BestPracticesSTBiocBook

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Welcome

Package: BestPracticesSTBiocBook **Authors:** First Last [aut, cre] **Compiled:** 2024-09-25 **Package version:** 0.98.0 **R version:** R version 4.4.1 (2024-06-14) **BioC version:** 3.20 **License:** MIT + file LICENSE

This is the website for the online book **'Best Practices for Spatial Transcriptomics Analysis with Bioconductor'**.

This book provides discussion and interactive examples on best practices for computational analysis workflows for spatial transcriptomics data, using the Bioconductor framework within R. The chapters contain details on individual analysis steps as well as complete example workflows, with interactive example datasets and R code.

The book is organized into several parts, including introductory materials, analysis steps, and example workflows.

Additional details on analysis workflows for non-spatial single-cell data as well as further introductory materials on R and Bioconductor can be found in the related book Orchestrating Single-Cell Analysis with Bioconductor (OSCA).

Docker image

A `Docker` image built from this repository is available here:

ghcr.io/lmweber/bestpracticesstbiocbook

Get started now

You can get access to all the packages used in this book in $<$ 1 minute, using this command in a terminal:

Listing 0.1 `bash`

```
docker run -it ghcr.io/lmweber/bestpracticesstbiocbook:devel R
```

RStudio Server

An RStudio Server instance can be initiated from the `Docker` image as follows:

Listing 0.2 `bash`

```
docker run \
    --volume <local_folder>:<destination_folder> \
    -e PASSWORD=OHCA \
    -p 8787:8787 \
    ghcr.io/lmweber/bestpracticesstbiocbook:devel
```

The initiated RStudio Server instance will be available at https://localhost:8787.

Session info

Click to expand

1 Introduction

1.1 Overview

Bioconductor

1.2 Contents

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1.3 Scope and who this book is for

Visium Data

Preprocessing

1.4 Bioconductor

Bioconductor

1.5 Additional resources

- Orchestrating Single-Cell Analysis with Bioconductor (OSCA)

- R for Data Science
- Data Carpentry Software Carpentry

- detailed guide
  YouTube videos
- Visium Data Preprocessing

1.6 Contributions

GitHub issues

References

2 Spatial transcriptomics

2.1 Overview

Method of the Year 2020

2.2 Sequencing-based platforms

2.2.1 10x Genomics Visium

10x Genomics Visium

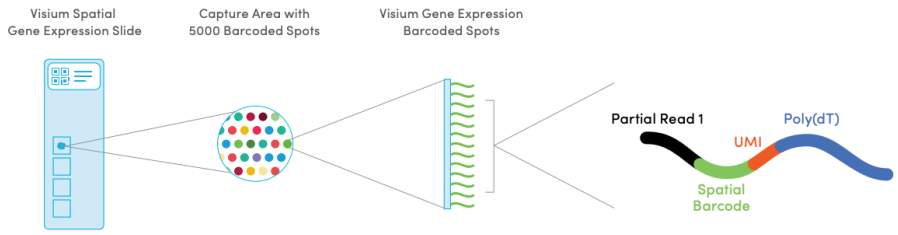

Figure 2.1: Schematic illustrating the 10x Genomics Visium platform. Source: 10x Genomics Visium

2.2.2 10x Genomics Visium HD

10x Genomics Visium HD

2.2.3 Curio Seeker

Curio Seeker

2.3 Molecule-based platforms

2.3.1 10x Genomics Xenium

10x Genomics

2.3.2 Vizgen MERSCOPE

Vizgen

2.3.3 NanoString CosMx

NanoString

References

3 Bioconductor data classes

3.1 Overview

3.2 SpatialExperiment class

SpatialExperiment

SingleCellExperiment

Bioconductor vignette

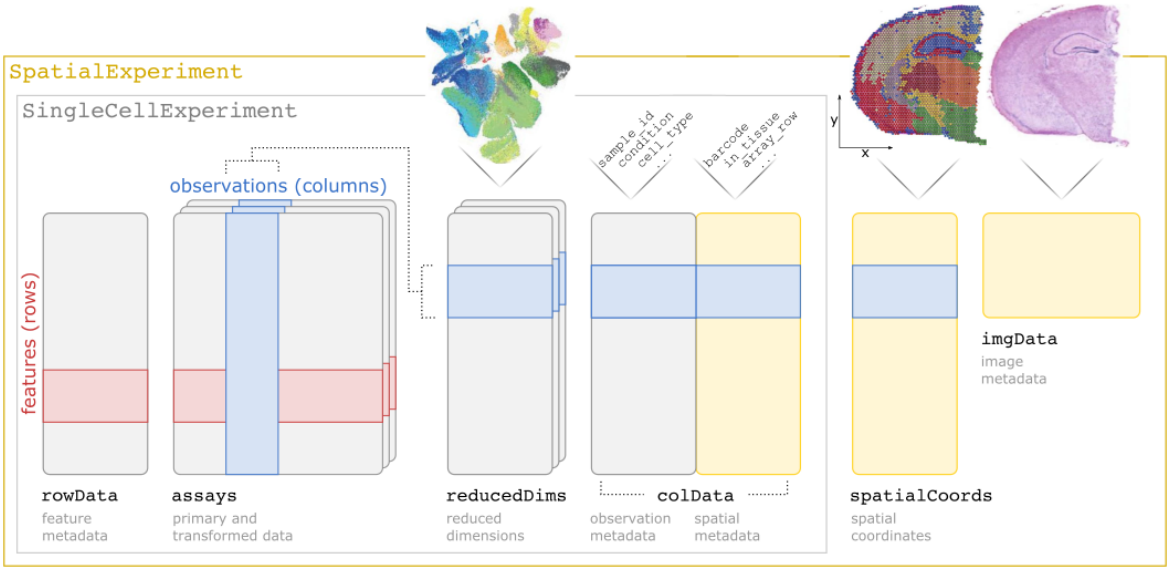

Figure 3.1: Overview of the `SpatialExperiment` data class for storing and manipulating spatial transcriptomics datasets within the Bioconductor framework.

3.3 Molecule-based data

3.3.1 MoleculeExperiment

Bioconductor package

3.3.2 SpatialFeatureExperiment

Bioconductor package

References

4 Analysis steps

[3]

4.1 Save data objects for re-use in later chapters

4.1.1 Human DLPFC dataset

```
# LOAD DATA

library(SpatialExperiment)
library(STexampleData)
spe <- Visium_humanDLPFC()

# save object
library(here)
# if (!dir.exists(here("outputs"))) dir.create(here("outputs"))
# saveRDS(spe, file = here("outputs/spe_load.rds"))
saveRDS(spe, file = here("spe_load.rds"))
```

```r
# QUALITY CONTROL (QC)

library(scater)
# subset to keep only spots over tissue
spe <- spe[, colData(spe)$in_tissue == 1]
# identify mitochondrial genes
is_mito <- grepl("(^MT-)|(^mt-)", rowData(spe)$gene_name)
# calculate per-spot QC metrics
spe <- addPerCellQC(spe, subsets = list(mito = is_mito))
# select QC thresholds
qc_lib_size <- colData(spe)$sum < 600
qc_detected <- colData(spe)$detected < 400
qc_mito <- colData(spe)$subsets_mito_percent > 28
qc_cell_count <- colData(spe)$cell_count > 10
# combined set of discarded spots
discard <- qc_lib_size | qc_detected | qc_mito | qc_cell_count
colData(spe)$discard <- discard
# filter low-quality spots
spe <- spe[, !colData(spe)$discard]

# save object
# saveRDS(spe, file = here("outputs/spe_qc.rds"))
saveRDS(spe, file = here("spe_qc.rds"))
```

```r
# NORMALIZATION

library(scran)
# calculate logcounts using library size factors
spe <- logNormCounts(spe)

# save object
# saveRDS(spe, file = here("outputs/spe_logcounts.rds"))
saveRDS(spe, file = here("spe_logcounts.rds"))
```

```r
# FEATURE SELECTION

# remove mitochondrial genes
spe <- spe[!is_mito, ]
# fit mean-variance relationship
dec <- modelGeneVar(spe)
# select top HVGs
top_hvgs <- getTopHVGs(dec, prop = 0.1)
```

```
# save object
# saveRDS(spe, file = here("outputs/spe_hvgs.rds"))
# saveRDS(top_hvgs, file = here("outputs/top_hvgs.rds"))
saveRDS(spe, file = here("spe_hvgs.rds"))
saveRDS(top_hvgs, file = here("top_hvgs.rds"))
```

```
# DIMENSIONALITY REDUCTION

# compute PCA
set.seed(123)
spe <- runPCA(spe, subset_row = top_hvgs)
# compute UMAP on top 50 PCs
set.seed(123)
spe <- runUMAP(spe, dimred = "PCA")
# update column names
colnames(reducedDim(spe, "UMAP")) <- paste0("UMAP", 1:2)

# save object
# saveRDS(spe, file = here("outputs/spe_reduceddims.rds"))
saveRDS(spe, file = here("spe_reduceddims.rds"))
```

```
# CLUSTERING

# graph-based clustering
set.seed(123)
k <- 10
g <- buildSNNGraph(spe, k = k, use.dimred = "PCA")
g_walk <- igraph::cluster_walktrap(g)
clus <- g_walk$membership
colLabels(spe) <- factor(clus)

# save object
# saveRDS(spe, file = here("outputs/spe_cluster.rds"))
saveRDS(spe, file = here("spe_cluster.rds"))
```