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

It should be noted that this package requires interactive use of scikit-learn(version >= 0.24.2) package in Python(version >= 3.7),so users are required to install Python and scikit-learn package in advance.If Python is not installed, we recommend installing anaconda at https://mirrors.tuna.tsinghua.edu.cn/anaconda/archive/.

After downloading and installing

#Check to see if there is a Python environment

Enter in cmd:

python(Enter key)

exiting python(ctrl + z),input:

#Check if a conda environment is available

conda --version

#Check if a package is available in conda and the version of the package

conda list scikit-learn

#If scikit-learn package does not exist,install the package

conda install scikit-learn

scAnno will first search python in the environment.If python exists,the program will call it directly internally. If not, the program will prompt users to enter a python path.

Using python in R

```
library(reticulate)
#Check python path
py.path <- Sys.which("python")

#Here I use conda, so specify the python in conda
py.path
```

```
##                                 python
## "F:\\PROGRA~3\\ANACON~1\\python.exe"
```

```
#Specify the python path
use_python(py.path)
```

```
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
```

```
#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.
pvalue.cut	Threshold for filtering cell type-specific markers. Default: 0.01

Parameters	Description
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,
      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`.

```
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
```

```
results$reference
```

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

```
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] 0.9614642 0.9552271 0.9107099 0.9571242 0.9730700 0.8099739 0.9492166
##  [8] 0.9368682 0.9080971 0.9336424 0.6240831 0.8557046 0.9601383 0.8311389
## [15] 0.8218028 0.7731653 0.8792571 0.9676490 0.8455279 0.9469511 0.9692730
## [22] 0.9220933 0.9120258 0.6453878 0.9683743 0.9072186 0.9325021 0.9689658
## [29] 0.8020389 0.9170581 0.8496184 0.4183305 0.7935830 0.9479270 0.8419836
```

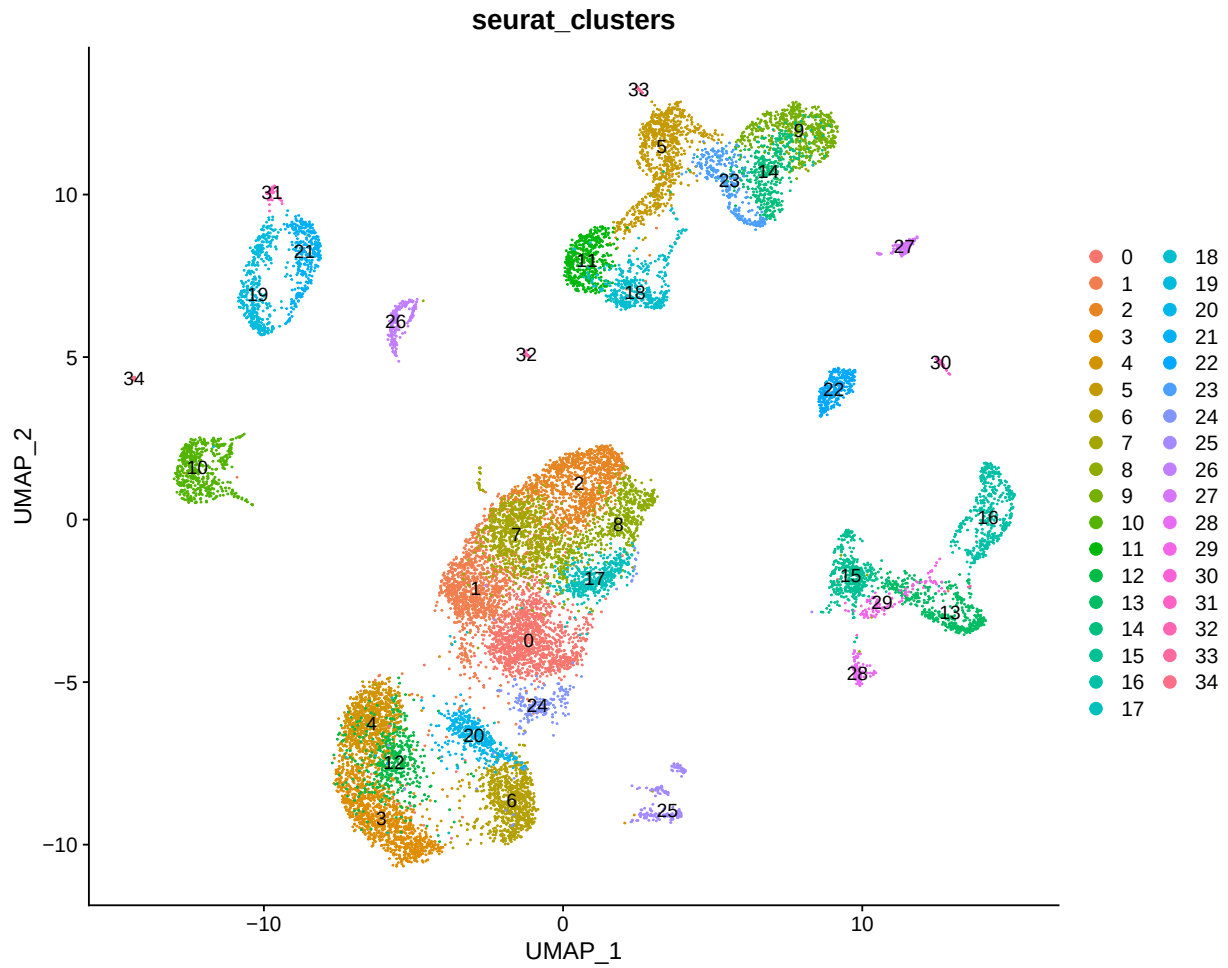
```
results$pvals
```

```
##            C0            C1            C2            C3            C4
##  0.000000e+00 7.893570e-303 1.869963e-247 2.585056e-305  0.000000e+00
##            C5            C6            C7            C8            C9
##  0.000000e+00 5.038966e-295 2.603847e-279 2.223778e-244  0.000000e+00
##           C10           C11           C12           C13           C14
##  0.000000e+00  0.000000e+00  0.000000e+00  0.000000e+00  0.000000e+00
##           C15           C16           C17           C18           C19
##  0.000000e+00  0.000000e+00  0.000000e+00  0.000000e+00  0.000000e+00
##           C20           C21           C22           C23           C24
##  0.000000e+00  0.000000e+00  0.000000e+00  0.000000e+00  0.000000e+00
##           C25           C26           C27           C28           C29
## 2.380358e-243  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

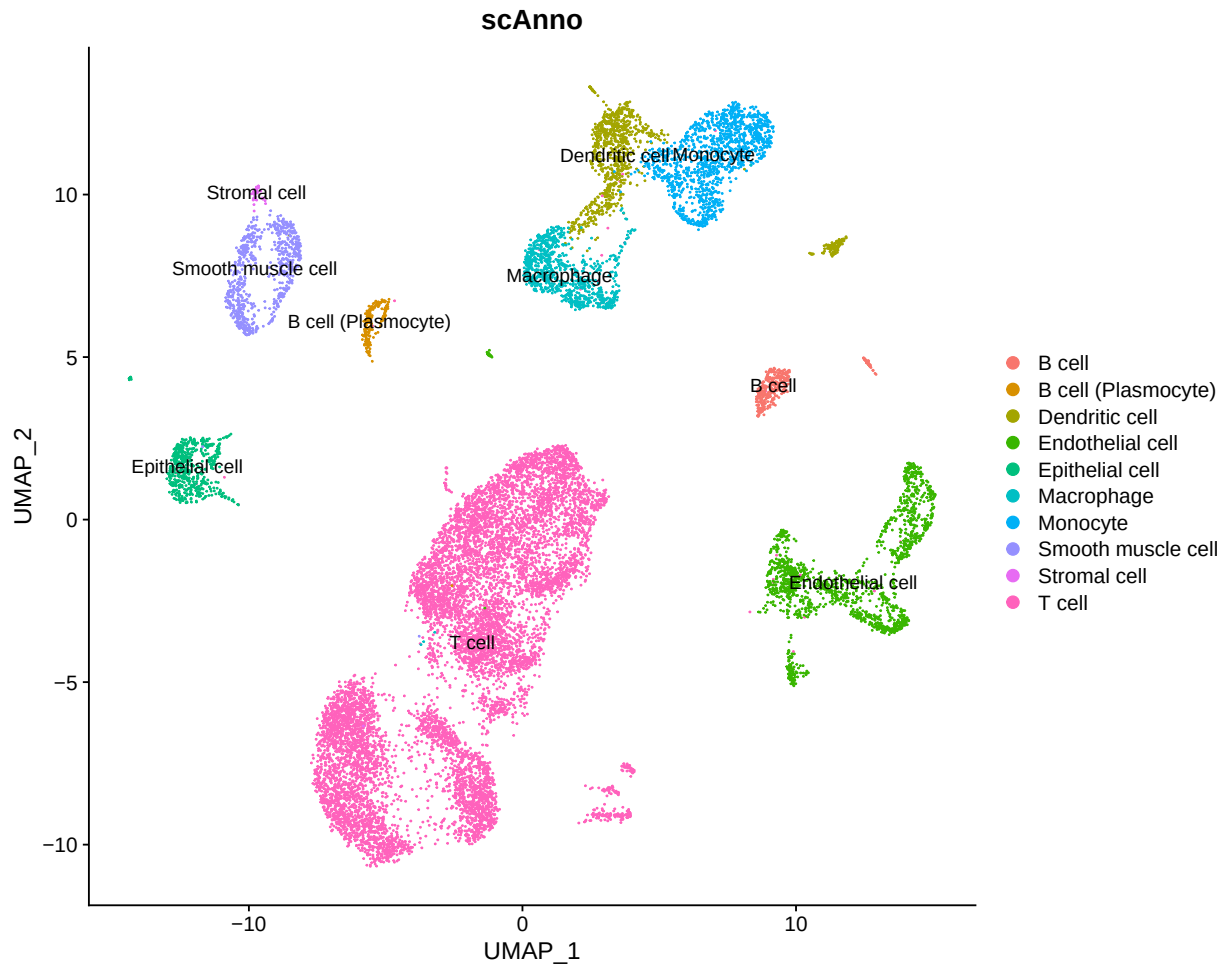
We plot query object in the two-dimensional space of UMAP based on the clustering information of seurat_cluster, which contains a total of 35 clusters.

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



scAnno annotation results visualization,the identity of each cell in the scAnno column of the metadata of query Seurat object.

```
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       dplyr_1.0.10       SeuratObject_4.1.3 Seurat_4.3.0
```

```
## [5] reticulate_1.26
##
## 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_0.3.3
##  [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] nlme_3.1-157           spatstat.explore_3.0-5 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_1.1.3
##  [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_1.1.0            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_1.6.2
##  [82] cowplot_1.1.1          tidyselect_1.2.0       parallelly_1.35.0
##  [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_1.9.0           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
```