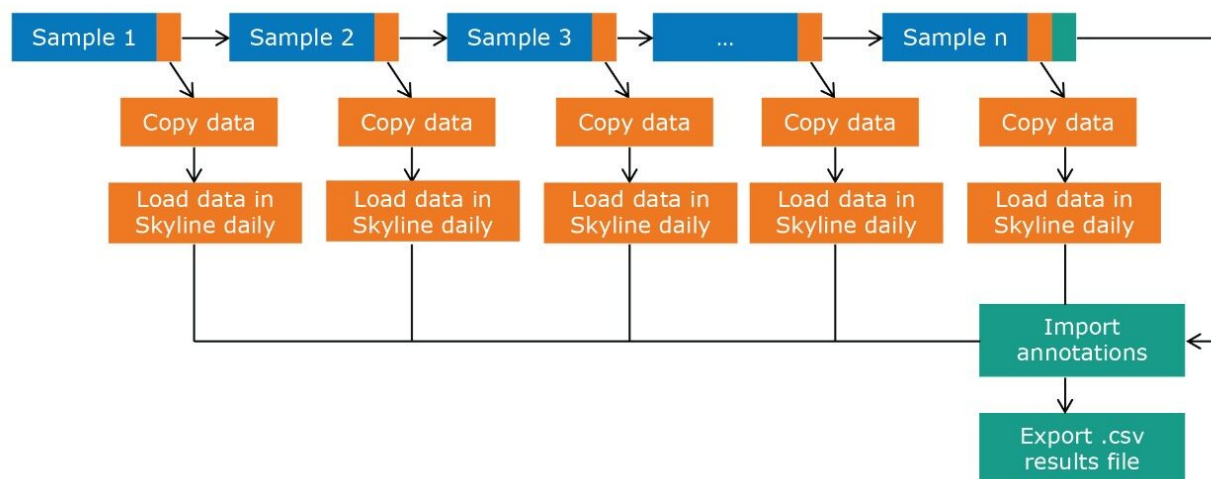


Figure 2. Symphony pipeline to automatically trigger the processing of MetaboQuan-R and LipidQuan data using Skyline. A results report compatible with MetaboAnalyst is generated. The pipeline consists of three tasks: 1) data transfer to the server/processing computer, 2) import and processing into Skyline, 3) import of the annotations (i.e., sample conditions such as healthy versus disease), and 4) export of the .csv results file. This .csv file is then directly compatible with MetaboAnalyst.

Build pipeline

Data keys and values:

#	Data key	Key type	Key condition	Key value
1	#rawdatapath	File	Exists	Provided by Symphony Client
2	#rawdatalocation	File	Exists	Provided by Symphony Client
3	#samplename	Text	None	Provided by Symphony Client
4	#xmlfilename	File	None	C:\MassLynx\project.PRO\Results.xml
5	#TargetLynxfilename	File	Exists	C:\MassLynx\project.PRO\samplelistname.qld
6	#xmloutputdirectory	Text	None	\\Processingcomputer\E\LipidQuan
7	#macroname	File	Exists	\\Processingcomputer\E\LipidQuan\TargetLynxXMLIntegration.xlsm
8	#xmlfilepath2	File	Exists	\\Processingcomputer\E\LipidQuan\Results.xml

Figure 3. Symphony pipeline builder interface that highlights the task library for MetaboQuan-R/LipidQuan analysis via TargetLynx with key user parameters.

Build pipeline

Data keys and values:

#	Data key	Key type	Key condition	Key value
1	#rawdatapath	File	Exists	Provided by Symphony Client
2	#rawdatalocation	File	Exists	Provided by Symphony Client
3	#samplename	Text	None	Provided by Symphony Client
4	#copydatapath	File	None	\\Server\Project\{samplename}.raw
5	#copydatapathforprocessing	File	None	\\Processingcomputer\E\Rawdata\{samplename}.raw
6	#SkylineProjectLocation	File	Exists	\\Processingcomputer\E\Skyline_template.sky
7	#reportnameinSkyline	Text	None	Metaboanalyst
8	#PathToSaveSkylineReport	File	None	\\Processingcomputer\E\Results.csv
9	#Pathtoannotationscsv	File	Exists	\\Processingcomputer\E\annotations.csv

Figure 4. Symphony pipeline builder interface that highlights the task library for MetaboQuan-R/LipidQuan analysis via Skyline with key user parameters.

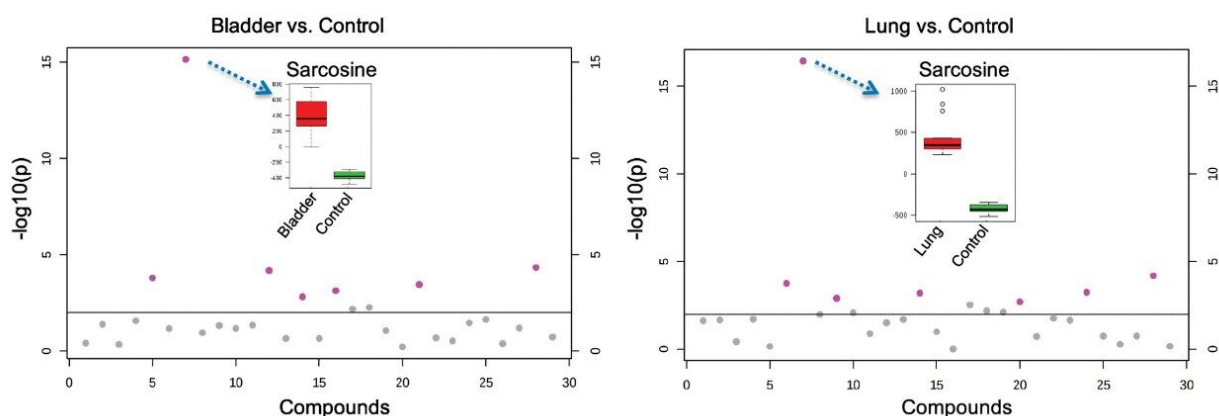


Figure 5. Example statistical analysis in MetaboAnalyst resulting from data that was processed through a Skyline-based Symphony pipeline.² A pair-wise comparison of the 28 amino acids from the MetaboQuan-R assay, which represents a lung and bladder cancer cohort, are shown. The pink dots represent amino acids that are significantly different between the two conditions (t-test, FDR cut-off at 1%) with sarcosine identified as being significantly over-expressed for both bladder and lung cancer subjects.

Conclusion

In this application brief, we outlined the application of the Symphony data pipeline software to automate the data processing for the MetaboQuan-R and LipidQuan-based assays. Symphony is triggered from the MassLynx sample list, enabling data transfer prior to loading and processing in TargetLynx and/or Skyline, which generates a report compatible with MetaboAnalyst. Symphony provides the capability of automating data transfer and processing, which ultimately increases the overall workflow productivity and is particularly applicable for large cohort/high-throughput analysis.

References

1. Chong, J. *et al.* MetaboAnalyst 4.0: Towards more Transparent and Integrative Metabolomics

Analysis. *Nucl. Acids Res.* (DOI: 10.1093/nar/gky310)

2. Waters Corporation. Rapid Molecular Profiling of a Bladder and Lung Cancer Human Plasma Cohort using MetaboQuan-R. 720006612EN, July 2019.

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