

How to import data

Contents

1.	Introduction.....	2
2.	Examples on Data sources and application areas.....	2
2.1.	RNA-seq data (Gene expression)	2
2.2.	Proteomics	2
2.3.	Single cell RNA-seq	2
2.4.	Metabolomics and Mass spec Data	3
2.5.	Flow cytometry	3
2.6.	DNA and RNA Sequencing (NGS).....	3
2.7.	ChIP-seq and ATAC-seq data	4
2.8.	TCGA data	4
3.	Supported data formats/arrays.....	4
4.	Aligned BAM files	5
5.	How to load and open files	5
6.	ThermoFisher (Affymetrix) files	6
6.1.	ThermoFisher (Affymetrix) CEL files (.cel).....	6
6.2.	Thermo Fisher (Affymetrix) Probe Set Files (.chp).....	7
7.	10X Genomics files	7
8.	Agilent Files.....	8
8.1.	Agilent Text Files.....	8
8.2.	Agilent GeneView files (.txt)	8
9.	Import Wizard for .txt, .csv and .tsv files	8
10.	Import Wizard for raw count data.....	10
11.	Qlucore Data Files (.gedata).....	11
11.1.	How to create a Qlucore Data File	11
11.2.	Formatting	12
11.3.	Layout.....	12
11.4.	Transposed data.....	13
12.	Simple Data File.....	13
13.	Compact text file (.csv)	13
14.	Load annotations.....	14

14.1. From a .txt or .csv file	14
14.2. Create a sample/variable annotation text file.....	15
14.3. From NetAffx.....	16
15. R users	16
16. Templates	16
Terminology.....	17
Decimal point and decimal comma.....	17
Disclaimer	17
Trademark List.....	17

1. INTRODUCTION

With the introduction of Qlucore Omics Explorer version 3.3 the program can be configured with separate modules. This document covers the import functionality of the Qlucore Omics Explorer Base module.

2. EXAMPLES ON DATA SOURCES AND APPLICATION AREAS

In this section we outline the data import options for common application areas.

2.1. RNA-SEQ DATA (GENE EXPRESSION)

For gene expression RNA-seq data there are several import options.

- a) Aligned BAM files. One for each sample/subject. Import details are available in section 4.
- b) A raw count matrix in a .tsv, .txt or .csv file. Import details are available in section 10.

2.2. PROTEOMICS

You will need quantified and normalized data in a file or in multiple files. The extension shall be .txt, .csv or .tsv. The can then be imported using the Wizard, see section 9.

Note: The Wizard can handle import of multiple files and it will work even if the files contain different variables.

If the data has not been log transformed as part of the normalization steps done before importing, it can be done in Qlucore Omics Explorer (in the Data tab).

2.3. SINGLE CELL RNA-SEQ

We support several options for import of single cell data:

- a) As aligned BAM files as described in section 4.
- b) As a matrix of data in a .txt, .tsv or .csv file (see section 9).
- c) For 10X Genomics data using the dedicated template. Data that has been process with the Cell Ranger pipeline is directly imported with the template that is named "10X Genomics assistant". Launch the Template browser and select the template and follow the steps. See section 7.

2.4. METABOLOMICS AND MASS SPEC DATA

You will need quantified and normalized data (concentrations, levels,...) in a file or in multiple files. The extensions shall be .txt, .csv or .tsv.

The data can then be imported using the Wizard, see section 9.

Note: The Wizard can handle import of multiple files and it will work even if the files contain different variables.

If the data has not been log transformed as part of the normalization steps done before importing, it can be done in Qlucore Omics Explorer (in the Data tab).

2.5. FLOW CYTOMETRY

The easiest way to export features (variables) describing each sample from gating software to QOE is by using .csv files. Counts of different populations is often a natural choice of features to export. However, any feature varying in a relevant way between samples can be used, for examples relative frequencies, mean fluorescence intensities (MFI's) or MFI ratios. You need to ensure that either each column or each row corresponds to a sample.

If you use FlowJo (<http://www.flowjo.com>) for manual gating you can use a synchronized group to do batch analysis of all samples (<http://docs.flowjo.com/d2/workspaces-and-samples/ws-groups/ws-gatecopying/>). Then use the Table Editor (<http://docs.flowjo.com/d2/tabular-reports/>) to define interesting features for export to a .csv file. To set the gates, you can either use one typical sample as a template, or you can first concatenate all the sample files into a new .fcs file and do the gating on the concatenated file. Using a concatenated file allows you to consider the data in all samples simultaneously.

The data can then be imported using the Wizard, see section 9.

After loading the data, you need to decide which normalization to use and if you should log-transform your data. These decisions should be based on which features you use and what type of patterns/deviations you want to detect in your data. General guidelines are given in the "How to work with flow cytometry data" that can be found on www.qlucore.com/documentation.

2.6. DNA AND RNA SEQUENCING (NGS)

For DNA-seq (and RNA-seq) data the NGS module is required for browsing the genome and detecting variants. DNA-seq data is imported by using the project manager and a set of different files are required to define a NGS project. The minimum requirements are:

- Aligned BAM files to define the sample(s)
- A reference genome (FAST file)
- A GTF file defining the transcripts

Details are presented in the Documentation and Help Manager that is shipped with the program.

2.7. CHIP-SEQ AND ATAC-SEQ DATA

The peak analysis support in QluCore Omics Explorer allows for comprehensive analysis of peak data such as ChIP-seq and ATAC-seq. The main components of the peak analysis processing are peak detection, consensus peaks and count matrix generation.

Peak analysis is an NGS feature and requires the NGS module to work. Peak analysis is always performed in the context of a QluCore NGS project created in the NGS Project Manager.

QluCore Omics Explorer supports Peak detection with the algorithm Macs2. This will generate one bed type file (narrowPeak or broadPeak) per peak bam file with each feature in it being a peak detected by the Macs2 algorithm.

QluCore Omics Explorer supports adding a group of input bam-files that can be used by the peak detection to get more accurate results. Although not strictly necessary, it is advisable in most cases to generate input data for ChIP-seq studies.

Details are presented in the Documentation and Help Manager that is shipped with the program.

2.8. TCGA DATA

From the Template browser you can access and start a template that downloads TCGA mRNA gene expression data from GDAC (Broad Institute). The template is called "TCGA RSEM". Start the template and follow the steps.

3. SUPPORTED DATA FORMATS/ARRAYS

This section applies to experiment data. Other sections will cover import of annotations (clinical information) and system biology related information such as gene sets and pathways.

The base module supports direct import of experiment data including normalization for:

- Aligned BAM files with RNA-seq data
- Thermo Fisher (Affymetrix) 3" and WT arrays, including Clariom arrays.
- 10X Genomics files from Cell Ranger pipeline
- Agilent mRNA arrays and Agilent microRNA arrays

For Illumina array data the recommended workflow is to normalize data with the GenomeStudio or BeadStudio software and then use the Wizard in QluCore Omics Explorer.

For data generated with other arrays/instruments or resulting from other type of sources, QluCore offers a wide range of import alternatives. If data is stored in a .txt, .csv or .tsv file the Import Wizard, see section 9, is a useful tool for efficient data import. **If you are uncertain of how your data is structured the Wizard is normally the best way to import data.**

In total 11 different data set file formats are supported in the base module:

- BAM files (.bam)
- Affymetrix CEL files (.cel)
- Affymetrix Probe Set Files (.chp)
- Agilent Text Files (.txt)
- Agilent Gene View files (.txt)

- Simple Data Files (.txt)
- Qlucore Data Files (.gedata)
- GEO Data Sets (.soft and .soft.gz) ¹
- GEO Series Matrix (.txt and .txt.gz) ²
- Compact Text Files (.txt; .csv)
- BioArray Software Environment Files (.base)
- 10X Genomics files from Cell Ranger pipeline (“count” and “aggr”)

4. ALIGNED BAM FILES

You can import and normalize RNA-seq data from aligned BAM file(s).

Select the **File** → **Open BAM** files menu item.

Use the Add button to select individual files. Or use the Add Folder button to select all BAM files in a folder.

Press OK to start loading the selected files. This can take a while.

A GTF (Gene Transfer Format) file is needed to describe where the genes are located in the reference genome. file. GTF files can be downloaded from ftp://ftp.ensembl.org/pub/current_gtf/.

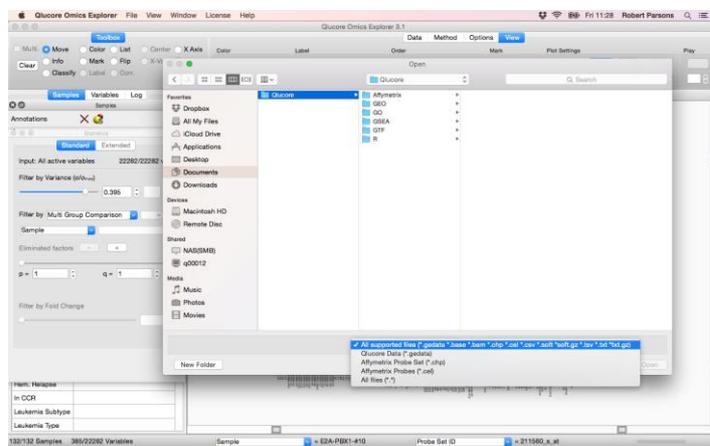
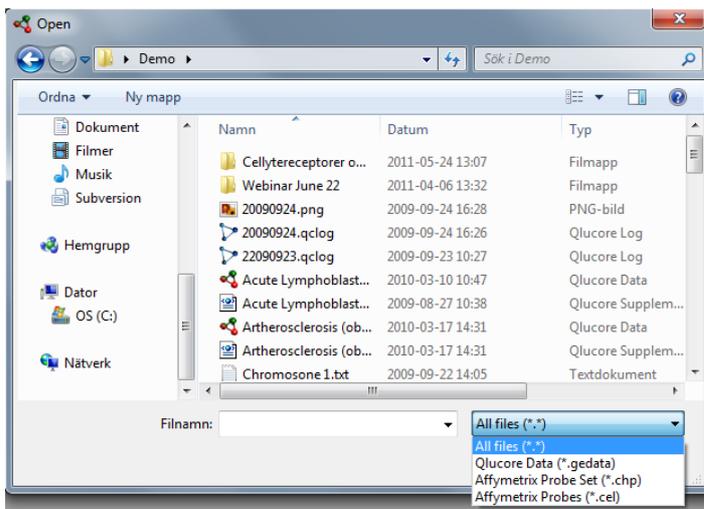
Note: The GTF shall based on the same reference genome as the one you have used for alignment.

The Normalization options (TMM, TPM and FPK) are described in the Reference manual.

5. HOW TO LOAD AND OPEN FILES

Files are loaded by using the File->Open menu. Then there are different filters that you can select. See the picture below. *Note that the picture might vary slightly depending on your operating system.*

^{1, 2} Note that it is only Data Sets from Gene Expression Omnibus in these formats that are supported. Not just any *.soft or *.soft.gz file.



It is also possible to load both variable and sample annotation files, see section 14.

6. THERMOFISHER (AFFYMETRIX) FILES

This applies to mRNA array based data. For miRNA data, only normalized data (as a .txt, .tsv or .csv file) is accepted, and the suggested workflow is to import the normalized data using the Wizard, see section 9.

6.1. THERMOFISHER (AFFYMETRIX) CEL FILES (.CEL)

The import and normalization will normally include four steps

1. Select files
2. Select normalization method
3. Select which annotations to import
4. Inspect the QC-report

To start the import: Select File->Open and select the files you would like to open. To select multiple files, use the shift key on the keyboard.

The data import process includes downloading information about the array from a ThermoFisher server. You need to be connected to Internet if you do not have this data stored locally.

After a short while you are asked to select normalization method. Three methods are provided

- RMA
- RMA Sketch
- Plier

The next step is to download annotations from the Thermo Fisher NetAffx server³. You can select which annotations to include in your data set.

Finally, the QC-report is displayed.

In OE, normalization and summarization will be performed on gene (transcript level) both for 3" arrays and WT arrays.

A wide range of different arrays for a wide range of species are supported, this includes for instance the newer Clariom arrays as well as older U133 arrays of various generations.

6.2. THERMO FISHER (AFFYMETRIX) PROBE SET FILES (.CHP)

Select **File->Open** and select the files you would like to open. To select multiple files, use the shift key on the keyboard.

You will be asked if you would like to download annotation data from Affymetrix NetAffx server automatically⁴. When loading probe set annotations from Affymetrix Whole Transcript arrays, such as Human Gene 1.0 ST, Omics Explorer automatically creates simplified annotations (Gene Symbol, Gene Title,...) in addition to the annotations in the Affymetrix annotation file. This makes it easier to interpret the results and to search for interesting genes.

7. 10X GENOMICS FILES

10X Genomics data is imported with the "Load 10x data" template that is reached from the Template Browser.

Any dataset containing results from the Cell Ranger "count" or "aggr" pipelines work.

You need to provide the path to the directory where data is stored. The directory **must** contain the following 3 files to make the template work:

- barcodes.tsv.gz
- features.tsv.gz

^{3,4} This requires that the user is registered at the Thermo Fisher(Affymetrix) website. See section 14.3 for more information about downloading this website.

- matrix.mtx.gz

8. AGILENT FILES

8.1. AGILENT TEXT FILES

Omics Explorer can import text files created with Agilent Feature Extraction Software. These files contain one array (sample) per file.

How to open Agilent text files

Select the **File**→**Open** menu item.

Select the Agilent Text File (*.txt) file filter.

Select the files and press Open. (Normally you select several files.)

In the Normalization Dialog, select a preprocessing scheme by pressing one of the four quick setting buttons.

- One-color mRNA
- One-color miRNA
- Two-color mRNA
- Two-color miRNA

Instead of using the above quick settings, you can select your own customized preprocessing. See the OE reference manual, section “Agilent .txt files”, for details.

8.2. AGILENT GENEVIEW FILES (.TXT)

Qlucore Omics Explorer can open Agilent GeneView files. These files contain miRNA expression levels. They are created with the Agilent Feature Extraction Software. Each file contains data from a single array. To select multiple files use the shift key on the keyboard.

Normalization

The data is normalized as recommended by Agilent:

- thresholding
- 2-logarithm
- percentile shift (optional, but recommended)

You can select the threshold value and the percentile. Recommended values are threshold = 1.0 and percentile = 75.

9. IMPORT WIZARD FOR .TXT, .CSV AND .TSV FILES

The Wizard supports import of data in many different formats. The starting point is that data is stored in a file with extension .txt, .csv or .tsv.

Example on different structures and layouts that are accepted, with header.

The samples can be in one or multiple files. If the samples are in multiple files, the file format for all samples must be identical. You reach the Wizard by the menu **File->Open with Wizard**.

Normally data to import shall be normalized. There is one exception and that is if the data is count data. Then the workflow provides options for normalization. See section 10.

The Wizard guides you through a number of steps where you define how the layout of the data shall be interpreted and imported. The Wizard is very flexible and can for example handle

- Samples as columns and variables as rows, or the transposed version
- Samples in multiple files, with or without the exact same number of variables in each file.
- Count and continuous data
- Various data separators [“,”, “;”, “tab” or other]
- Data on every i^{th} column or every i^{th} row.
- File header or not
- Data that shall not be imported
- Various indications of missing values [empty cell, n/a, NA,...]
- Annotations

If you are uncertain of how your data is structured, then the Wizard is the best option for data import.

Note: The Import Wizard is an automatic import method for very many different types of files. Make sure to verify that the import results in the expected number of samples and variables.

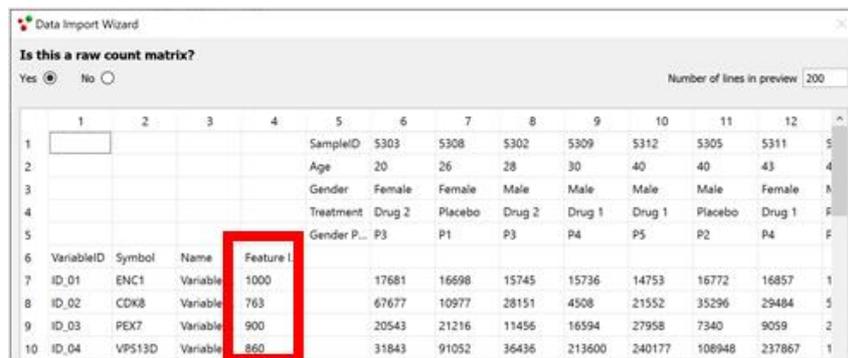
10. IMPORT WIZARD FOR RAW COUNT DATA

The starting point is that data is stored in a matrix in a file with extension .txt, .csv or .tsv.

In the second step in the Wizard you define that you have count data and you will then be asked to select normalization method. Available Normalization methods are TMM, FPKM and TPM. More information on these methods is provided in the reference manual. You also need to provide the length of each feature (gene lengths in base pairs for gene data), either as a variable annotation or as a separate text file with one row per feature (i.e. no header row) and two columns, feature Id and feature length. The feature lengths are only used in the normalization formula and not for anything else. Advanced users can thus use the feature length as a per-feature normalization weight to customize the normalization.

The Wizard guides you through 10 steps where you define how the layout of the data shall be interpreted and imported.

Note: When you select variable annotations to import the feature lengths annotation shall be included.



	1	2	3	4	5	6	7	8	9	10	11	12
1					SampleID	5303	5308	5302	5309	5312	5305	5311
2					Age	20	26	28	30	40	40	43
3					Gender	Female	Female	Male	Male	Male	Male	Female
4					Treatment	Drug 2	Placebo	Drug 2	Drug 1	Drug 1	Placebo	Drug 1
5					Gender P...	P3	P1	P3	P4	P5	P2	P4
6	VariableID	Symbol	Name	Feature L								
7	ID_01	ENC1	Variable	1000		17681	16698	15745	15736	14753	16772	16857
8	ID_02	CDK8	Variable	763		67677	10977	28151	4508	21552	35296	29484
9	ID_03	PEX7	Variable	900		20543	21216	11456	16594	27958	7340	9059
10	ID_04	VPS13D	Variable	860		31843	91052	36436	213600	240177	108948	237867

Example of count matrix - the column marked in red contains the gene lengths.

11. Qlucore Data Files (.GEDATA)

The Qlucore Data file format has been designed to make it easy to create a file by copying and pasting in a spreadsheet application. Data resulting from other tools and applications can easily be formatted and imported into Qlucore Omics Explorer using this file format. A Qlucore Data File contains a complete data frame, including sample and variable annotations.⁵

There are three important things to note when a file shall be created

1. It is a tab-separated text file.
2. The file shall have the file name extension .gedata.
3. Data shall be formatted as described below

11.1. HOW TO CREATE A Qlucore Data File

There are several ways to start the creation of the data file (.gedata).

a) If you have installed OE on your computer you can create a new data file template by right clicking on your desktop and selecting New and Qlucore Omics Explorer. This will generate a new file on your desktop. If you right click on the new file you can open the file with Excel or another spreadsheet application. Then you need to copy and paste data from your original data file into the new file. A guide on how to do this follows below in the section Formatting.

b) Open your original data file and include rows and columns as described in the section Formatting.

⁵ A Qlucore data file does not contain information about the colors used to represent the values of a sample attribute in a plot, or the default variable annotation used when matching variable name lists with variable annotations in the data frame. That information is stored in supplementary data files.

11.2. FORMATTING

Copy and paste the data into the spreadsheet, following the layout below. You can use any spreadsheet application, such as Excel or OpenOffice. When you are done and have formatted data as below, go through the following three steps.

1. Select 'Save as' in the spread sheet application
2. Save the spread sheet as a tab delimited text file.
3. Rename the file by changing the file name extension from the default .txt to .gedata.

11.3. LAYOUT

The layout of the spreadsheet is best explained through an example. The data below contains

1. The measured data (cells are blue)
2. Sample annotation IDs: Array ID, Age, Sex (cells are pink)
3. Sample annotations (cells are yellow)
4. Variable annotation IDs: VarID and Symbol (cells are light blue).
5. Variable annotations (cells are green)

In a spread sheet application, this data frame should be arranged as follows:

Qlucore	gedata	version 1.0						
4	samples	with	3	attributes				
5	variables	with	2	annotations				
		Array ID	5301	5302	5303	5304		
		Age	34	22	47	41		
		Sex	Female	Male	Male	Male		
varID	Symbol							
3140	MR1		2.28	-1.23		0.45		
622	BDH1		1.04	0	-0.03	0		
7551	ZNF3		-0.67	3.14	2.18	0.53		
1537	CYC1		-1.34	2.34	-0.3	2.73		
961	CD47		2.73	1.07	0.83	-1.52		

Always included

Note: The start of the file shall include the text and information as indicated by row 1 to 5. For your own data you need to adjust the number of samples, variables and annotations.

11.4. TRANSPOSED DATA

The example given above has samples as columns and variables as rows. For some data it is more natural to have the opposite, i.e. samples as rows and variables as columns.

Qlucore OE is capable to import .gedata files with data organized in this way if the word “transposed” is added to cell D1, see the table below. Note that the table is not complete.

Qlucore	gedata	version 1.0	transposed			
5	samples	with	2	attributes		
4	variables	with	3	annotations		
		Variable ID	101	102	103	104

12. SIMPLE DATA FILE

The simple data file is a tab separated text file with the extension .txt. The table below shows how data and identifiers should be organized. The rows are variables and the columns are samples. The first column should include a unique variable identifier (green cells) and the first row a unique sample identifier (yellow cells). Data is stored in the blue cells.

	5301	5302	5303	5304	
3140	2.28	-1.23		0.45	
622	1.04	0	-0.03	0	
7551	-0.67	3.14	2.18	0.53	
1537	-1.34	2.34	-0.3	2.73	
961	2.73	1.07	0.83	-1.52	

If there is a text in the upper left cell, that information will be used as the variable annotation header, otherwise the name will be *Variable ID*. The sample annotation header, corresponding to the sample annotation in the first row, will be the *Sample ID*.

Normally you would like to complement your data import with annotations, see chapter 10.

13. COMPACT TEXT FILE (.CSV)

Omics Explorer supports compact text files. These contain data, sample annotations and variable annotations for multiple samples in a single file. If compared to a “.gedata” file, see chapter 7, the compact text file is lacking a file header and has samples as rows. The layout of the files is described below. The last variable annotation (in the example below the second

row) will be used as variable id. Note that the name of this annotation is not included in the file; after loading the file the annotation will be called Variable Id.

The file can have either the extension “.txt” or “.csv”, both options work.

		Symbol	ENC1	CDK8	PEX7	SNN	DLX6
Array	Age	Gender	201314_at	204831_at	205420_at	218032_at	221289_at
5301	34	Female	2.28	1.04	-0.67	-1.34	2.73
5302	22	Male	-1.23	0	3.14	2.34	1.07
5303	47	Male	1.45	-0.03	2.18	-0.3	0.83
5304	41	Female	0.45	0	0.53	2.73	-1.52

How to open a compact text file

Select the **File** → **Open** menu item.

Select the file and press Open.

If the file contains only one variable annotation you will be asked to enter the index of the first data column (in the example above it is column 4).

14. LOAD ANNOTATIONS

Annotations are information about your samples and variables. It can for instance be clinical information about patients. The description below is a summary of options. More details are provided in the How to load annotations document and the section with the same name in the Documentation and Help manager in the program itself.

Note. To import annotations a data set, need to be loaded.

14.1. FROM A .TXT OR .CSV FILE

There are two options. Using the Annotation Wizard or importing a file of the format specified below.

Using the Annotation Wizard

Select the **File** → **Import** → **Sample Annotations via Wizard** or the **File** → **Import** → **Variable Annotations via Wizard** menu item.

The Annotation Wizard is launched and by following the instructions it is possible to import data from *.txt, *.csv or *.tsv files. The Wizard will assist you to pick out the data you need.

Direct From a .txt or .csv file

You can add sample and variable annotations to an existing data set by importing an annotation text file. These files are tab- or comma-delimited text files. The file name extension is .txt if the file is tab-delimited and .csv if the file is comma-delimited.

How to import a sample/variable annotation text file

Select the **File** → **Import** → **Sample Annotations** or the **File** → **Import** → **Variable Annotations** menu item.

Select the Tab-separated Text (*.txt) or the Comma-separated Text (*.csv) file filter.

Select the file and press Open.

Select the annotations you want to import and press OK.

When importing an annotation text file, the samples/variables in the file will be matched with the samples/variables in the data frame using the annotation in the first column of the annotation file and the sample/variable ID annotation in the data set (This can be changed in the Data tab). In this way the ordering of the rows in the annotation file and the data frame does not matter.

14.2. CREATE A SAMPLE/VARIABLE ANNOTATION TEXT FILE

Place the data in a spreadsheet, following the layout below. You can use any spreadsheet application, such as Excel or OpenOffice. Save the spreadsheet as a tab- or comma-delimited text file. Use the file name extension .txt if the file is tab-delimited and .csv if the file is comma-delimited. We recommend using tab-delimited text files.

The following layout should be used:

Probe Set	Transcript	Gene Symbol	Chromosome	Entrez Gene
1007_s_at	U48705	DDR1	chr6p21.3	780
1053_at	M87338	RFC2	chr7q11.23	5982
117_at	X51757	HSPA6	chr1q23	3310
121_at	X69699	PAX8	chr2q12-q14	7849
1255_g_at	L36861	GUCA1A	chr6p21.1	2978
1294_g_at	L13852	UBA7	chr3p21	7318

The first row contains the name of each annotation. Each of the remaining rows contains the value of each annotation for one sample/variable. The first column should contain an annotation that matches the sample/variable ID in the data set.

This table should be saved as a tab-separated text file with file name extension .txt or as a comma-separated text file with file name extension .csv.

14.3. FROM NETAFFX

You can download variable annotations for Affymetrix arrays from the Affymetrix NetAffx server and add them to the data set.

The downloaded annotation files will be stored on your computer, and can be used without having to download them from the server again. When you select to download annotations, you will be notified if updated annotations are available on the Affymetrix server.

When downloading probe set annotations for the Affymetrix whole transcript arrays, Omics Explorer will create simplified annotations (Gene Symbol, Gene Title,...) in addition to the annotations in the downloaded annotation file.

How to download and import annotations from the Affymetrix NetAffx server ⁶

Select the **File** → **Download** → **Affymetrix Probe Set Annotations** menu item.

Select the array type and press OK .

Select the annotations you want to import and press OK.

15. R USERS

Two R-scripts are shipped with Qlucore Omics Explorer. They make it easy to convert data back and forth from R.

The script function definition files are located in the user's Documents folder. The path is Documents/Qlucore/R-import/, where the two files read.gedata.1.1.R and write.gedata.1.1.R can be found.

For detailed description on how to use the scripts see the "R to .gedata" section in the Documentation and Help system.

16. TEMPLATES

Templates are scripts that enables a pre-configured set of operations to be executed in the program. Templates are based on Python.

Templates can be opened from **File** → **Execute Template**.

The Template Browser (**File** → **Template Browser**) shows an overview of all templates available at the location(s) specified in the Template section of the Preferences.

Current standard templates include import of TCGA data and 10X Genomics Single cell data.

⁶ The first time you use this function, you will also be asked to provide: your *Affymetrix user name* and your Thermo Fisher (*Affymetrix*) *password* and the folder where the downloaded annotation files will be stored. If you do not have an Affymetrix user name and password, you can get one by pressing the Register button. This will take you to the registration page on the Affymetrix web site.

TERMINOLOGY

Samples: We use samples to describe units such as patients, persons, animals, treatments, dates,...

Variables: We use variables to describe quantities that have been measured for each samples, such as Gene Expression Levels, Protein concentrations, answers to a question in a questionnaire,...

Annotation: A description of a sample or a variable. One sample or variable can be described by one or many annotations.

DECIMAL POINT AND DECIMAL COMMA

Both separators are supported in all tab separated .txt files and .csv files.

DISCLAIMER

The contents of this document are subject to revision without notice due to continuous progress in methodology, design, and manufacturing.

Qlucore shall have no liability for any error or damages of any kind resulting from the use of this document.

Qlucore Omics Explorer is only intended for research purposes.

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