Gene-Relevance

immediate

November 6, 2019

Contents

1 Single Cell RNA-Sequencing data and gene relevance

Libraries

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

```
In [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.

```
In [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
            paste(collapse = '') %>% IRdisplay::display_html()
```

379 cells seems sufficient to see something!

```
In [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`:

```
In [137]: allen <- as(allen, 'SingleCellExperiment')
          rowData(allen)$Symbol <- rownames(allen)
          rowData(allen)$EntrezID <- AnnotationDbi::mapIds(org.Mm.eg.db::org.Mm.eg.
          rowData(allen)$Uniprot <- AnnotationDbi::mapIds(org.Mm.eg.db::org.Mm.eg.d
          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_fpkm 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`.

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

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

```
In [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 rasonably highly variable genes and no spike-ins:

```
In [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.

In [75]:
```
#reducedDim(allen_hvg, 'pca') <- irlba::prcomp_irlba(t(assay(allen, 'logcc
```

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

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

```
Warning message in DiffusionMap(allen_hvg, distance = ., knn_params = list(method =
“You have 4972 genes. Consider passing e.g. n_pcs = 50 to speed up computation.”
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“You have 4972 genes. Consider passing e.g. n_pcs = 50 to speed up computation.”
```
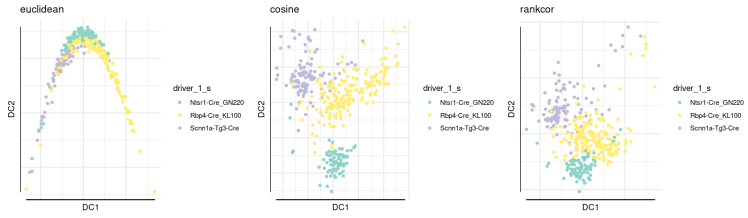
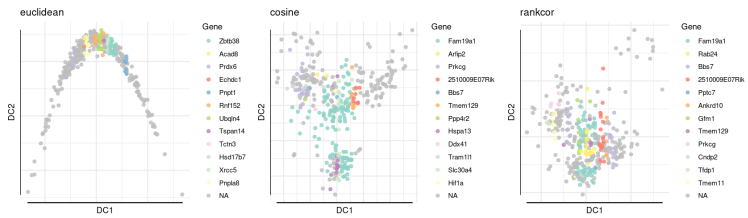

In [77]:
```
options(repr.plot.width = 14, repr.plot.height = 4)
dms %>%
    imap(function(dm, dist) plot(dm, 1:2, col_by = 'driver_1_s') + ggtitle
    cowplot::plot_grid(plotlist = ., nrow = 1)
```



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

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

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

```
Fam19a1 Prkcg 2510009E07Rik Bbs7 Tmem129
```

```
In [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() %>%
          )) %>% httr::content(type = 'text/tab-separated-values', encoding = 'utf-
```

```
Parsed with column specification:
cols(
  Entry = col_character(),
  Gene names = col_character(),
  Tissue specificity = col_character()
)
```

A spec_tbl_df: 9 × 3

Entry	Gene names	Tissue specificity
<chr>	<chr>	<chr>
Q8K221	Arfip2	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, cereb
Q8K2G4	Bbs7 Bbs2l1	NA
Q2NKI4	Prkcg Prkcc	NA
A0A1B0GSM3	Arfip2 mCG_19713	NA
Q3UN66	Prkcg Prkcc mCG_18472	NA