

Gene-Relevance

immediate

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1 Single Cell RNA-Sequencing data and gene relevance

Libraries

We need of course `destiny`, `scran` for preprocessing, and some tidyverse niceties.

```
[114]: library(conflicted)
library(destiny)
suppressPackageStartupMessages(library(scran))
library(purrr)
library(ggplot2)
```

Data

Let's use data from the `scRNAseq[1]` package. If necessary, install it via `BiocManager::install('scRNAseq')`.

[1] Risso D, Cole M (2019). [scRNAseq: A Collection of Public Single-Cell RNA-Seq Datasets](#).

```
[8]: # The parts of the help we're interested in
help('scRNAseq-package', package = 'scRNAseq') %>% repr::repr_html()
  ↳ %>%
  stringr::str_extract_all(stringr::regex('<p>The dataset.*?</p>',
  ↳ dotall = TRUE)) %>% unlist() %>%
  paste(collapse = ' ') %>% IRdisplay::display_html()
```

379 cells seems sufficient to see something!

```
[136]: data('allen', package = 'scRNAseq')
```

Preprocessing

We'll mostly stick to the [scran vignette](#) here. Let's add basic information to the data and choose what to work with.

As scran expects the raw counts in the counts assay, we rename the more accurate RSEM counts to counts:

```
[137]: allen <- as(allen, 'SingleCellExperiment')
rowData(allen)$Symbol <- rownames(allen)
rowData(allen)$EntrezID <- AnnotationDbi::mapIds(org.Mm.eg.db::org.Mm.
  eg.db, rownames(allen), 'ENTREZID', 'ALIAS')
rowData(allen)$Uniprot <- AnnotationDbi::mapIds(org.Mm.eg.db::org.Mm.
  eg.db, rownames(allen), 'UNIPROT', 'ALIAS', multiVals = 'list')
assayNames(allen)[assayNames(allen) == 'rsem_counts'] <- 'counts'
isSpike(allen, 'ERCC') <- grepl('^ERCC-', rownames(allen))
allen
```

'select()' returned 1:many mapping between keys and columns

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```
class: SingleCellExperiment
dim: 20908 379
metadata(2): SuppInfo which_qc
assays(4): tophat_counts cufflinks_fpkms counts rsem_tpm
rownames(20908): 0610007P14Rik 0610009B22Rik ... Zzef1 Zzz3
rowData names(3): Symbol EntrezID Uniprot
colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR2139336
colData names(22): NREADS NALIGNED ... Animal.ID passes_qc_checks_s
reducedDimNames(0):
spikeNames(1): ERCC
```

Now we can use it to renormalize the data. We normalize the counts using the spike-in size factors and logarithmize them into logcounts.

```
[138]: allen <- computeSpikeFactors(allen)
      allen <- normalize(allen)
```

We also use the spike-ins to detect highly variable genes more accurately:

```
[139]: decomp <- decomposeVar(allen, trendVar(allen, parametric = TRUE))
      rowData(allen)$hvg_order <- order(decomp$bio, decreasing = TRUE)
```

We create a subset of the data containing only reasonably highly variable genes and no spike-ins:

```
[140]: allen_hvg <- subset(allen, hvg_order <= 5000L & !isSpike(allen))
```

Let's create a Diffusion map. For rapid results, people often create a PCA first, which can be stored in your `SingleCellExperiment` before creating the Diffusion map or simply created implicitly using `DiffusionMap(..., n_pcs = <number>)`.

However, even with many more principal components than necessary to get a nicely resolved Diffusion Map, the close spatial correspondence between diffusion components and genes are lost.

```
[75]: #reducedDim(allen_hvg, 'pca') <- irlba::prcomp_irlba(t(assay(allen, _  
  ↪ 'logcounts')), 50)$x
```

The chosen distance metric has big implications on your results, you should try at least cosine and rankcor.

```
[76]: set.seed(1)  
dms <- c('euclidean', 'cosine', 'rankcor') %>% #, 'l2'  
  set_names() %>%  
  map(~ DiffusionMap(allen_hvg, distance = ., knn_params = _  
  ↪ list(method = 'covertree')))
```

Warning message in `DiffusionMap(allen_hvg, distance = ., knn_params = list(method = "covertree"))`:

“You have 4972 genes. Consider passing e.g. `n_pcs = 50` to speed up computation.”

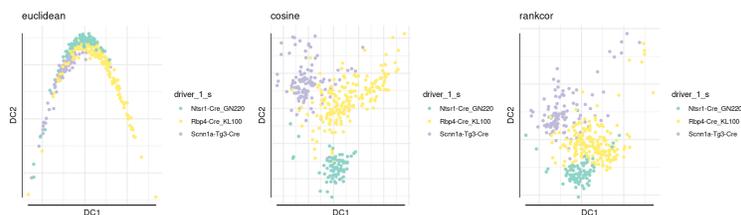
Warning message in `DiffusionMap(allen_hvg, distance = ., knn_params = list(method = "covertree"))`:

“You have 4972 genes. Consider passing e.g. `n_pcs = 50` to speed up computation.”

Warning message in `DiffusionMap(allen_hvg, distance = ., knn_params = list(method = "covertree"))`:

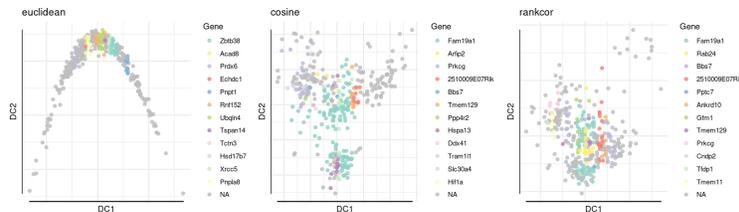
“You have 4972 genes. Consider passing e.g. `n_pcs = 50` to speed up computation.”

```
[77]: options(repr.plot.width = 14, repr.plot.height = 4)  
dms %>%  
  imap(function(dm, dist) plot(dm, 1:2, col_by = 'driver_1_s') +  
  ↪ ggtitle(dist)) %>%  
  cowplot::plot_grid(plotlist = ., nrow = 1)
```



```
[78]: grs <- map(dms, gene_relevance)
```

```
[79]: options(repr.plot.width = 14, repr.plot.height = 4)
gms <- imap(grs, function(gr, dist) plot(gr, iter_smooth = 0) +
  ggtitle(dist))
cowplot::plot_grid(plotlist = gms, nrow = 1)
```



As you can see, despite the quite different embedding, the rankcor and Cosine diffusion Maps display a number of the same driving genes.

```
[116]: gms[-1] %>% map(~ .$ids[1:10]) %>% purrr::reduce(intersect) %>%
  cat(sep = ' ')
```

Fam19a1 Prkcg 2510009E07Rik Bbs7 Tmem129

```
[176]: httr::GET('https://www.uniprot.org/uniprot/', query = list(
  columns = 'id,genes,comment(TISSUE SPECIFICITY)',
  format = 'tab',
  query = rowData(allen)$Uniprot[gms$cosine$ids[1:6]] %>% unlist()
) %>% paste(collapse = ' or ')) %>% httr::content(type = 'text/tab-separated-values', encoding =
  'utf-8', )
```

Parsed with column specification:

```
cols(
  Entry = col_character(),
  Gene names = col_character(),
  Tissue specificity = col_character()
)
```

	Entry <chr>	Gene names <chr>	Tissue specificity <chr>
Aspec_tbl_df: 9×3	Q8K221	Arfp2	NA
	Q6GQU0	NA	NA
	Q7TPG8	Tafa1 Fam19a1	TISSUE SPECIFICITY: Expressed in the hippocampus and
	Q8K304	Tmem129	NA
	P63318	Prkcg Pkcc Pkcg Prkcc	TISSUE SPECIFICITY: Expressed in the cerebellum, cerebr
	Q8K2G4	Bbs7 Bbs2l1	NA
	Q2NKI4	Prkcg Prkcc	NA
	A0A1B0GSM3	Arfp2 mCG_19713	NA
	Q3UN66	Prkcg Prkcc mCG_18472	NA