

Bulk RNA-seq Cheatsheet

The tables below consist of valuable functions or commands that will help you through this module.

Each table represents a different library/tool and its corresponding commands.

Please note that these tables are not intended to tell you all the information you need to know about each command.

The hyperlinks found in each piece of code will take you to the documentation for further information on the usage of each command.

Base R

Read the Base R package documentation [here](#).

Library/Package	Piece of Code	What it's called	What it does
Base R	<code>list.files()</code>	List files	Produces a character vector of files or directories in the specified directory
Base R	<code>names()</code>	Names	Gets or sets the names of an object
Base R	<code>colnames()</code>	Column names	Gets or sets the column names of a matrix or data frame
Base R	<code>all.equal()</code>	All equal	Checks if two R objects are nearly equal
Base R	<code>attr()</code>	Object Attributes	Gets or sets the attributes of an object
Base R	<code>rowSums()</code>	Row Sums	Returns the sum of the rows in a numeric matrix-like object (i.e.. a matrix, data.frame, etc.)
Base R	<code>relevel()</code>	Relevel	Reorders the levels of a factor as specified
Base R	<code>summary()</code>	Object Summary	Returns a result summary of an object
Base R	<code>as.data.frame()</code>	Data Frame	Checks if an object is a data.frame, and transforms the object into one, if possible

DESeq2

Read the `DESeq2` package documentation [here](#), and the package vignette by Love, Anders, and Huber [here](#).

Library/Package	Piece of Code	What it's called	What it does
<code>DESeq2</code>	<code>vst()</code>	Variance Stabilizing Transformation	Applies variance stabilizing transformation to data (log2-like scale)
<code>DESeq2</code>	<code>DESeqDataSet()</code>	<code>DESeqDataSet</code> constructor that can take a <code>SummarizedExperiment</code>	Creates a <code>DESeqDataSet</code> object
<code>DESeq2</code>	<code>DESeqDataSetFromMatrix()</code>	<code>DESeqDataSet</code> constructor	Creates a <code>DESeqDataSet</code> object from a matrix of count data
<code>DESeq2</code>	<code>DESeq()</code>	Differential Expression Analysis Based on the Negative Binomial Distribution	Estimates size factors, estimates dispersion, and performs negative binomial fitting and Wald statistics as steps in the default <code>DESeq2</code> differential expression analysis
<code>DESeq2</code>	<code>plotPCA()</code>	PCA plot	Produces a principal component analysis plot for transformed data. It can be used to visually inspect the data, which might allow an analyst to identify batch effects.
<code>DESeq2</code>	<code>counts()</code>	Counts	Returns count matrix from <code>DESeqDataSet</code> object
<code>DESeq2</code>	<code>results()</code>	Results	Returns the results table from a <code>DESeq2</code> analysis
<code>DESeq2</code>	<code>assay()</code>	Assay	Returns matrix from the <code>assay</code> slot of a <code>DESeqDataSet</code> object

FastQC and fastp

Read the `FastQC` documentation [here](#) and the `fastp` documentation [here](#).

Library/Package	Piece of Code	What it's called	What it does
<code>fastp</code>	<code>fastp</code>	FASTQ preprocessor	Preprocesses FASTQ files through adapter trimming, quality filtering, length filtering, and a number of additional options
<code>FastQC</code>	<code>fastqc</code>	FASTQC (Quality Control)	Performs quality control checks on raw sequence data and outputs a QC(quality control) report

ggplot2

Read the `ggplot2` package documentation [here](#). A vignette on the usage of the `ggplot2` package can be found [here](#).

Library/Package	Piece of Code	What it's called	What it does
<code>ggplot2</code>	<code>ggsave()</code>	GG Save	Saves the last plot in working directory
<code>ggplot2</code>	<code>last_plot()</code>	Last plot	Returns the last plot produced
<code>ggplot2</code>	<code>geom_point()</code>	Geom point	Creates a scatterplot (when added to the <code>ggplot()</code> function)
<code>ggplot2</code>	<code>xlab() ; ylab()</code>	X Axis Labels; Y Axis Labels	Modifies the labels on the x axis and on the y axis, respectively
<code>ggplot2</code>	<code>coord_fixed()</code>	Cartesian Coordinates with Fixed Aspect Ratio	Coerces the coordinates on the plot to represent a fixed specified ratio

tximeta and SummarizedExperiment

Read the `tximeta` package documentation [here](#), and the package vignette by Love *et al.* [here](#).

Read the `SummarizedExperiment` package documentation [here](#), and the package vignette by Morgan *et al.* [here](#).

Library/Package	Piece of Code	What it's called	What it does
<code>tximeta</code>	<code>tximeta()</code>	tximeta	Imports transcript-level estimates, attaches transcriptome annotation, and returns a <code>SummarizedExperiment</code> object
<code>tximeta</code>	<code>makeLinkedTxome()</code>	Make Linked Transcriptome	Sets up transcriptome annotation to be used by the <code>tximeta()</code> function (Only necessary if <code>tximeta()</code> fails to find annotation, like for non-human, non-mouse species data)
<code>tximeta</code>	<code>summarizeToGene()</code>	Summarize to Gene	Takes a <code>SummarizedExperiment</code> that was set up by <code>tximeta</code> and summarizes transcript data to the gene-level
<code>SummarizedExperiment</code>	<code>rowData()</code> <code>colData()</code>	Col/Row Data	Accesses the row or column data from a <code>SummarizedExperiment</code> object
<code>SummarizedExperiment</code>	<code>assay()</code> <code>assayNames()</code>	Assay or AssayNames	Accesses the assay data or the names of the assays from <code>SummarizedExperiment</code> object

stringr, readr, dplyr, pheatmap

Documentation for each of these packages can be accessed by clicking the package name in the table below.

Library/Package	Piece of Code	What it's called	What it does
<code>stringr</code>	<code>word()</code>	Word	Extracts words from a character vector
<code>readr</code>	<code>write_rds()</code>	Write RDS	Writes data to a .RDS output file
<code>dplyr</code>	<code>pull()</code>	Pull	Extracts a variable (column) as a vector
<code>pheatmap</code>	<code>pheatmap()</code>	Pretty heatmap	Plots clustered heatmaps

Salmon

Read the Salmon tool documentation [here](#).

Tool	Piece of Code	What it's called	What it does
Salmon	<code>salmon index</code>	Salmon index	Builds a transcriptome index which is required for Salmon quantification (from the command line)
Salmon	<code>salmon quant</code>	Salmon quantification	Runs Salmon's quantification of transcript expression (from the command line)

Useful command line commands:

```
rstudio@e549475e8bdb:~/kitematic$ ls
ccd1-training-waiver.DOCX  docker-install  intro-to-R-tidyverse  LICENSE.md  machine-learning  README.md  RNA-seq  scRNA-seq
rstudio@e549475e8bdb:~/kitematic$ cd RNA-seq
rstudio@e549475e8bdb:~/kitematic/RNA-seq$ cd data
rstudio@e549475e8bdb:~/kitematic/RNA-seq/data$ mkdir command_line_example
rstudio@e549475e8bdb:~/kitematic/RNA-seq/data$ cd command_line_example/
rstudio@e549475e8bdb:~/kitematic/RNA-seq/data/command_line_example$ wget -O bash_example.sh https://raw.githubusercontent.com/GameServerManagers/LinuxGSM/master/linuxgsm.sh
--2019-07-24 20:46:35-- https://raw.githubusercontent.com/GameServerManagers/LinuxGSM/master/linuxgsm.sh
Resolving raw.githubusercontent.com (raw.githubusercontent.com)... 151.101.200.133
Connecting to raw.githubusercontent.com (raw.githubusercontent.com)|151.101.200.133|:443... connected.
HTTP request sent, awaiting response... 200 OK
Length: 11826 (12K) [text/plain]
Saving to: 'bash_example.sh'
bash_example.sh      100%[=====>] 11.55K  --.-KB/s   in 0.007s

2019-07-24 20:46:35 (1.62 MB/s) - 'bash_example.sh' saved [11826/11826]

rstudio@e549475e8bdb:~/kitematic/RNA-seq/data/command_line_example$ bash bash_example.sh
fetching serverlist.csv...OK
Usage: bash_example.sh [option]

Installer - Linux Game Server Managers - Version v19.9.0
https://linuxgsm.com

Commands
install      | Select server to install.
servername  | Enter name of game server to install. e.g bash_example.sh csgoserver.
list         | List all servers available for install.
rstudio@e549475e8bdb:~/kitematic/RNA-seq/data/command_line_example$ echo This is the end of the command line demonstration
This is the end of the command line demonstration
rstudio@e549475e8bdb:~/kitematic/RNA-seq/data/command_line_example$ >
```

Command to list the files or subdirectories in the current directory

Command to change into the subdirectory "RNA-seq"

Make a new directory named "command_line_example" in the current directory

Now 'cd' into that new directory

'wget' downloads a file from the internet (in this case a bash script) and saves it in the current directory

The '-O' flag in our previous command allows us to name the file we retrieve

bash command runs a bash script

echo command prints the string that follows

Feel free to give these commands a try on your own! (Note that our example begins in the training-module directory.)