

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](#)

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	<code>plotReducedDim()</code>	Plot reduced dimensions	Plot a given reduced dimension slot from a <code>SingleCellExperiment</code> object by its name
scater	<code>plotUMAP()</code>	Plot UMAP	Plot the "UMAP"-named reduced dimension slot from a <code>SingleCellExperiment</code> object
scater	<code>plotExpression()</code>	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
miQC	<code>mixtureModel()</code>	Mixture model	Fit a <code>miQC</code> mixture model to a <code>SingleCellExperiment</code> object for use in filtering
miQC	<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
miQC	<code>plotMetrics()</code>	Plot metrics	Plot percent of mitochondrial reads against the number of unique genes found for each cell
miQC	<code>plotModel()</code>	Plot model	<code>miQC::plotMetrics()</code> with the <code>miQC</code> fitted model overlaid
miQC	<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	<code>MultiBatchPCA()</code>	Multi-batch PCA	Perform PCA across multiple gene expression matrices, weighted by batch size
batchelor	<code>fastMNN()</code>	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	<code>HarmonyMatrix()</code>	Perform harmony integration on a matrix	Perform integration with <code>harmony</code> on either a matrix of principle components or gene expression, returning a matrix of batch-corrected principal components

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

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	<code>map()</code>	map	Apply a function across each element of list; return a list
purrr	<code>imap()</code>	imap	Apply a function across each element of list and its index/names; return a list
purrr	<code>map2()</code>	map2	Apply a function across each element of two lists at a time; return a list

Library/Package	Piece of Code	What it's called	What it does
purrr	<code>reduce()</code>	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
ggplot2	<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
ggplot2	<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
ggplot2	<code>guides()</code>	Guides	Function to customize legend ("guide") appearance
ggplot2	<code>facet_grid()</code>	Facet grid	Plot individual panels using specified variables to subset the data across rows and/or columns of a grid

Library/Package	Piece of Code	What it's called	What it does
ggplot2	<code>vars()</code>	Vars	Helper function to specify variables to <code>facet_grid()</code> or <code>facet_wrap()</code>
ggplot2	<code>theme_bw()</code>	Black and white theme	Display <code>ggplot</code> with gridlines but a white background
ggplot2	<code>theme()</code>	Theme	Customize elements of a <code>ggplot</code> plot theme
ggplot2	<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 TRUE/FALSE 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

Library/Package	Piece of Code	What it's called	What it does
tibble	<code>as_tibble()</code>	As tibble	Convert an object to a tibble
stringr	<code>str_detect()</code>	String detect	Returns TRUE/FALSE if a string contains a given substring
stringr	<code>str_starts()</code>	String starts	Returns TRUE/FALSE if a string starts with a given substring

Seurat and SCE object conversion

The Seurat documentation provides a [vignette about converting objects](#) between SCE and Seurat formats.

In addition, we provide some code examples below for how you can accomplish these conversions. For all code examples below, it is assumed that the `SingleCellExperiment` library has been loaded into your R environment:

```
library(SingleCellExperiment)
```

Converting from Seurat to SCE

The following example code assumes you have a Seurat object called `seurat_obj`.

```
# Convert Seurat object to SCE object
sce_object <- Seurat::as.SingleCellExperiment(seurat_obj)
```

By default, all assays present in the Seurat object will be ported into the new SCE object.

To only specify that certain assays are retained, you can optionally provide the argument `assays`, as in: `assays = c("assays", "to", "keep")`.

Converting from SCE to Seurat

The following example code assumes you are starting with an SCE object called `sce_object`.

The function `Seurat::as.Seurat()` can be used to convert an SCE object into a Seurat object and takes the following arguments:

- The SCE object to convert
- Optional named arguments with the following defaults:
 - `counts = "counts"` specifies that the SCE object contains a "counts" assay of normalized counts that should be included during conversion.

- If there is no "counts" assay in the SCE object, set this argument as `counts = NULL` or rename accordingly, e.g. `logcounts = "whatever_assay_name_you_are_using"`.
- `data = "logcounts"` specifies that the SCE object contains a "logcounts" assay of normalized counts that should be included during conversion.
 - If there is no "logcounts" assay in the SCE object, set this argument as `logcounts = NULL` or rename accordingly, e.g. `logcounts = "whatever_assay_name_you_are_using"`.
- `assay = NULL` specifies that, by default, all assays will be converted.
 - To specify that an additional assay besides "counts" or "logcounts" should be converted, include it here as in `assay = "additional_assay_name"`.
- `project = "SingleCellExperiment"` specifies that the Seurat object being created will have this associated project name. You can override this with any string of interest, e.g. `project = "sample_XYZ"`.

```
# Convert SCE object to Seurat object, assuming both
# `counts` and `logcounts` assays are present
seurat_object <- Seurat::as.Seurat(sce_object)
```

Approaches from ScPCA

In addition, this [documentation from the ScPCA](#) introduces how to convert SCE objects to Seurat objects. Although this documentation was written for ScPCA datasets, the steps generally apply to any SCE object. We also offer a conversion function `sce_to_seurat()` as part of our [scpcaTools\(\)](#) package, which holds utilities used in the ScPCA workflow.

Again, although this function was written to convert SCE objects from ScPCA, it should generally work for most SCE objects, although it will only retain a single assay (raw "counts") in the new SCE object, and it will not retain reduced dimension representations (e.g., PCA or UMAP).

Therefore, this function is mostly useful at the early stages of processing before you have normalized counts and and calculated reduced dimensions.

You can obtain this package using the `remotes` package, which may also need to be installed first:

```
# Install `remotes`, as needed:
install.packages("remotes")

# Install the current version of `scpcaTools`
remotes::install_github("AlexsLemonade/scpcaTools")

# Now, you can use the function, specifying the argument `assay` for which
seurat_object <- scpcaTools::sce_to_seurat(sce_object)
```