GSE71585_count

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R Markdown

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```
##github. (2020). GitHub. Retrieved from https://github.com/MarcElosua/SPOTlight/tree/spotlight-0.1.7

##Sometimes in GSE, the single cell data saved as CSV format
#Download the csv file from GSE
#Bring the packages
library(SingleCellExperiment)
```

```
## Loading required package: SummarizedExperiment
```

```
## Loading required package: MatrixGenerics
```

```
## Loading required package: matrixStats
```

```
##
## Attaching package: 'MatrixGenerics'
```

```
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars
```

```
## Loading required package: GenomicRanges
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which.max, which.min
```

```
## Loading required package: S4Vectors
```

```
##
## Attaching package: 'S4Vectors'
```

```
## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname
```

```
## Loading required package: IRanges
```

```
##
## Attaching package: 'IRanges'
```

```
## The following object is masked from 'package:grDevices':
##
##     windows
```

```
## Loading required package: GenomeInfoDb
```

```
## Loading required package: Biobase
```

```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
##
## Attaching package: 'Biobase'
```

```
## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians
```

```
## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
```

```
library(Seurat)
```

```
## Attaching SeuratObject
```

```
## Attaching sp
```

```
##
## Attaching package: 'Seurat'
```

```
## The following object is masked from 'package:SummarizedExperiment':
##
##     Assays
```

```
library(tidyverse)
```

```
## ── Attaching packages ─────────────────────────────────────── tidyverse 1.3.2 ──
```

```
## ✓ ggplot2 3.3.6     ✓ purrr   0.3.4
## ✓ tibble  3.1.8     ✓ dplyr   1.0.9
## ✓ tidyr   1.2.0     ✓ stringr 1.4.0
## ✓ readr   2.1.2     ✓ forcats 0.5.1
## ── Conflicts ─────────────────────────────────────────────────────────────────────────── tidyverse_conflicts() ──
## ✖ dplyr::collapse()   masks IRanges::collapse()
## ✖ dplyr::combine()    masks Biobase::combine(), BiocGenerics::combine()
## ✖ dplyr::count()      masks matrixStats::count()
## ✖ dplyr::desc()       masks IRanges::desc()
## ✖ tidyr::expand()     masks S4Vectors::expand()
## ✖ dplyr::filter()     masks stats::filter()
## ✖ dplyr::first()      masks S4Vectors::first()
## ✖ dplyr::lag()        masks stats::lag()
## ✖ ggplot2::Position() masks BiocGenerics::Position(), base::Position()
## ✖ purrr::reduce()     masks GenomicRanges::reduce(), IRanges::reduce()
## ✖ dplyr::rename()     masks S4Vectors::rename()
## ✖ dplyr::slice()      masks IRanges::slice()
```

```
library(Matrix)
```

```
##
## Attaching package: 'Matrix'
##
## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack
##
## The following object is masked from 'package:S4Vectors':
##
##     expand
```

```
library(scales)
```

```
##
## Attaching package: 'scales'
##
## The following object is masked from 'package:purrr':
##
##     discard
##
## The following object is masked from 'package:readr':
##
##     col_factor
```

```
library(cowplot)
library(RCurl)
```

```
##
## Attaching package: 'RCurl'
##
## The following object is masked from 'package:tidyr':
##
##     complete
```

```
library(devtools)
```

```
## Loading required package: usethis
```

```
library(Matrix)
library(data.table)
```

```
##
## Attaching package: 'data.table'
##
## The following objects are masked from 'package:dplyr':
##
##     between, first, last
##
## The following object is masked from 'package:purrr':
##
##     transpose
##
## The following object is masked from 'package:SummarizedExperiment':
##
##     shift
##
## The following object is masked from 'package:GenomicRanges':
##
##     shift
##
## The following object is masked from 'package:IRanges':
##
##     shift
##
## The following objects are masked from 'package:S4Vectors':
##
##     first, second
```

```
library(Seurat) #sc data package
library(SeuratData)
```

```
## ── Installed datasets ─────────────────────────────────────────────────────── SeuratData v0.2.2 ──
## ✓ stxBrain 0.1.1
##
## ──────────────────────────────────────────────────── Key ────────────────────────────────────────
──────────────────────
## ✓ Dataset loaded successfully
## › Dataset built with a newer version of Seurat than installed
## ❓ Unknown version of Seurat installed
```

```
library(dplyr) #data structure
library(gt)
library(SPOTlight)
library(igraph)
```

```
##
## Attaching package: 'igraph'
##
## The following objects are masked from 'package:dplyr':
##
##     as_data_frame, groups, union
##
## The following objects are masked from 'package:purrr':
##
##     compose, simplify
##
## The following object is masked from 'package:tidyr':
##
##     crossing
##
## The following object is masked from 'package:tibble':
##
##     as_data_frame
##
## The following object is masked from 'package:GenomicRanges':
##
##     union
##
## The following object is masked from 'package:IRanges':
##
##     union
##
## The following object is masked from 'package:S4Vectors':
##
##     union
##
## The following objects are masked from 'package:BiocGenerics':
##
##     normalize, path, union
##
## The following objects are masked from 'package:stats':
##
##     decompose, spectrum
##
## The following object is masked from 'package:base':
##
##     union
```

```
library(RColorBrewer)
library(NMF)
```

```
## Loading required package: pkgmaker
## Loading required package: registry
##
## Attaching package: 'pkgmaker'
##
## The following object is masked from 'package:S4Vectors':
##
##     new2
##
## Loading required package: rngtools
## Loading required package: cluster
## NMF - BioConductor layer [OK] | Shared memory capabilities [NO: windows] | Cores 15/16
##
## Attaching package: 'NMF'
##
## The following objects are masked from 'package:igraph':
##
##     algorithm, compare
##
## The following object is masked from 'package:S4Vectors':
##
##     nrun
```

```
library(magrittr)
```

```
##
## Attaching package: 'magrittr'
##
## The following object is masked from 'package:purrr':
##
##     set_names
##
## The following object is masked from 'package:tidyr':
##
##     extract
##
## The following object is masked from 'package:GenomicRanges':
##
##     subtract
```

```
#Generate single-cell RNA seq data
count <- read.csv("D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/GSE71585_RefSeq_counts.csv", header=T,row.name
s = 1) #Gene name is in rows, cell number is in column, counts in grid
temp1 <- as.matrix(count) #Convert it as matrix
#Save sparse matrix(sparse matrix is the most of the value is 0)
sparse.gbm <- Matrix(temp1 , sparse = T )
#Create matrix file
writeMM(obj = sparse.gbm, file="matrix_n.mtx")
```

```
## NULL
```

```
#Create features and barcode file
write(x = rownames(sparse.gbm), file = "features_n.tsv")
write(x = colnames(sparse.gbm), file = "barcodes_n.tsv")
#Create Seuratboject
getwd()
```

```
## [1] "D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv"
```

```
data_dir <- 'D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv'
test1<-  Read10X(data.dir = data_dir,gene.column = 1, unique.features = TRUE)
test_seurat <- CreateSeuratObject(counts = test1, project = "seurat", min.cells = 3, min.features = 200)
```

```
## Warning: Feature names cannot have underscores ('_'), replacing with dashes
## ('-')
```

```
#Customaize the format
test_seurat <- FindVariableFeatures(test_seurat, selection.method = "vst", nfeatures = 2000)

#I have to run RunPCA, RunUMAP to create object for spotlight
set.seed(0812)
test_seurat <- Seurat::SCTransform(test_seurat, verbose = FALSE) %>%
  Seurat::RunPCA(., verbose = FALSE) %>%
  Seurat::RunUMAP(., dims = 1:30, verbose = FALSE)
```

```
## Warning: Invalid name supplied, making object name syntactically valid. New
## object name is Seurat..SCTransform.RNA; see ?make.names for more details on
## syntax validity
```

```
## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R-native UWOT using the c
osine metric
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session
```

```
#I have to run FindNeighbors, FindClusters to create object for spotlight
test_seurat <- FindNeighbors(test_seurat, dims=1:10)
```

```
## Computing nearest neighbor graph
## Computing SNN
```

```
test_seurat <- FindClusters(test_seurat,resolution=0.5)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 1809
## Number of edges: 50497
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9325
## Number of communities: 15
## Elapsed time: 0 seconds
```

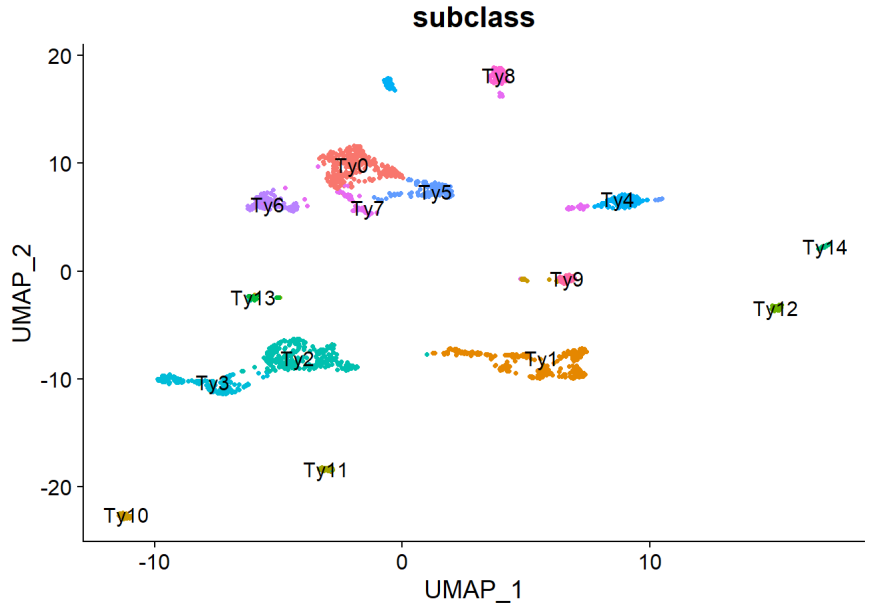
```
#Use levels to find out how many clusters in Seurat object
d<-levels(test_seurat)
d
```

```
##  [1] "0"  "1"  "2"  "3"  "4"  "5"  "6"  "7"  "8"  "9"  "10" "11" "12" "13" "14"
```

```
#Use for loop to name each cluster easily
dl<-length(d)-1 #Cluseter starts at 0
nx<-c()
for (i in 0:dl){
  cc<-paste0('Ty',i)
  nx<-c(nx,cc)
}

#In this case, cell type will be just number, but if you can give cell name

#Name the levels with cell type
new.cluster.ids <- nx
names(new.cluster.ids) <- levels(test_seurat) #Match the levels with cell type
test_seurat <- RenameIdents(test_seurat, new.cluster.ids) #Change the level of  each ident to cell type

#Add the subclass chapter in meta.data
test_seurat[["subclass"]] <- as.character(Idents(object = test_seurat))

saveRDS(object = test_seurat,
        file = here::here("D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/test_seurat_n.rds"))

#Find markers
cluster_markers_all <- Seurat::FindAllMarkers(object = test_seurat,
                                              assay = "SCT",
                                              slot = "data",
                                              verbose = TRUE,
                                              only.pos = TRUE)
```

```
## Calculating cluster Ty0
## Calculating cluster Ty1
## Calculating cluster Ty2
## Calculating cluster Ty3
## Calculating cluster Ty4
## Calculating cluster Ty5
## Calculating cluster Ty6
## Calculating cluster Ty7
## Calculating cluster Ty8
## Calculating cluster Ty9
## Calculating cluster Ty10
## Calculating cluster Ty11
## Calculating cluster Ty12
## Calculating cluster Ty13
## Calculating cluster Ty14
```

```
saveRDS(object = cluster_markers_all,
        file = here::here("D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/markers_sc_n.rds"))

#================================================

#Now apply the SeuratObject in the SPotlight vignette

#install.packages("devtools") #Install it if you need
#devtools::install_github("https://github.com/MarcElosua/SPOTlight/tree/spotlight-0.1.7") #Download the sample_data
#sample_data will saved in "D:WRWR-4.2.1WlibraryWSPOTlight" by default setting

#Load data
path_to_data <- system.file(package = "SPOTlight")
test_seurat <- readRDS(glue::glue("D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/test_seurat_n.rds"))

if (! "stxBrain" %in% SeuratData::AvailableData()[, "Dataset"]) {
  # If dataset not downloaded proceed to download it
  SeuratData::InstallData("stxBrain")
}

# Load data
anterior_a <- SeuratData::LoadData("stxBrain", type = "anterior1")

#check the cluster
Seurat::DimPlot(test_seurat,
                group.by = "subclass",
                label = TRUE) + Seurat::NoLegend()
```




```
#check the cell type
test_seurat@meta.data %>%
  dplyr::count(subclass) %>%
  gt::gt(.[-1, ]) %>%
  gt::tab_header(
    title = "Cell types present in the reference dataset",
  ) %>%
  gt::cols_label(
    subclass = gt::html("Cell Type")
  )
```

```
## Warning in !is.null(rowname_col) && rowname_col %in% colnames(data_tbl):
## 'length(x) = 2 > 1' in coercion to 'logical(1)'
```

Cell types present in the reference dataset	
Cell Type	n
Ty0	297
Ty1	292
Ty10	43
Ty11	40

Cell types present in the reference dataset	
Ty12	36
Ty13	28
Ty14	23
Ty2	286
Ty3	159
Ty4	145
Ty5	126
Ty6	117
Ty7	99
Ty8	74
Ty9	44

```
#I already found the markers at line 77
#Load markers
cluster_markers_all<- readRDS(file = "D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/markers_sc_n.rds")

#SPOTlight Decomposition
set.seed(0812)
spotlight_ls <- spotlight_deconvolution(
  se_sc = test_seurat,
  counts_spatial = anterior_a@assays$Spatial@counts,
  clust_vr = "subclass", # Variable in sc_seu containing the cell-type annotation
  cluster_markers = cluster_markers_all, # Dataframe with the marker genes
  cl_n = 100, # number of cells per cell type to use
  hvg = 3000, # Number of HVG to use
  ntop = NULL, # How many of the marker genes to use (by default all)
  transf = "uv", # Perform unit-variance scaling per cell and spot prior to factorzation and NLS
  method = "nsNMF", # Factorization method
  min_cont = 0 # Remove those cells contributing to a spot below a certain threshold
)
```

```
## [1] "Preparing Gene set"
## [1] "Normalizing count matrix"
## [1] "Seeding initial matrices"
## [1] "Training..."
## [1] "Time to train NMF model was 36.98mins"
## [1] "Deconvoluting spots"
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  |==================================================================|  99%
  |
  |==================================================================| 100%
```
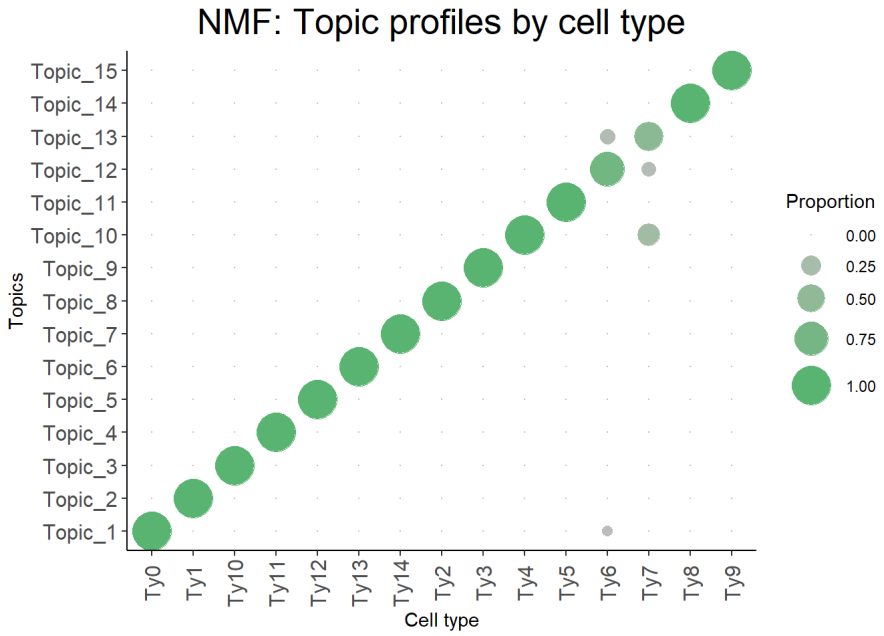
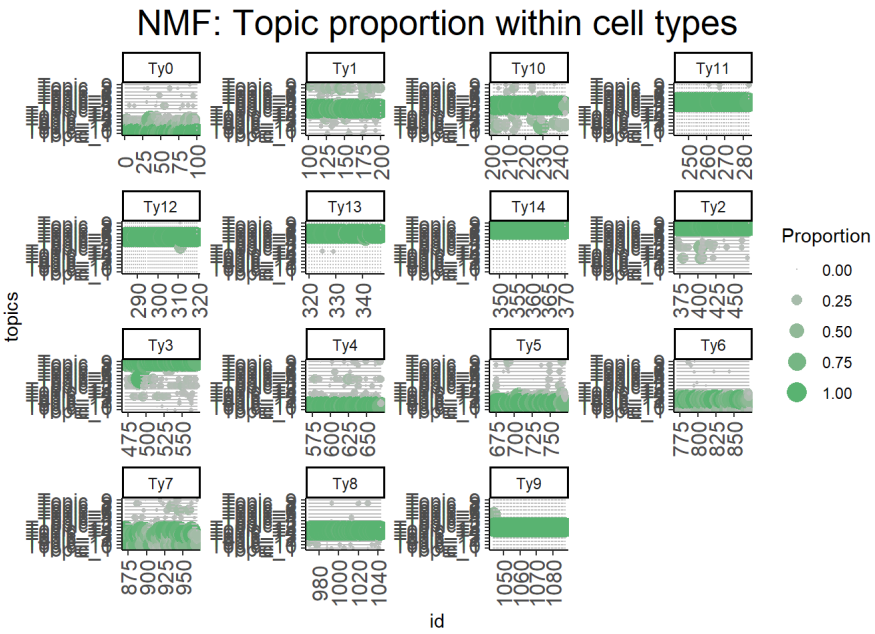

```
saveRDS(object = spotlight_ls, file = here::here("D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/spotlight_ls_n.
rds"))

#Read RDS object
spotlight_ls <- readRDS(file = here::here("D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/spotlight_ls_n.rds"))

nmf_mod <- spotlight_ls[[1]]
decon_mtrx <- spotlight_ls[[2]]

#Assess deconvolution
h <- NMF::coef(nmf_mod[[1]])
rownames(h) <- paste("Topic", 1:nrow(h), sep = "_")
topic_profile_plts_a <- SPOTlight::dot_plot_profiles_fun(
  h = h,
  train_cell_clust = nmf_mod[[2]])

topic_profile_plts_a[[2]] + ggplot2::theme(
  axis.text.x = ggplot2::element_text(angle = 90),
  axis.text = ggplot2::element_text(size = 12))
```




```
#Look at the individual topic profiles
topic_profile_plts_a[[1]] + theme(axis.text.x = element_text(angle = 90),
                                  axis.text = element_text(size = 12))
```




```
#Look at which genes are the most important
basis_spotlight_a <- data.frame(NMF::basis(nmf_mod[[1]]))

colnames(basis_spotlight_a) <- unique(stringr::str_wrap(nmf_mod[[2]], width = 30))

basis_spotlight_a %>%
  dplyr::arrange(desc(Ty0)) %>%
  round(., 5) %>%
  DT::datatable(., filter = "top")
```

```
## Warning in instance$preRenderHook(instance): It seems your data is too big
## for client-side DataTables. You may consider server-side processing: https://
## rstudio.github.io/DT/server.html
```

Show 10 entries Search:

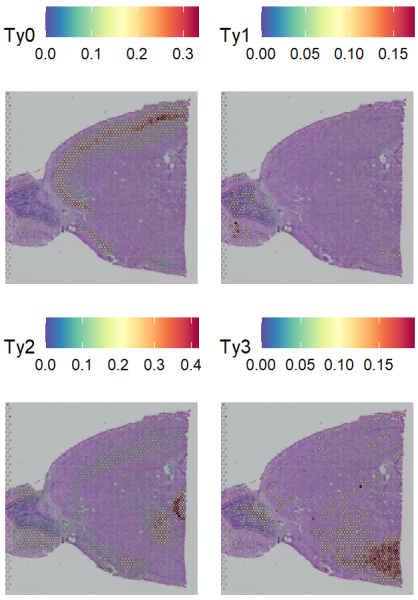
	Ty0	Ty1	Ty10	Ty11	Ty12	Ty13	Ty14	Ty2	Ty3	Ty4
Camk4	0.00074	0.00002	0	0	0	0	0	0	0	0.00023
Krt12	0.00067	0	0	0	0	0	0	0	0	0
Nrxn1	0.00065	0.00028	0	0.00002	0	0.00019	0	0	0.00005	0.00017
Plcxd2	0.00064	0.00011	0	0.00002	0	0	0	0	0	0
Rgs4	0.00063	0	0	0	0	0	0	0	0	0.00004
Coch	0.00063	0	0	0	0	0	0	0	0	0
Nrsn1	0.00062	0.00006	0	0.00015	0	0	0	0.00015	0.00002	0.00005
Foxp1	0.00061	0	0.00011	0	0	0	0	0	0	0.00013
Cacnb4	0.00059	0.00009	0	0.00012	0	0	0	0.00021	0.00027	0.00036
Lrrc4c	0.00058	0.00006	0	0	0	0.00002	0	0.00004	0	0.00013

Showing 1 to 10 of 12,080 entries Previous 1 2 3 4 5 … 1,208 Next

```
#Visualization
# This is the equivalent to setting min_cont to 0.04
decon_mtrx_sub <- decon_mtrx[, colnames(decon_mtrx) != "res_ss"] #Exclude res_ss column
decon_mtrx_sub[decon_mtrx_sub < 0.08] <- 0 #Convert the value that smaller that 0.08 to o
decon_mtrx <- cbind(decon_mtrx_sub, "res_ss" = decon_mtrx[, "res_ss"]) #Add res_ss column again
rownames(decon_mtrx) <- colnames(anterior_a) #Give the spot information

#I guess, with single cell data, we can classify the Ty9, but there is no such cell type in tissue which is used in reference
#I used different single cell data in this project, so probably it can happen

decon_df_a <- decon_mtrx %>%
  data.frame() %>%
  tibble::rownames_to_column("barcodes")

anterior_a@meta.data <- anterior_a@meta.data %>%
  tibble::rownames_to_column("barcodes") %>%
  dplyr::left_join(decon_df_a, by = "barcodes") %>%
  tibble::column_to_rownames("barcodes")

#See the Specific cell-types plot
#View the whole cell type
nx
```

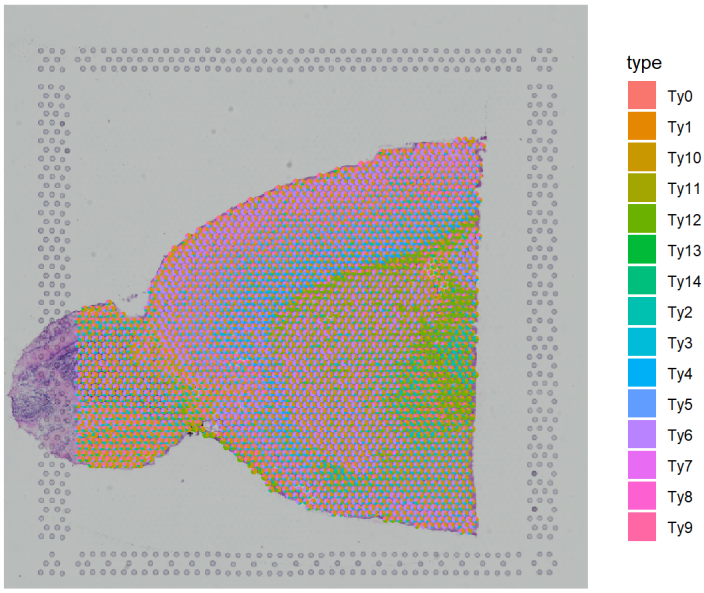
```
##  [1] "Ty0"  "Ty1"  "Ty2"  "Ty3"  "Ty4"  "Ty5"  "Ty6"  "Ty7"  "Ty8"  "Ty9"
## [11] "Ty10" "Ty11" "Ty12" "Ty13" "Ty14"
```

```
#Select cell types
nxs<-nx[1:4] #In this case, Ty1 to Ty4

#Draw the plot
Seurat::SpatialFeaturePlot(
  object = anterior_a,
  features = c(nxs),
  ncol = 2, #Set the display
  alpha = c(0.1, 1)) #Set the opacity
```




```
#Spatial_scatterpie plot will show spots containing at least one of cell types we select
#In this case, Spatial scatterpies for all cell types
cell_types_all_a <- colnames(decon_mtrx)[which(colnames(decon_mtrx) != "res_ss")]
SPOTlight::spatial_scatterpie(se_obj = anterior_a,
                              cell_types_all = cell_types_all_a,
                              img_path = "D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/tissue_lowres_image.pn
g",
                              pie_scale = 0.4)
```

```
## Warning in SPOTlight::spatial_scatterpie(se_obj = anterior_a, cell_types_all =
## cell_types_all_a, : Using slice anterior1
```




```
#Select cell types
nxs1<-nx[1:2] #In this case, Ty1 to Ty4

#Spatial scatterpies for specific cell type
SPOTlight::spatial_scatterpie(se_obj = anterior_a,
                              cell_types_all = cell_types_all_a,
                              img_path = "D:/pankyung/intern_back_up/sub/GSE71585_RefSeq_counts (1).csv/tissue_lowres_image.pn
g",
                              cell_types_interest = nxs1, #Show spots which contain at least Ty0 or Ty1
                              pie_scale = 0.8)
```

```
## Warning in SPOTlight::spatial_scatterpie(se_obj = anterior_a, cell_types_all =
## cell_types_all_a, : Using slice anterior1
```