

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

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 SCE object
SingleR	<code>SingleR()</code>	Annotate scRNA-seq data	Combines <code>trainSingleR()</code> and <code>classifySingleR()</code> to assign cell types to an SCE object from an annotated reference dataset

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
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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 other differential expression functions

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

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

Library/Package	Piece of Code	What it's called	What it does
DESeq2	<code>results()</code>	Extract results from a DESeq analysis	Extract results from a fitted DESeq object into a data frame
DESeq2	<code>resultsNames()</code>	Extract results names	Return coefficient names from a fitted DESeq object
DESeq2	<code>lfcShrink()</code>	Shrink log2 fold changes	Add shrunken log2-fold changes to a results table produced by <code>DESeq2::results()</code> from a fitted DESeq object

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

When converting between Seurat and SCE objects, it's helpful to know how the different object types store and refer to similar information.

The table below shows different aspects of single-cell objects and how to access the associated data, assuming the default names for each type of single-cell object.

There are several differences between Seurat and SCE objects that are useful to be aware of when converting them.

Importantly, the term "assay" refers to different things in SCE vs. Seurat objects:

- In an SCE object, an assay is a matrix of counts, with default names "counts" for raw counts and "logcounts" for normalized counts.
- In a Seurat object, an assay instead refers to an *experiment*. The default Seurat assay is called "RNA", and it is analogous to the "main experiment" in an SCE object, which is not given a particular name.
- The Seurat count matrices are stored within a given assay (experiment) and have default names of "counts" for raw counts and "data" for normalized counts.

In addition, by default, SCE reduced dimension names are capitalized (e.g., "PCA"), and Seurat reduced dimension names are in lower case (e.g., "pca").

Always bear in mind that your object(s) may be named differently from the defaults as described here!

Data aspect	SCE	Seurat
Raw counts matrix	<code>counts(sce_object)</code>	<code>seurat_obj[["RNA"]]<code>@counts</code></code>
Normalized counts matrix	<code>logcounts(sce_object)</code>	<code>seurat_obj[["RNA"]]<code>@data</code></code>
Reduced dimension: PCA matrix	<code>reducedDim(sce_object, "PCA")</code>	<code>seurat_obj\$<code>pca</code>@cell.embeddings</code>
Reduced dimension: UMAP matrix	<code>reducedDim(sce_object, "UMAP")</code>	<code>seurat_obj\$<code>umap</code>@cell.embeddings</code>
Cell-level metadata	<code>colData(sce_object)</code>	<code>seurat_obj@<code>meta.data</code></code>
Feature (gene)-level metadata	<code>rowData(sce_object)</code>	<code>seurat_obj[["RNA"]]<code>@meta.features</code></code>
Miscellaneous additional metadata	<code>metadata(sce_object)</code>	<code>seurat_obj@<code>misc</code></code>

Below, 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 (experiments) present in the Seurat object will be ported into the new SCE object. Recall, in Seurat, an assay refers to an *experiment* which may be associated with multiple count matrices. To only specify that certain assays are retained, you can optionally provide the argument `assay` with Seurat assay names to retain in the SCE object, for example:

```
# Convert Seurat object to SCE object, retaining only the 'RNA' experiment
(assay)
sce_object <- Seurat::as.SingleCellExperiment(seurat_obj, assay = "RNA")
```

Specifying `assay` is mostly useful if there are alternative experiments, for example from CITE-Seq data, present in the Seurat object that you do not want to retain during SCE conversion.

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. `counts = "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 `data = NULL` or rename accordingly, e.g. `data = "whatever_assay_name_you_are_using"`.
 - `assay = NULL` specifies that, by default, all assays (experiments) will be converted. If there are multiple assays and you wish to only convert, for example, the "RNA" assay, set this argument as `assay = "RNA"`.
 - `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)

# Convert SCE object to Seurat object, where the SCE object
```

```
# contains a `counts` but not a `logcounts` assay  
seurat_object <- Seurat::as.Seurat(sce_object, data = NULL)
```

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. It's worth noting that the example code provided at that link 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 example code is mostly useful at the early stages of processing before you have performed normalization and calculated reduced dimensions.