scAnno

Introduction

scAnno is an automated annotation tool for single-cell RNA sequencing datasets primarily based on the single cell cluster levels, using a joint deconvolution strategy and logistic regression.

Dependencies

- R version >= 3.5.0.
- R packages: Seurat, dplyr, reticulate, MASS, irlba, future, progress, parallel, glmnet, knitr, rmarkdown, devtools

```
library(scAnno)
```

```
##       Seurat
```

```
## Attaching SeuratObject
```

```
##       dplyr
```

```
## 
##    'dplyr'
```

```
## The following objects are masked from 'package:stats':
## 
##     filter, lag
```

```
## The following objects are masked from 'package:base':
## 
##     intersect, setdiff, setequal, union
```

```
##       reticulate
```

```
#Import human cell type reference profile.
data(Human_cell_landscape)

#Import protein coding gene(19814 genes) to filter reference expression profile.
data(gene.anno)

#Import TCGA bulk data in pan-cancer.
data(tcga.data.u)

#A liver tissue data set to be annotated.
data(GSE136103)
```

Set parameters

Parameters	Description
query	Seurat object, which need to be annotated.
ref.expr	Reference gene expression profile.
ref.anno	Cell type information of reference profile, corresponding to the above `ref.expr`.
save.markers	Specified the filename of makers need to be saved.Default: markers.
cluster.col	Column name of clusters to be annotated in meta.data slot of query Seurat object. Default: seurat_clusters.
factor.size	Factor size for scaling the weight of gene expression. Default: 0.1.
seed.num	Number of seed genes of each cell type for recognizing candidate markers, only used when method = 'co.exp'. Default: 10.
redo.markers	Re-search candidate markers or not. Default: FALSE.
gene.anno	Gene annotation data.frame. Default: gene.anno.
permut.num	Number of permutations for estimating p-values of annotations. Default: 100.
show.plot	Show annotated results or not. Default: TRUE.
verbose	Show running messages or not. Default: TRUE.
tcga.data.u	bulk RNA-seq data of pan-cancer in TCGA.

Preparing data for input

```
# Seurat object, which need to be annotated.
obj.seu <- GSE136103

#Seurat object of reference gene expression profile.
ref.obj <- Human_cell_landscape

#Reference gene expression profile.
ref.expr <- GetAssayData(ref.obj, slot = 'data') %>% as.data.frame

#Cell type information of reference profile, corresponding to the above `ref.expr`.
ref.anno <- Idents(ref.obj) %>% as.character
```

scRNA-seq data annotation

```
results = scAnno(query = obj.seu,
      ref.expr = ref.expr,
      ref.anno = ref.anno,
      save.markers = "markers",
      cluster.col = "seurat_clusters",
      factor.size = 0.1,
      pvalue.cut = 0.01,
      seed.num = 10,
      redo.markers = FALSE,
      gene.anno = gene.anno,
      permut.num = 100,
```

```r
      show.plot = FALSE,
      verbose = TRUE,
      tcga.data.u = tcga.data.u
      )
```

```
## [INFO] Checking the legality of parameters
## [INFO] 30 cell types in reference, 35 clusters in query objects
## [INFO] Searching candidate marker genes...
## [INFO] Deconvolution by using RLM method
## [INFO] Logistic regression for cell-type predictions, waiting...
## [INFO] Merging the scores of both models, and assign annotations to clusters
## [INFO] Estimating p-values for annotations...
## [INFO] Finish!
```

Results

Details of the results is described in the table below.

output	details
query	Seurat object, which need to be annotated.
reference	Seurat object of reference gene expression profile.
pred.label	Cell types corresponding to each cluster.
pred.score	The prediction score for each cluster,corresponding to `pred.label`.
pvals	Significance level of the predicted scores, corresponding to `pred.score`.

```r
results$query
```

```
## An object of class Seurat
## 21898 features across 16036 samples within 1 assay
## Active assay: RNA (21898 features, 2830 variable features)
##  2 dimensional reductions calculated: pca, umap
```

```r
results$reference
```

```
## An object of class Seurat
## 17020 features across 5561 samples within 1 assay
## Active assay: RNA (17020 features, 0 variable features)
```

```r
results$pred.label
```

```
##                    C0                    C1                    C2
##              "T cell"              "T cell"              "T cell"
##                    C3                    C4                    C5
##              "T cell"              "T cell"      "Dendritic cell"
##                    C6                    C7                    C8
##              "T cell"              "T cell"              "T cell"
##                    C9                   C10                   C11
##            "Monocyte"     "Epithelial cell"          "Macrophage"
##                   C12                   C13                   C14
##              "T cell"    "Endothelial cell"            "Monocyte"
##                   C15                   C16                   C17
##    "Endothelial cell"    "Endothelial cell"              "T cell"
##                   C18                   C19                   C20
##          "Macrophage"  "Smooth muscle cell"              "T cell"
##                   C21                   C22                   C23
##  "Smooth muscle cell"              "B cell"            "Monocyte"
##                   C24                   C25                   C26
##              "T cell"              "T cell" "B cell (Plasmocyte)"
##                   C27                   C28                   C29
##      "Dendritic cell"    "Endothelial cell"    "Endothelial cell"
##                   C30                   C31                   C32
##              "B cell"        "Stromal cell"    "Endothelial cell"
##                   C33                   C34
##      "Dendritic cell"     "Epithelial cell"
```

```
results$pred.score
```

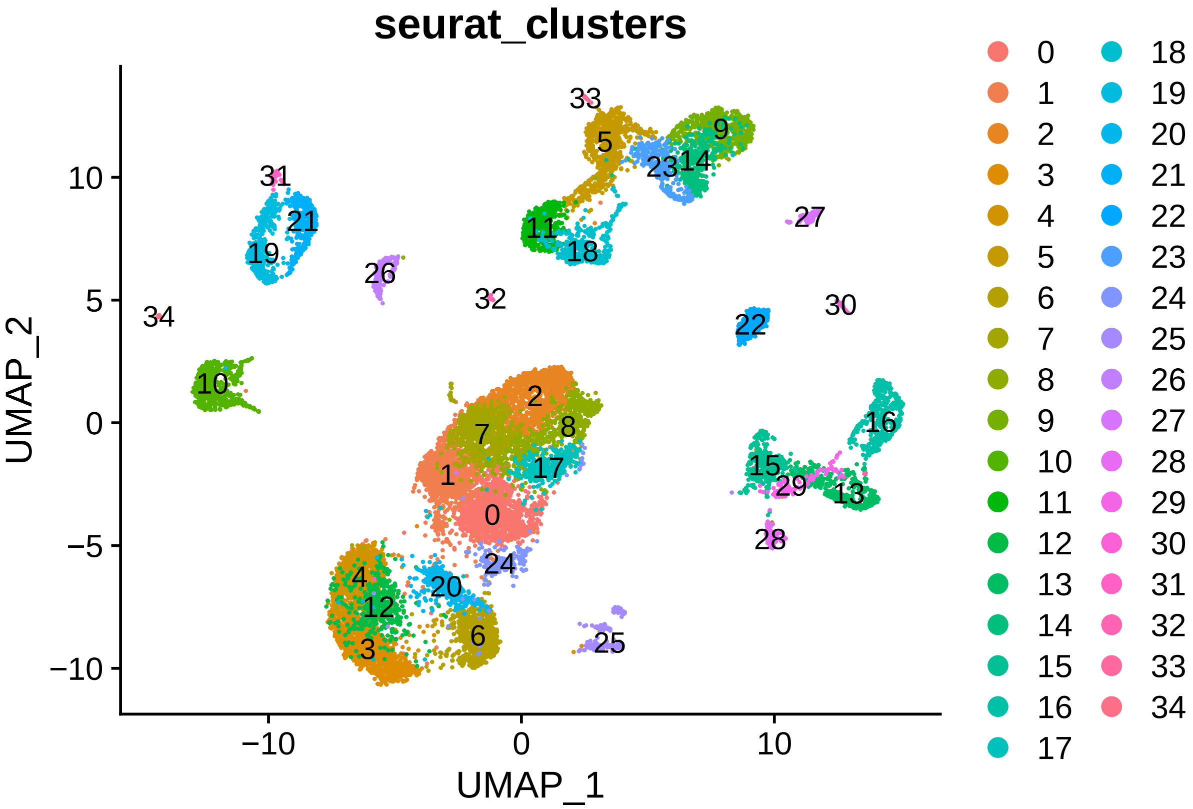
```
##  [1] 0.9999973 0.9994229 0.9982720 1.0000000 1.0000000 0.9987077 0.9999760
##  [8] 0.9988584 0.9987471 0.9997311 0.8919368 0.9956672 0.9999959 0.9989456
## [15] 0.9688465 0.9806819 0.9985180 1.0000000 0.9925140 0.9997775 1.0000000
## [22] 0.9999727 0.9987806 0.6087062 1.0000000 0.9990268 0.9992328 1.0000000
## [29] 0.9986609 0.9993443 0.9852378 0.6264032 0.9825261 1.0000000 1.0000000
```

```
results$pvals
```

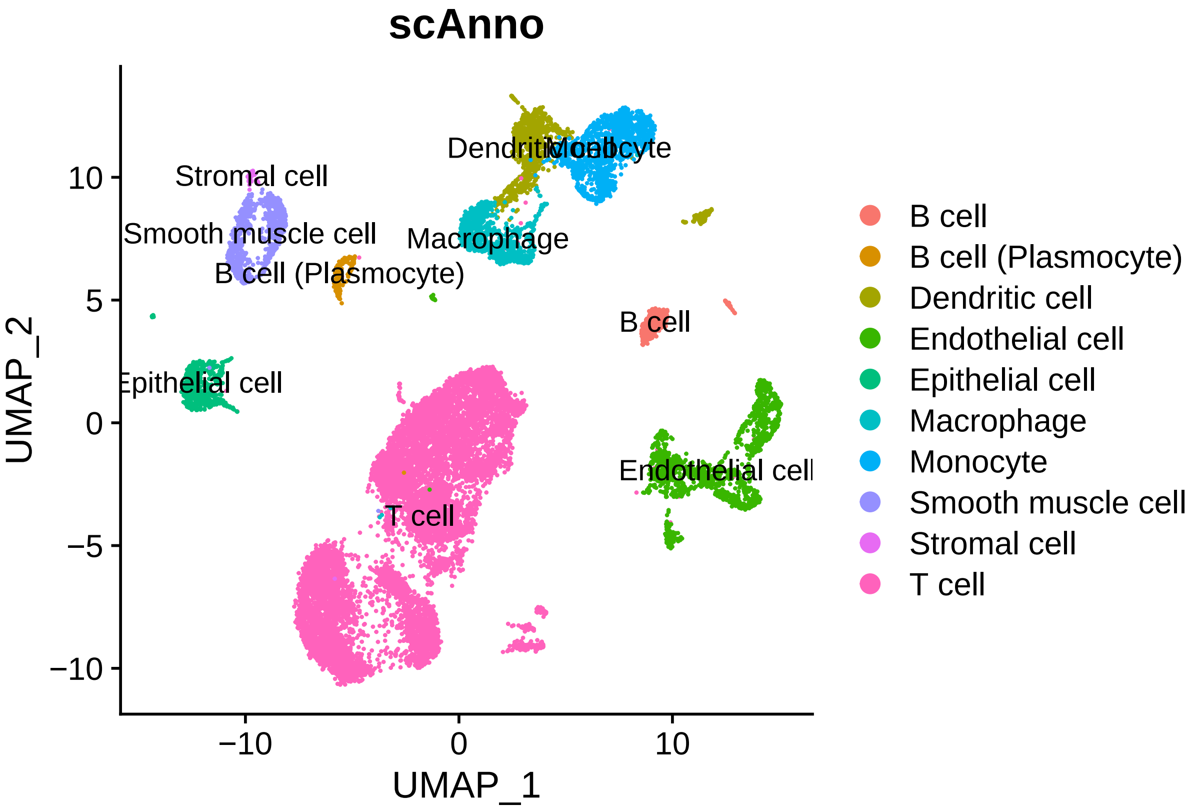
```
##           C0           C1           C2           C3           C4           C5
## 1.742825e-15 4.596242e-15 3.069306e-14 1.734694e-15 1.734694e-15 0.000000e+00
##           C6           C7           C8           C9          C10          C11
## 1.807108e-15 1.175180e-14 1.411631e-14 0.000000e+00 0.000000e+00 0.000000e+00
##          C12          C13          C14          C15          C16          C17
## 1.746914e-15 0.000000e+00 0.000000e+00 0.000000e+00 0.000000e+00 1.734694e-15
##          C18          C19          C20          C21          C22          C23
## 0.000000e+00 0.000000e+00 1.734694e-15 0.000000e+00 0.000000e+00 0.000000e+00
##          C24          C25          C26          C27          C28          C29
## 1.734694e-15 8.894709e-15 0.000000e+00 0.000000e+00 0.000000e+00 0.000000e+00
##          C30          C31          C32          C33          C34
## 0.000000e+00 0.000000e+00 0.000000e+00 0.000000e+00 0.000000e+00
```

Visualization

```
DimPlot(results$query, group.by = "seurat_clusters", label = TRUE)
```



```
DimPlot(results$query, group.by = 'scAnno', label = TRUE)
```



sessionInfo()

```
## R version 4.1.1 (2021-08-10)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=Chinese (Simplified)_China.936
## [2] LC_CTYPE=Chinese (Simplified)_China.936
## [3] LC_MONETARY=Chinese (Simplified)_China.936
## [4] LC_NUMERIC=C
## [5] LC_TIME=Chinese (Simplified)_China.936
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] scAnno_1.0.0       reticulate_1.26    dplyr_1.0.10       SeuratObject_4.1.3
## [5] Seurat_4.3.0
##
## loaded via a namespace (and not attached):
##   [1] Rtsne_0.16             colorspace_2.0-3       deldir_1.0-6
##   [4] ellipsis_0.3.2         ggridges_0.5.4         rstudioapi_0.14
##   [7] spatstat.data_3.0-1    farver_2.1.1           leiden_0.4.3
##  [10] listenv_0.9.0          ggrepel_0.9.2          fansi_1.0.3
##  [13] codetools_0.2-19       splines_4.1.1          knitr_1.42
##  [16] polyclip_1.10-4        jsonlite_1.8.3         ica_1.0-3
##  [19] cluster_2.1.3          png_0.1-7              uwot_0.1.14
##  [22] shiny_1.7.4            sctransform_0.3.5      spatstat.sparse_3.0-0
##  [25] compiler_4.1.1         httr_1.4.5             Matrix_1.5-1
##  [28] fastmap_1.1.0          lazyeval_0.2.2         cli_3.6.0
##  [31] later_1.3.0            prettyunits_1.1.1      htmltools_0.5.4
##  [34] tools_4.1.1            igraph_1.3.5           gtable_0.3.1
##  [37] glue_1.6.2             RANN_2.6.1             reshape2_1.4.4
##  [40] Rcpp_1.0.9             scattermore_0.8        vctrs_0.5.0
##  [43] spatstat.explore_3.0-5 nlme_3.1-157           progressr_0.13.0
##  [46] lmtest_0.9-40          spatstat.random_3.0-1  xfun_0.36
##  [49] stringr_1.5.0          globals_0.16.2         mime_0.12
##  [52] miniUI_0.1.1.1         lifecycle_1.0.3        irlba_2.3.5.1
##  [55] goftest_1.2-3          future_1.32.0          MASS_7.3-57
##  [58] zoo_1.8-11             scales_1.2.1           hms_1.1.2
##  [61] promises_1.2.0.1       spatstat.utils_3.0-2   parallel_4.1.1
##  [64] RColorBrewer_1.1-3     yaml_2.3.6             pbapply_1.7-0
##  [67] gridExtra_2.3          ggplot2_3.4.1          stringi_1.7.8
##  [70] highr_0.10             rlang_1.0.6            pkgconfig_2.0.3
##  [73] matrixStats_0.62.0     evaluate_0.20          lattice_0.20-45
##  [76] ROCR_1.0-11            purrr_0.3.4            tensor_1.5
##  [79] labeling_0.4.2         patchwork_1.1.2        htmlwidgets_1.6.1
##  [82] cowplot_1.1.1          tidyselect_1.2.0       parallelly_1.34.0
##  [85] RcppAnnoy_0.0.20       plyr_1.8.7             magrittr_2.0.3
##  [88] R6_2.5.1               generics_0.1.3         DBI_1.1.3
##  [91] withr_2.5.0            pillar_1.8.1           fitdistrplus_1.1-8
##  [94] survival_3.3-1         abind_1.4-5            sp_1.5-1
##  [97] tibble_3.1.8           future.apply_1.10.0    crayon_1.5.2
## [100] KernSmooth_2.23-20     utf8_1.2.2             spatstat.geom_3.0-3
## [103] plotly_4.10.1          rmarkdown_2.20         progress_1.2.2
## [106] grid_4.1.1             data.table_1.14.4      digest_0.6.30
## [109] xtable_1.8-4           tidyr_1.2.1            httpuv_1.6.6
## [112] munsell_0.5.0          viridisLite_0.4.1
```