Using vizAPA: a minimal tutorial

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Overview

This tutorial takes a PACdataset object storing a list of poly(A) sites as input and describes some simple but commonly used functions of vizAPA.

Demo PACdataset

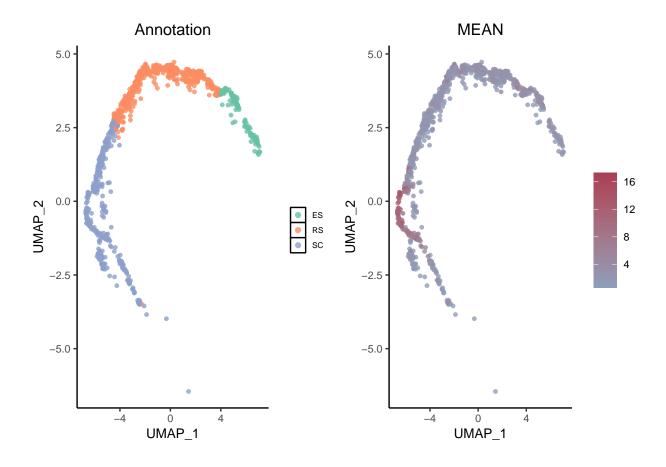
In the package of vizAPA, there is a demo PACdataset object of mouse sperm cells, containing 974 pAs [poly(A) sites] from 413 genes. This PACdataset has been annotated, with both pAs' and cells' meta data.

```
library(vizAPA)
data(scPACds, package='vizAPA')
# summary of the PACdataset
movAPA::summary(scPACds)
## PAC# 974
## sample# 955
## summary of expression level of each PA
     Min. 1st Qu. Median
                              Mean 3rd Qu.
                                              Max.
##
         1
                72
                       957
                              3151
                                      3636
                                             96363
## summary of expressed sample# of each PA
     Min. 1st Qu. Median
##
                              Mean 3rd Qu.
##
      1.00
           64.25 452.50 452.05 810.00 955.00
## gene# 413
##
       nPAC
## 3UTR 974
# cell meta data
head(scPACds@colData)
```

```
orig.ident nCount_RNA nFeature_RNA RNA_snn_res.0.5
##
## AAACCTGAGCTTATCG
                                    23617
                                                   5061
                          gene
                                                                      9
## AAACCTGGTTGAGTTC
                                     19555
                                                   4802
                          gene
## AAACCTGTCAACGAAA
                                                   5009
                                                                      8
                          gene
                                    23467
## AAACGGCACAGGTTT
                          gene
                                     28832
                                                   5484
                                                                      8
## AAACGGTCATTTGGG
                                                   4819
                                                                      8
                          gene
                                    18931
## AAACGGGTCCTCATTA
                                    15734
                                                   3855
                          gene
##
                    seurat_clusters celltype
                                                    UMAP 1
                                                                UMAP 2
## AAACCTGAGCTTATCG
                                  9
                                           RS 0.361751856 4.528803031
                                          RS -0.119255482 4.563224952
## AAACCTGGTTGAGTTC
                                  9
## AAACCTGTCAACGAAA
                                  8
                                          RS 3.023034156 4.074635188
## AAACGGCACAGGTTT
                                  8
                                          RS 3.322863163 3.81046788
                                  8
## AAACGGGTCATTTGGG
                                               4.73071772 3.419416826
## AAACGGGTCCTCATTA
                                  8
                                          ES 5.306060375 3.274766843
##
                             barcode
## AAACCTGAGCTTATCG AAACCTGAGCTTATCG
## AAACCTGGTTGAGTTC AAACCTGGTTGAGTTC
## AAACCTGTCAACGAAA AAACCTGTCAACGAAA
## AAACGGCACAGGTTT AAACGGCACAGGTTT
## AAACGGGTCATTTGGG AAACGGGTCATTTGGG
## AAACGGTCCTCATTA AAACGGGTCCTCATTA
```

Since the dataset already contains cell coordinates of UMAP, it is easy to view the 2D-embeddings of this dataset with vizAPA.

```
vizUMAP(scPACds, group='celltype', xcol='UMAP_1', ycol='UMAP_2')
## vizUMAP: group=celltype, x=UMAP 1, y=UMAP 2
```



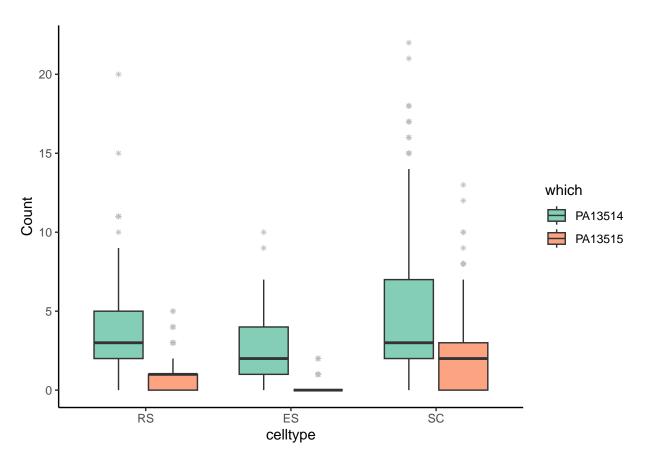
vizStats to summarize APA usages across cell categories

vizStats draws different types of plots, including boxplot, violin plot, dot plot, and bubble plot, to show coordinates and expression (pA count or APA ratio) of given pAs or pAs in a gene across different conditions (e.g., cell types).

Here is an example to plot a boxplot to show the expression levels of pAs in a given gene across cell types. We chose an example gene Odf4 (252868, ENSMUSG00000032921) for demonstration.

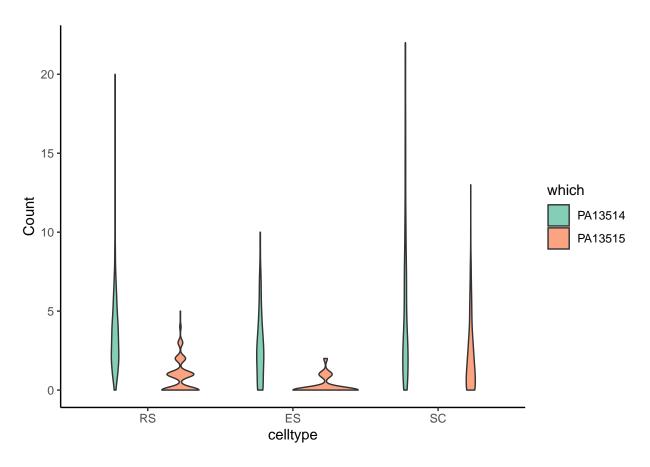
```
## first, we check the gene column in the anno slot
head(scPACds@anno[, c('chr', 'strand', 'coord', 'gene', 'ftr')])
##
             chr strand
                            coord
                                       gene
                                            ftr
## PA6062
           chr17
                         6607918 100040531 3UTR
## PA15501 chr17
                         6648938 100040531 3UTR
## PA6073
                        13376977 100041352 3UTR
           chr17
                        13377115 100041352 3UTR
## PA6074
           chr17
## PA6072
                      + 13377893 100041352 3UTR
           chr17
## PA15866 chr17
                      - 14964150 100041639 3UTR
## the gene ID is entrez id, so we use 252868 instead of Odf4
gene='252868'
```

plot a boxplot to compare the pA usage of this gene in different cell types
vizStats(scPACds, group='celltype', gene=gene, PAs=NULL, figType="box")

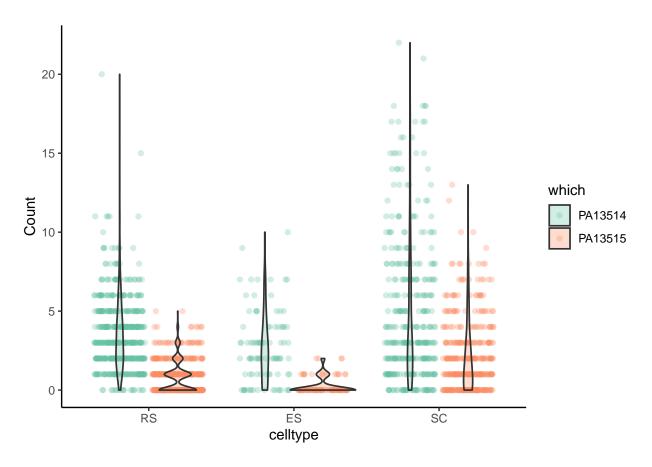


Plot other types of plots.

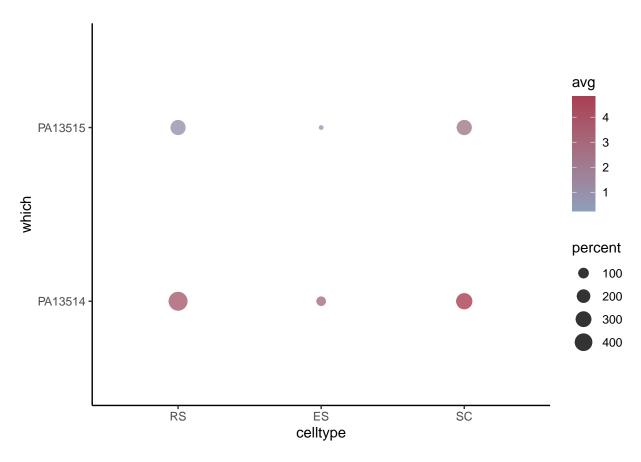
```
# violin plot
vizStats(scPACds, group='celltype', gene=gene, PAs=NULL, figType="violin")
```



violin plot with dots
vizStats(scPACds, group='celltype', gene=gene, PAs=NULL, figType="dot")

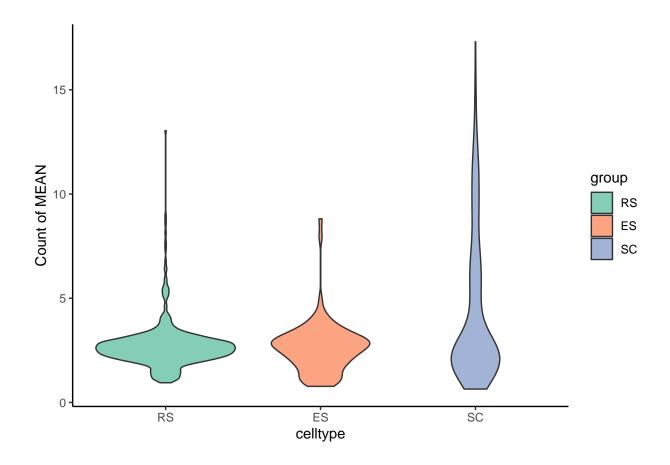


bubble plot
vizStats(scPACds, group='celltype', gene=gene, PAs=NULL, figType="bubble")



If no pA or gene is provided, then it is to plot the mean of all pAs (if it is a pA matrix) or genes (if it is a gene or APA index matrix) in the PACds. Here the PACds is a pA-expression matrix, so vizStats plots the mean value of all pAs across cell types.

```
vizStats(scPACds, group='celltype', figType="violin")
```



vizUMAP to plot 2D-embeddings

gene

gene

AAACGGGTCCTCATTA

vizumap plots a UMAP plot where each point is a cell and it's positioned based on the cell embedding determined by the reduction technique.

```
gene='252868'
# First, we check the coordinate labels of the 2D-embedding.
\# For this data, the labels are UMAP_1 and UMAP_2.
colnames(scPACds@colData)
## [1] "orig.ident"
                          "nCount RNA"
                                             "nFeature_RNA"
                                                                "RNA_snn_res.0.5"
## [5] "seurat_clusters" "celltype"
                                             "UMAP_1"
                                                                "UMAP_2"
## [9] "barcode"
head(scPACds@colData)
                     orig.ident nCount_RNA nFeature_RNA RNA_snn_res.0.5
##
## AAACCTGAGCTTATCG
                                     23617
                                                    5061
                                                                        9
                           gene
## AAACCTGGTTGAGTTC
                                     19555
                                                    4802
                           gene
## AAACCTGTCAACGAAA
                                                    5009
                                                                        8
                           gene
                                     23467
## AAACGGCACAGGTTT
                           gene
                                     28832
                                                    5484
                                                                        8
## AAACGGTCATTTGGG
                                                                        8
                                                    4819
```

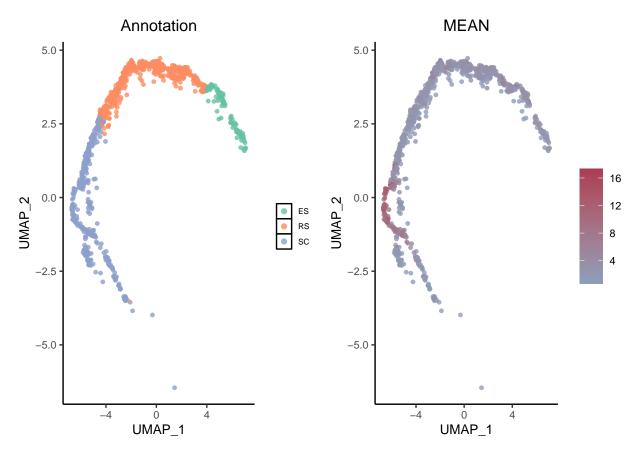
3855

18931

15734

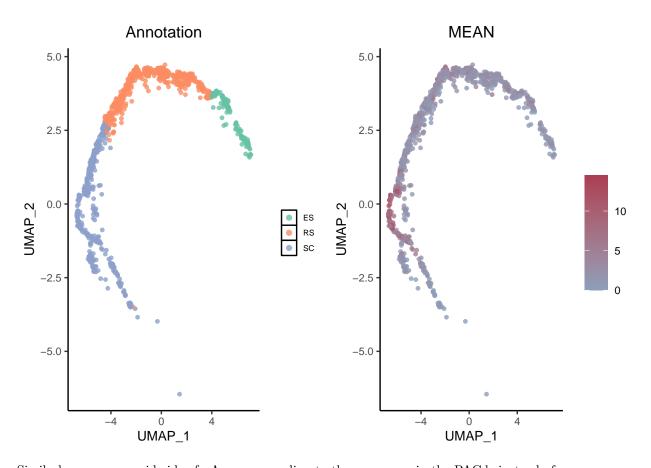
```
##
                    seurat_clusters celltype
                                                    UMAP 1
                                                                UMAP 2
## AAACCTGAGCTTATCG
                                  9
                                              0.361751856 4.528803031
                                          RS
                                  9
                                             -0.119255482 4.563224952
  AAACCTGGTTGAGTTC
  AAACCTGTCAACGAAA
                                  8
                                              3.023034156 4.074635188
  AAACGGCACAGGTTT
                                  8
                                               3.322863163
                                                           3.81046788
  AAACGGGTCATTTGGG
                                  8
                                          ES
                                                4.73071772 3.419416826
## AAACGGGTCCTCATTA
                                  8
                                              5.306060375 3.274766843
##
                             barcode
## AAACCTGAGCTTATCG AAACCTGAGCTTATCG
  AAACCTGGTTGAGTTC AAACCTGGTTGAGTTC
## AAACCTGTCAACGAAA AAACCTGTCAACGAAA
## AAACGGCACAGGTTT AAACGGCACAGGTTT
## AAACGGTCATTTGGG AAACGGGTCATTTGGG
## AAACGGTCCTCATTA AAACGGGTCCTCATTA
# Plot the UMAP plot showing cell clusters and another UMAP plot overlaying
# with the mean expression value of pAs in each cell.
vizUMAP(scPACds, group='celltype', xcol='UMAP_1', ycol='UMAP_2')
```

vizUMAP: group=celltype, x=UMAP_1, y=UMAP_2



Providing a gene id or a list of genes in the gene column of the PACdataset, we can plot a UMAP overlaying with the mean expression value of the gene(s).

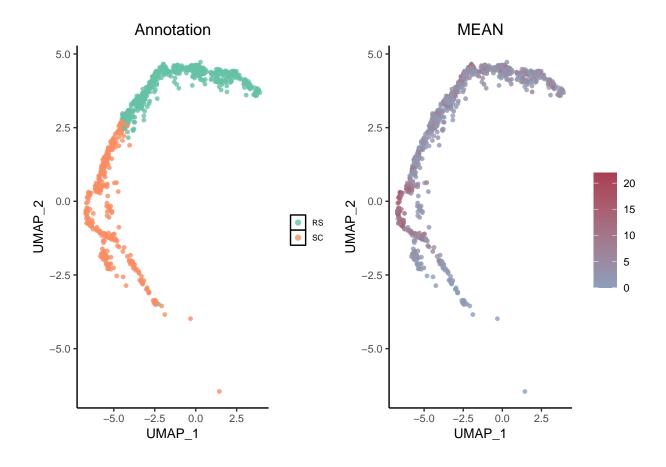
vizUMAP: group=celltype, x=UMAP_1, y=UMAP_2



Similarly, we can provide ids of pAs corresponding to the rownames in the PACds instead of genes.

```
# here we only overlay one pA in Odf4 gene
# here only show two cell types by specifying selGroup
PAids='PA13514'
vizUMAP(scPACds, group='celltype', xcol='UMAP_1', ycol='UMAP_2', selGroups=c('SC','RS'), PAs=PAids)
```

vizUMAP: group=celltype, x=UMAP_1, y=UMAP_2



vizAPAmarkers to visualize APA markers across cell categories

getAPAmarkers to get APA markers

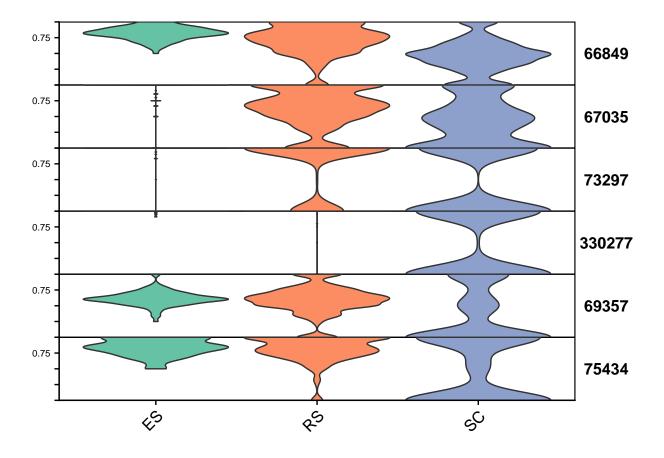
An APA marker is an APA gene with differential APA usage between two pAs in the 3'UTR of the gene. Here we calculate the relative usage of distal pA (RUD) to represent the APA usage of each gene. An larger RUD means the longer 3'UTR. Note: getAPAindexPACds only implements the RUD index in movAPA, users can use movAPA::movAPAindex for more types of APA index.

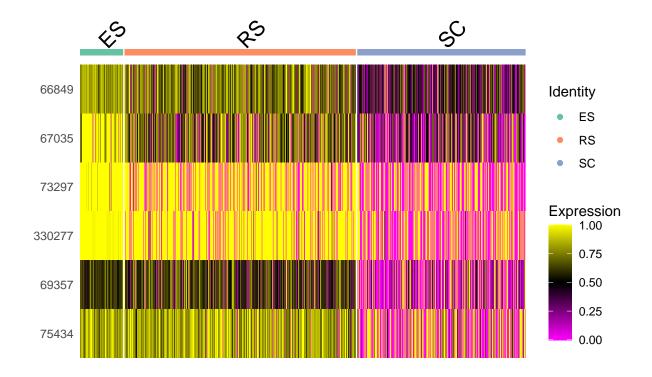
```
# First, calculate the RUD index for each gene.
# Only genes with 3'UTR APA can be used for RUD calculation.
iPACds=getAPAindexPACds(scPACds, choose2PA='PD')
head(iPACds[, 1:10])
## PAC# 6
## sample# 10
## AAACCTGAGCTTATCG AAACCTGGTTGAGTTC AAACCTGTCAACGAAA AAACGGGCACAGGTTT AAACGGGTCATTTGGG ...
  groups:
  @colData...[10 x 9]
                    orig.ident nCount_RNA nFeature_RNA RNA_snn_res.0.5
##
## AAACCTGAGCTTATCG
                                     23617
                                                   5061
                          gene
  AAACCTGGTTGAGTTC
                          gene
                                     19555
                                                   4802
##
                    seurat clusters celltype
                                                    UMAP_1
                                                                UMAP 2
                                           RS 0.361751856 4.528803031
## AAACCTGAGCTTATCG
```

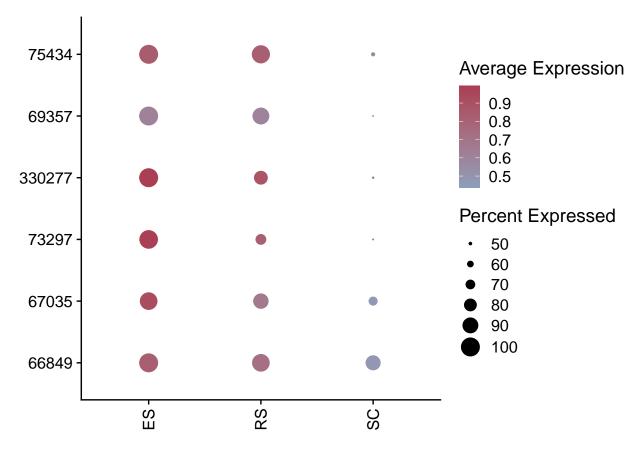
```
## AAACCTGGTTGAGTTC
                                          RS -0.119255482 4.563224952
##
                             barcode
## AAACCTGAGCTTATCG AAACCTGAGCTTATCG
## AAACCTGGTTGAGTTC AAACCTGGTTGAGTTC
## @counts...[6 x 10]
## 2 x 10 sparse Matrix of class "dgCMatrix"
## 100040531 0.4871795 0.5510204 0.3888889 0.6206897 0.8 0.3333333 0.5434783
## 100041352 .
##
## 100040531 0.6027778 0.4788732 0.4814815
## 100041352 .
## @colData...[10 x 9]
                    orig.ident nCount_RNA nFeature_RNA RNA_snn_res.0.5
##
## AAACCTGAGCTTATCG
                                    23617
                                                  5061
                          gene
## AAACCTGGTTGAGTTC
                                    19555
                                                  4802
                                                                      9
                          gene
                                                                UMAP_2
##
                    seurat_clusters celltype
                                                   UMAP_1
## AAACCTGAGCTTATCG
                                  9 RS 0.361751856 4.528803031
## AAACCTGGTTGAGTTC
                                  9
                                          RS -0.119255482 4.563224952
                             barcode
## AAACCTGAGCTTATCG AAACCTGAGCTTATCG
## AAACCTGGTTGAGTTC AAACCTGGTTGAGTTC
## @anno...[6 x 1]
                  gene
## 100040531 100040531
## 100041352 100041352
## @supp...[2]
## dataType row
Then we obtain APA markers by wilcox.test for each pair of cell types.
## obtain APA markers by wilcox.test for each pair of cell types
m=getAPAmarkers(iPACds, group='celltype', everyPair = TRUE)
## PACds row = gene, PACds dataType = ratio
## It seems that PACds is APA ratio, will apply wilcox-test on the APA index to get DE APA events (each
## show marker numbers
table(m$cluster1, m$cluster2)
##
        ES RS SC
##
     ES 0 0 6
##
##
     RS 33 0
               3
     SC 0 0 0
## show marker details
head(m)
##
                  p_val avg_log2FC pct.1 pct.2
                                                  p_val_adj cluster1 cluster2
           7.500958e-33  0.3242110 1.000 0.873 3.097896e-30
## 66849
           9.399927e-28  0.4355351  0.957  0.678  3.882170e-25
                                                                            SC
## 67035
                                                                   F.S
```

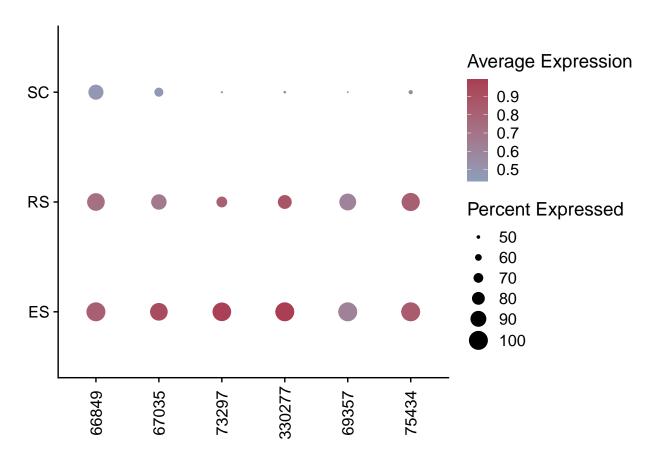
```
## 732971 7.115414e-19 0.4493426 0.989 0.449 2.938666e-16
                                                          ES
                                                                  SC
## 3302771 7.989574e-17 0.4478787 1.000 0.466 3.299694e-14
                                                          ES
                                                                  SC
## 69357
         ES
                                                                  SC
## 754341
         2.128367e-05  0.3191785  1.000  0.518  8.790158e-03
                                                          ES
                                                                  SC
          rowid
## 66849
          66849
          67035
## 67035
## 732971
          73297
## 3302771 330277
## 69357
          69357
## 754341
          75434
```

vizAPAMarkers to visualize multiple APA markers

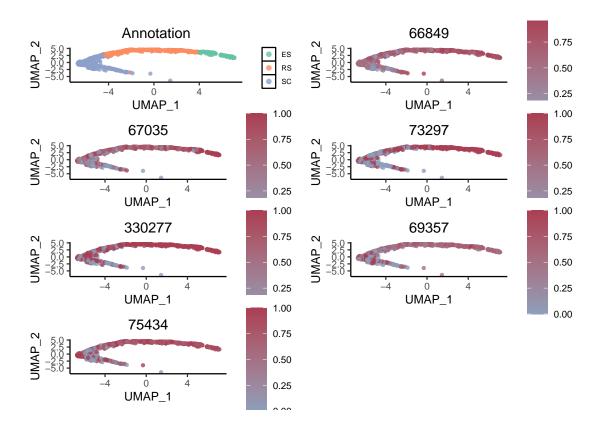








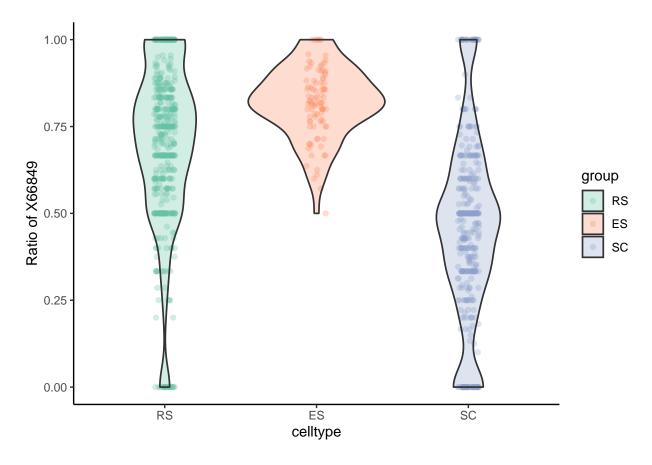
Next, we can plot UMAP for these APA markers.



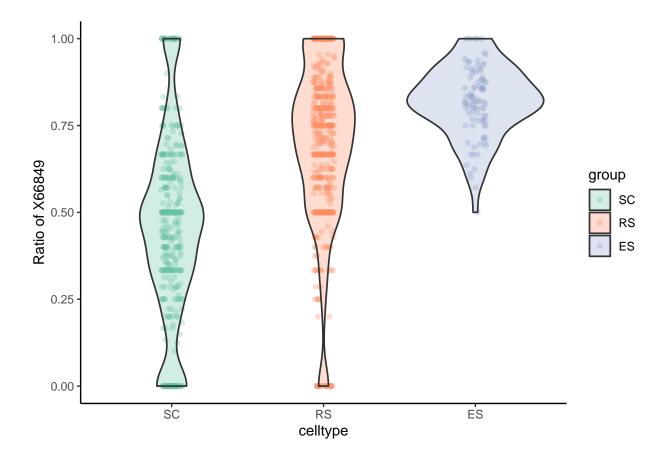
Visualize single APA marker

vizAPAMarkers is used for visualize multiple APA markers. When only one marker is provided, we can use vizStats instead.

```
vizStats(iPACds, group='celltype', figType="dot", gene=m$rowid[1])
```



To change the cell type order
vizStats(iPACds, group='celltype', figType="dot", gene=m\$rowid[1], selGroups=c('SC','RS','ES'))



APA markers for one cell type

The above examples detect markers between every pair of cell types. It is also possible to compare one cell type with all other cells.

```
# Detect markers between ES and all other cells.
m=getAPAmarkers(scPACds, group='celltype', cluster1='ES')
## PACds row = PA, PACds dataType = count
## Warning: it seems that PACds is pA count, will get DE pAs (each row is a pA).
## If you want APA markers, please use getAPAindexPACds() to transform your PACds to APA ratio.
head(m)
##
                  p_val avg_log2FC pct.1 pct.2
                                                   p_val_adj cluster1 cluster2
           7.867291e-71
                          3.046038 1.000 0.311 7.662741e-68
                                                                        non-ES
## PA2955
## PA13906 4.219130e-60
                          1.330360 0.828 0.147 4.109432e-57
                                                                   ES
                                                                        non-ES
           1.747775e-56
                          2.707196 0.989 0.454 1.702333e-53
                                                                   ES
                                                                        non-ES
## PA9536
## PA6248
           8.215108e-56
                          2.500012 1.000 0.558 8.001515e-53
                                                                   ES
                                                                        non-ES
## PA4014
           5.989791e-55
                          2.873583 1.000 0.588 5.834056e-52
                                                                   ES
                                                                        non-ES
## PA7661
           9.854346e-55
                          3.625598 1.000 1.000 9.598133e-52
                                                                        non-ES
##
             rowid
## PA2955
            PA2955
## PA13906 PA13906
```

```
## PA9536  PA9536
## PA6248  PA6248
## PA4014  PA4014
## PA7661  PA7661

table(m$cluster1, m$cluster2)

##
## non-ES
## ES 124
```

vizTracks to plot gene model, pAs and BAM tracks

One unique feature of vizAPA is plotting IGV-like plot, including gene models, pA positions and BAM coverages.

Prepare BAM files

The BAM files and the corresponding index (.bai) files for the following analysis can be downloaded from the GitHub site of vizAPA: mouse.sperm.bam. For demonstration, these BAM files contain only three genes [66514(Asrgl1), 21463 (Tcp11),27058(Srp9)] extracted from the original BAM (accession number: GSM2803334).

Load genome annotation to an annoHub

In vizAPA, the genome annotation is used for the track plots to show gene models in a genomic region. The genome annotation could be retrieved from several sources, including gff3/gtf file, TxDb, EnsDb, BioMart, and OrganismDb. Users can provide one or more annotation sources.

We can make an annoHub object storing different annotation sources, which can be used by many functions in vizAPA. In the following, we used the TxDB annotation for demonstration.

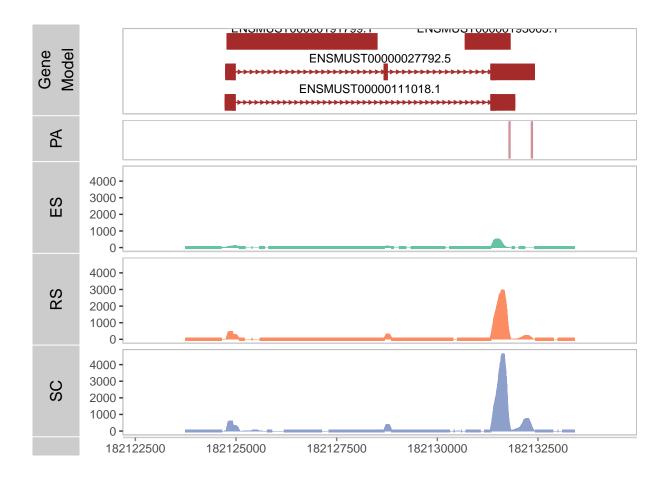
```
annoSource=new("annoHub")
library(TxDb.Mmusculus.UCSC.mm10.knownGene, quietly = TRUE)
txdb=TxDb.Mmusculus.UCSC.mm10.knownGene
annoSource=addAnno(annoSource, txdb)
annoSource

## @annos [annotation sources]:
## txdb=TxDb
## @defaultAnno:
## txdb
```

Plot tracks for a specified gene

Having prepared the PACdataset, annoHub, and BAM files, we can easily plot an example gene (here is the Srp9 gene), with gene model, pA coordinates, and BAM coverages.

```
## Plot tracks for region: chr1:+:182123737:182133407
## Get gene model track from annoSource[ txdb ]...
## Get PACds track...
## chr1:+:182123737:182133407
## Get BAM tracks...
```



Session information

The session information records the versions of all the packages used in the generation of the present document.

sessionInfo()

```
## R version 4.2.2 (2022-10-31 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 22621)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=Chinese (Simplified)_China.utf8
## [2] LC_CTYPE=Chinese (Simplified)_China.utf8
## [3] LC_MONETARY=Chinese (Simplified)_China.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=Chinese (Simplified)_China.utf8
##
## attached base packages:
                           graphics grDevices utils
## [1] stats4
                 stats
                                                         datasets methods
## [8] base
##
```

```
## other attached packages:
   [1] TxDb.Mmusculus.UCSC.mm10.knownGene_3.10.0
   [2] GenomicFeatures 1.50.2
##
   [3] AnnotationDbi_1.60.0
##
   [4] Biobase_2.58.0
##
  [5] GenomicRanges 1.50.1
   [6] GenomeInfoDb 1.34.9
##
   [7] IRanges_2.32.0
##
   [8] S4Vectors_0.36.0
##
   [9] BiocGenerics_0.44.0
## [10] vizAPA_0.1.0
##
## loaded via a namespace (and not attached):
     [1] utf8_1.2.2
##
                                     spatstat.explore_3.0-5
##
     [3] reticulate_1.30
                                     tidyselect_1.2.0
##
     [5] movAPA_0.2.0
                                     RSQLite_2.2.18
##
                                     grid_4.2.2
     [7] htmlwidgets_1.5.4
##
                                     Rtsne 0.16
     [9] BiocParallel 1.32.1
##
    [11] munsell_0.5.0
                                     codetools_0.2-18
    [13] ica_1.0-3
                                     interp_1.1-3
##
   [15] future_1.30.0
                                     miniUI_0.1.1.1
  [17] withr_2.5.0
                                     spatstat.random_3.0-1
##
  [19] colorspace_2.0-3
                                     progressr 0.12.0
##
   [21] filelock 1.0.2
                                     OrganismDbi_1.40.0
  [23] highr_0.9
##
                                     knitr 1.41
  [25] rstudioapi_0.14
                                     Seurat 4.3.0
##
                                     tensor_1.5
   [27] ROCR_1.0-11
##
   [29] ggsignif_0.6.4
                                     listenv_0.9.0
  [31] MatrixGenerics_1.10.0
                                     labeling_0.4.2
  [33] GenomeInfoDbData_1.2.9
                                     polyclip_1.10-4
##
   [35] bit64_4.0.5
                                     farver_2.1.1
##
   [37] parallelly_1.33.0
                                     vctrs_0.5.1
   [39] generics_0.1.3
                                     xfun_0.35
##
   [41] biovizBase_1.46.0
                                     BiocFileCache_2.6.0
##
    [43] R6_2.5.1
                                     AnnotationFilter 1.22.0
##
  [45] spatstat.utils_3.0-1
                                     bitops_1.0-7
   [47] cachem 1.0.6
                                     reshape 0.8.9
##
  [49] DelayedArray_0.24.0
                                     assertthat_0.2.1
   [51] promises_1.2.0.1
                                     BiocIO_1.8.0
##
##
  [53] scales_1.2.1
                                     nnet_7.3-18
  [55] gtable_0.3.1
                                     globals_0.16.2
##
   [57] goftest_1.2-3
                                     ggbio_1.46.0
   [59] ensembldb_2.22.0
                                     rlang_1.0.6
##
  [61] splines_4.2.2
                                     rtracklayer_1.58.0
  [63] rstatix_0.7.1
                                     lazyeval_0.2.2
##
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