

Advanced scRNA-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.

You may also be interested in the following additional cheatsheets:

- Download the PDF for the [Introduction to R and Tidyverse cheatsheet](#)
- Download the PDF for the [Introduction to Single-Cell RNA sequencing cheatsheet](#)
- Download the PDF for the [SCE and Seurat object comparison cheatsheet](#)

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. Please be aware that the documentation will generally provide information about the given function's most current version (or a recent version, depending on how often the documentation site is updated). This will usually (but not always!) match what you have installed on your machine. If you have a different version of R or other R packages, the documentation may differ from what you have installed.

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scater

Read the [scater package documentation](#), and a [vignette on its usage](#).

Library/Package	Piece of Code	What it's called	What it does
scater	plotReducedDim(.)	Plot reduced dimensions	Plot a given reduced dimension slot from a <code>SingleCellExperiment</code> object by its name
scater	plotUMAP(.)	Plot UMAP	Plot the "UMAP"-named reduced dimension slot from a <code>SingleCellExperiment</code> object
scater	plotExpression(.)	Plot expression	Plot expression values for all cells in a <code>SingleCellExperiment</code> object, using the <code>logcounts</code> assay by default

miQC

Read the [miQC package documentation](#), and a [vignette on its usage](#).

Library/Package	Piece of Code	What it's called	What it does
<code>miQC</code>	<code>mixtureModel(.)</code>	Mixture model	Fit a <code>miQC</code> mixture model to a <code>SingleCellExperiment</code> object for use in filtering
<code>miQC</code>	<code>filterCells(.)</code>	Filter cells	Filter cells from a <code>SingleCellExperiment</code> object based on a <code>miQC</code> model, returning a filtered <code>SingleCellExperiment</code> object
<code>miQC</code>	<code>plotMetrics(.)</code>	Plot metrics	Plot percent of mitochondrial reads against the number of unique genes found for each cell
<code>miQC</code>	<code>plotModel(.)</code>	Plot model	<code>miQC::plotMetrics()</code> with the <code>miQC</code> fitted model overlaid
<code>miQC</code>	<code>plotFiltering(.)</code>	Plot filtering	Plot percent of mitochondrial reads against the number of unique genes found, coloring points based on whether they will be filtered out or not

batchelor and harmony

Read the [batchelor](#) [package documentation](#), and a [vignette on its usage](#).

Read the [harmony](#) [package documentation](#), and a [vignette on its usage](#).

Library/Package	Piece of Code	What it's called	What it does
batchelor	MultiBatchPCA(.)	Multi-batch PCA	Perform PCA across multiple gene expression matrices, weighted by batch size
batchelor	fastMNN(.)	Fast mutual nearest neighbors correction	Perform integration on an SCE object with mutual nearest neighbors using the <code>fastMNN</code> algorithm, returning an SCE object with batch-corrected principal components
harmony	RunHarmony(.)	Perform integration with the <code>harmony</code> algorithm on a matrix of single-cell genomics cell embeddings	Perform integration with the <code>harmony</code> algorithm, returning a matrix of batch-corrected principal components

SingleR

Read the [SingleR](#) [package documentation](#), and an [e-book on its usage](#).

Library/Package	Piece of Code	What it's called	What it does
SingleR	<code>trainSingleR(.)</code>	Train the SingleR classifier	Build a <code>SingleR</code> classifier model object from an annotated reference dataset
SingleR	<code>classifySingleR(.)</code>	Classify cells with SingleR	Use a <code>SingleR</code> model object to assign cell types to the cells in an <code>SCE</code> object
SingleR	<code>SingleR(.)</code>	Annotate scRNA-seq data	Combines <code>trainSingleR()</code> and <code>classifySingleR()</code> to assign cell types to an <code>SCE</code> object from an annotated reference dataset

pheatmap and EnhancedVolcano

Read the [pheatmap](#) [package documentation](#).

Read the [EnhancedVolcano](#) [package documentation](#), and [vignette on its usage](#).

Library/Package	Piece of Code	What it's called	What it does
pheatmap	<code>pheatmap(.)</code>	Pretty heatmap	Plot a (pretty!) clustered heatmap
EnhancedVolcano	<code>EnhancedVolcano(.)</code>	Enhanced volcano	Plot a volcano plot to visualize differential expression analysis results

DESeq2 and pseudo-bulking functions

Read the [DESeq2 package documentation](#), and a [vignette on its usage](#).

Library/Package	Piece of Code	What it's called	What it does
scuttle	aggregateAcrossCells(.)	Aggregate data across groups of cells	Sum counts for each combination of features across groups of cells, commonly used to <i>pseudo-bulk</i> SCE counts
DESeq2	DESeqDataSet(.)	DESeq Dataset	Establish a DESeq object from a pseudo-bulked SingleCellExperiment object or a bulk SummarizedExperiment object
DESeq2	estimateSizeFactors(.)	Estimate size factors	Estimate size factors which are used to normalize counts for differential expression analysis
DESeq2	rlog(.)	Apply a regularized log transformation	Log2-transform counts in a DESeq object for differential expression analysis
DESeq2	plotPCA(.)	Sample PCA plot for transformed data	Plot sample PCA from a log-transformed DESeq object to check for batch effects
DESeq2	DESeq(.)	Perform differential expression analysis	Perform differential expression: Estimate size factors, transform data, estimate dispersions, and perform testing.
DESeq2	plotDispEsts(.)	Plot dispersion estimates	Plot dispersion estimates from a fitted DESeq object to evaluate model fit

DESeq2	results()	Extract results from a DESeq analysis	Extract results from a fitted DESeq object into a data frame
DESeq2	resultsNames()	Extract results names	Return coefficient names from a fitted DESeq object
DESeq2	lfcShrink()	Shrink log2 fold changes	Add shrunken log2-fold changes to a results table produced by DESeq2::results()

tidyverse functions

purrr functions

Read the [purrr package documentation](#) and a [vignette on its usage](#), and download the [purrr package cheatsheet](#).

Library/Package	Piece of Code	What it's called	What it does
purrr	map()	map	Apply a function across each element of list; return a list
purrr	imap()	imap	Apply a function across each element of list and its index/names; return a list
purrr	map2()	map2	Apply a function across each element of two lists at a time; return a list
purrr	reduce()	Reduce	Reduce a list to a single value by applying a given function

Note that `purrr::map()` functions can take advantage of R's new (as of version 4.1.0) [anonymous function syntax](#):


```
# One-line syntax:
\(x) # function code goes here #

# Multi-line syntax:
\(x) {
  # function code goes      #
  # inside the curly braces #
}

# Example: Use an anonymous function with `purrr::map()`
# to get the colData's rownames for each SCE in `list_of_sce_objects`
purrr::map(
  list_of_sce_objects,
  \(x) rownames(colData(x))
)
```

ggplot2 functions

Read the [ggplot2 .package documentation](#) and an [overall reference for ggplot2 functions](#), and download the [ggplot2 .package cheatsheet](#).

Library/Package	Piece of Code	What it's called	What it does
<code>ggplot2</code>	<code>geom_bar()</code>	Barplot	Creates a barplot of counts for a given categorical variable when added as a layer to a <code>ggplot()</code> object
<code>ggplot2</code>	<code>scale_fill_brewer()</code>	Add brewer fill scale	Apply a Brewer "fill" color palette to a categorical variable in a <code>ggplot()</code> object
<code>ggplot2</code>	<code>guides()</code>	Guides	Function to customize legend ("guide") appearance
<code>ggplot2</code>	<code>facet_grid()</code>	Facet grid	Plot individual panels using specified variables to subset the data across rows and/or columns of a grid
<code>ggplot2</code>	<code>vars()</code>	Vars	Helper function to specify variables to <code>facet_grid()</code> or <code>facet_wrap()</code>
<code>ggplot2</code>	<code>theme_bw()</code>	Black and white theme	Display <code>ggplot</code> with gridlines but a white background
<code>ggplot2</code>	<code>theme()</code>	Theme	Customize elements of a <code>ggplot</code> plot theme
<code>ggplot2</code>	<code>element_text()</code>	Element text	Customize textual elements of a <code>ggplot</code> theme

dplyr , **tidyr** , **stringr** , and **tibble** functions

Read the full documentation and download cheatsheets (where available) for these **tidyverse** packages at the following links:

- [dplyr documentation](#) and [dplyr cheatsheet](#)
- [tidyr documentation](#) and [tidyr cheatsheet](#)
- [stringr documentation](#) and [stringr cheatsheet](#)
- [tibble documentation](#)

Library/Package	Piece of Code	What it's called	What it does
dplyr	<code>pull()</code>	Pull	Extract a single column from a data frame into a stand-alone vector
dplyr	<code>count()</code>	Count	Count the number of observations in each group of a data frame
dplyr	<code>left_join()</code>	Left join	Joins two data frames together, retaining only rows present in the first ("left") argument to the function
dplyr	<code>relocate()</code>	Relocate	Change column order in a data frame by relocating one or more columns
dplyr	<code>case_when()</code>	Case when	Return a value based on a set of <code>TRUE / FALSE</code> comparisons; a vectorized <code>if-else</code>
tidyr	<code>pivot_longer()</code>	Pivot longer	Convert a "wide" format data frame to a "long" format data frame
tibble	<code>as_tibble()</code>	As tibble	Convert an object to a tibble
stringr	<code>str_detect()</code>	String detect	Returns <code>TRUE / FALSE</code> if a string contains a given substring
stringr	<code>str_starts()</code>	String starts	Returns <code>TRUE / FALSE</code> if a string starts with a given substring

Pathway analysis

msigdb

Read the [msigdbr](#) [package documentation](#) and [its vignette](#).

Library/Package	Piece of Code	What it's called	What it does
<code>msigdbr</code>	<code>msigdbr_species()</code>	List <code>msigdbr</code> - supported species	Lists the species <code>msigdbr</code> supports
<code>msigdbr</code>	msigdbr(.)	Retrieve gene set	Retrieves gene sets and member genes in long data frame format

`clusterProfiler` and `enrichplot`

Read the [clusterProfiler](#) [package documentation \(PDF\)](#).

Library/Package	Piece of Code	What it's called	What it does
<code>clusterProfiler</code>	GSEA(.)	Gene Set Enrichment Analysis (GSEA)	Performs a universal gene set enrichment analysis on given preranked (sorted) named vector of statistics, where the names in the vector are gene identifiers of gene sets
<code>enrichplot</code>	gseaplot(.)	GSEA plot	Produces a plot displaying the distribution of gene set and enrichment score

`AUCell` and `GSEABase`

Read the [AUCell](#) [package documentation](#) and [its vignette](#).

Library/Package	Piece of Code	What it's called	What it does
<code>GSEABase</code>	<code>GeneSet(.)</code>	Gene set	Constructs a gene set as a <code>GeneSet</code> object for use with <code>AUCell</code>
<code>GSEABase</code>	<code>GeneSetCollection(.)</code>	Gene set collection	Constructs a collection of gene sets as a <code>GeneSetCollection</code> object for use with <code>AUCell</code>
<code>AUCell</code>	<code>AUCell_buildRankings(.)</code>	Build cell rankings	Builds a ranking of genes for each cell that is used to calculate the recovery curve
<code>AUCell</code>	<code>AUCell_calcAUC(.)</code>	Calculate AUC	Calculates the area under the recovery curve (AUC) for each gene set in each cell
<code>AUCell</code>	<code>AUCell_exploreThresholds(.)</code>	Explore thresholds	Calculates thresholds in AUC values that can be used to assign cells; optionally makes assignments and produces histograms

bluster

Read the [bluster](#) [package documentation](#) and vignettes on its usage:

- [Flexible clustering for Bioconductor](#)
- [Assorted clustering diagnostics](#)

Library/Package	Piece of Code	What it's called	What it does
<code>bluster</code>	<code>clusterRows(.)</code>	Cluster rows of a matrix	Perform clustering using a variety of algorithms on a matrix-like object
<code>bluster</code>	<code>KmeansParam(.)</code>	K-means clustering parameters	Set up parameters to run clustering using <code>kmeans ()</code> within <code>bluster::clusterRows ()</code>
<code>bluster</code>	<code>NNGraphParam(.)</code>	Graph-based clustering parameters	Set up parameters for nearest-neighbor (NN) graph-based clustering algorithms within <code>bluster::clusterRows ()</code>
<code>bluster</code>	<code>approxSilhouette(.)</code>	Approximate silhouette width	Calculate an approximate silhouette width for each cell given a set of clusters
<code>bluster</code>	<code>neighborPurity(.)</code>	Compute neighborhood purity	Calculate neighborhood purity for each cell given a set of clusters
<code>bluster</code>	<code>bootstrapStability(.)</code>	Assess cluster stability by bootstrapping	Generate cluster bootstrap replicates to estimate cluster robustness to sampling noise