3.6 Tutorial-6: Differential Gene analysis in longitudinal data (CNP0001102)

This tutorial allows users to identify differential expressed genes in direction of time-points. As an example single cell data from Zhu et al. 2020 downloaded from CNP0001102. Metadata is downloaded from Supplementary table and curated version can be found in the metadata. The dataset consists of 5 Covid-19 donors, 2 Flu donors with longitudinal data and 3 controls. To explore differetial expressed gened in each celltype of each donor we used hurdle model based modeling on input data to retrieve the DEGs. To infer DEGs in each celltype towards time progression (timepoints considered as continuous if more than 2), please follow following steps.

3.6.1 Load data and clinical metadata (Time ~ 30sec)

Single cell object CNP0001102

```
#Single cell object CNP0001102
pbmc <- readRDS("data/CNP0001102_Final_nCoV_0716_upload.RDS")
#Add column Sample
pbmc@meta.data$Sample <- pbmc@meta.data$batch
```

3.6.2 Clinical annotations Table S1. Clinical data of the enrolled subjects (Time ~ 5sec)

```
metadata <- read.csv("data/CNP0001102-annotation.csv", stringsAsFactors = F)
```

3.6.3 Load library and run (Time ~15 min)

```
#Load Library
library("PALMO")
library("tidyverse")

#Create PALMO object
palmo_obj <- createPALMOobject(anndata=metadata, data=pbmc)

#Assign Sample, PTID and Time parameters
palmo_obj <- annotateMetadata(data_object=palmo_obj,
                              sample_column= "Sample",
                              donor_column= "Participant",
                              time_column= "Day")

#Sample overlap and final matrix
palmo_obj <- mergePALMOdata(data_object=palmo_obj, datatype="singlecell")

#Perform longitudinal differential analysis
palmo_obj <- sclongitudinalDEG(data_object=palmo_obj, scassay="RNA",
                               group_column="cell_type")
>Fitting a ZLM model for donorID:  COV-1 ...
>Fitting a ZLM model for donorID:  COV-2 ...
>Fitting a ZLM model for donorID:  COV-3 ...
>Fitting a ZLM model for donorID:  COV-4 ...
>Fitting a ZLM model for donorID:  COV-5 ...
>Fitting a ZLM model for donorID:  IAV-1 ...
>Fitting a ZLM model for donorID:  IAV-2 ...
```
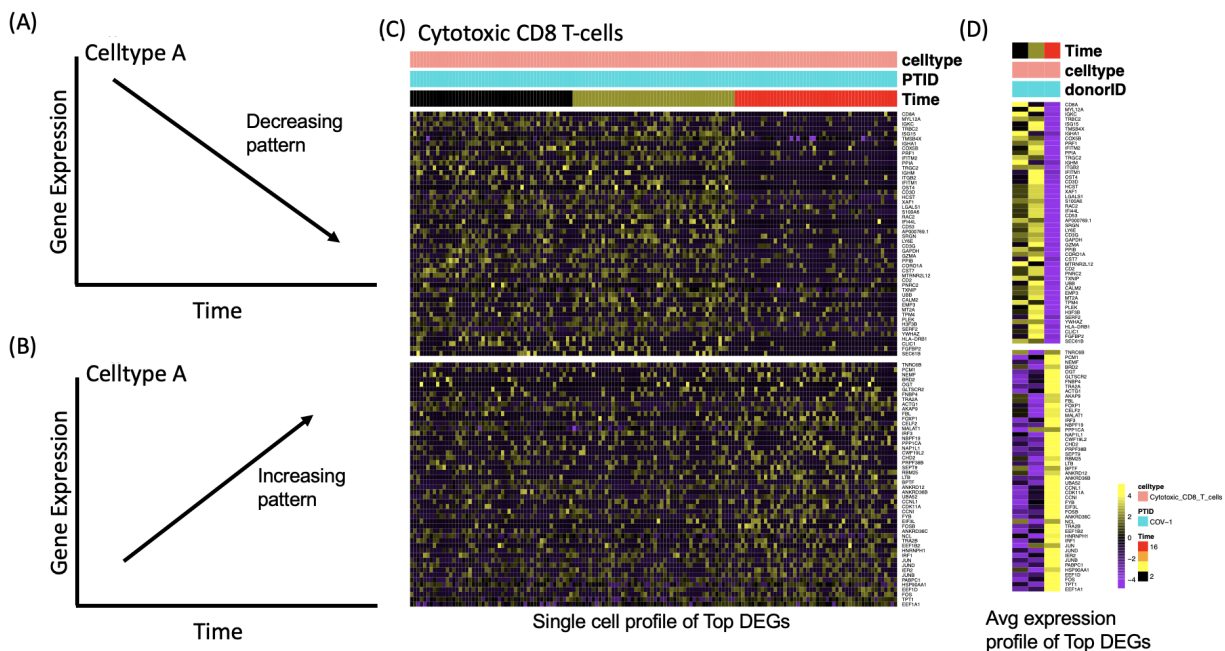
Check output folder for tabular results and visualization.

```
#Plots can be seen in output directory output
DEGres <- palmo_obj@result$degs
head(DEGres[order(DEGres$coef, decreasing = T),])
#primerid contrast       nomP     coef        adjP donorID celltype dir
#IGHG4   TimeD9 1.701579e-26 3.056092 1.453999e-23   IAV-2   Plasma upregulated at D9
#JCHAIN  TimeD9 8.759407e-32 2.647757 2.245474e-28   IAV-2   Plasma upregulated at D9
#IGHG3   TimeD9 8.470810e-22 2.485250 2.412769e-19   IAV-2   Plasma upregulated at D9
#IGLC2   TimeD9 1.352490e-16 2.289544 8.890022e-15   IAV-2   Plasma upregulated at D9
#IGHG1   TimeD9 1.292885e-16 2.219146 8.608601e-15   IAV-2   Plasma upregulated at D9
#SYNE2   TimeD4 7.249010e-13 2.209541 1.215659e-09   COV-4 XCL+_NKs upregulated at D4


#Or interested celltypes
celltype_oi <- c("Activated CD4 T cells", "Naive B cells")
palmo_obj <- sclongitudinalDEG(data_object=palmo_obj, scassay="RNA",
                       group_column="cell_type",
                       group_oi = celltype_oi)
```

General analysis schema and differential results in each donor over timepoints in celltype Cytotoxic CD8 T-cells using PALM shown below.

(A) Celltype A — Decreasing pattern

(B) Celltype A — Increasing pattern

(C) Cytotoxic CD8 T-cells — Single cell profile of Top DEGs

(D) Avg expression profile of Top DEGs

6 Session info

```
sessionInfo()
#> R version 4.0.3 (2020-10-10)
#> Platform: x86_64-apple-darwin17.0 (64-bit)
#> Running under: macOS Catalina 10.15.7
#>
#> Matrix products: default
#> BLAS:   /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
#> LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
#>
#> locale:
#> [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
#>
#> attached base packages:
#> [1] grid      stats     graphics  grDevices utils     datasets  methods
#> [8] base
#>
#> other attached packages:
#> [1] PALMO_0.99.0
#>
#> loaded via a namespace (and not attached):
#>    [1] readxl_1.3.1               backports_1.2.0
#>    [3] circlize_0.4.11           plyr_1.8.6
#>    [5] igraph_1.2.8              lazyeval_0.2.2
#>    [7] splines_4.0.3             listenv_0.8.0
#>    [9] scattermore_0.7           GenomeInfoDb_1.24.2
#>   [11] ggplot2_3.3.5             digest_0.6.28
#>   [13] htmltools_0.5.2           fansi_0.5.0
#>   [15] magrittr_2.0.1            tensor_1.5
#>   [17] cluster_2.1.0             ROCR_1.0-11
#>   [19] ComplexHeatmap_2.4.3      globals_0.14.0
#>   [21] readr_1.4.0               modelr_0.1.8
#>   [23] matrixStats_0.61.0        colorspace_2.0-2
#>   [25] rvest_0.3.6               blob_1.2.1
#>   [27] ggrepel_0.9.1             haven_2.3.1
#>   [29] xfun_0.25                 dplyr_1.0.7
#>   [31] RCurl_1.98-1.2            crayon_1.4.2
#>   [33] jsonlite_1.7.2            lme4_1.1-25
#>   [35] spatstat_1.64-1           spatstat.data_2.1-0
#>   [37] survival_3.2-7            zoo_1.8-9
#>   [39] glue_1.5.0                polyclip_1.10-0
#>   [41] gtable_0.3.0              zlibbioc_1.34.0
#>   [43] XVector_0.28.0            leiden_0.3.9
#>   [45] DelayedArray_0.14.1       GetoptLong_1.0.4
#>   [47] SingleCellExperiment_1.10.1 future.apply_1.8.1
#>   [49] shape_1.4.5               BiocGenerics_0.34.0
#>   [51] abind_1.4-5               scales_1.1.1
#>   [53] pheatmap_1.0.12           DBI_1.1.0
#>   [55] miniUI_0.1.1.1            Rcpp_1.0.7
#>   [57] viridisLite_0.4.0         xtable_1.8-4
#>   [59] clue_0.3-57               reticulate_1.22
#>   [61] stats4_4.0.3              htmlwidgets_1.5.4
```

```
#>  [63] httr_1.4.2                    RColorBrewer_1.1-2
#>  [65] ellipsis_0.3.2                Seurat_4.0.0
#>  [67] factoextra_1.0.7.999          ica_1.0-2
#>  [69] farver_2.1.0                  pkgconfig_2.0.3
#>  [71] uwot_0.1.10                   dbplyr_1.4.4
#>  [73] deldir_1.0-6                  utf8_1.2.2
#>  [75] tidyselect_1.1.1              rlang_0.4.12
#>  [77] reshape2_1.4.4                later_1.3.0
#>  [79] munsell_0.5.0                 cellranger_1.1.0
#>  [81] tools_4.0.3                   generics_0.1.1
#>  [83] broom_0.7.2                   ggridges_0.5.3
#>  [85] evaluate_0.14                 stringr_1.4.0
#>  [87] fastmap_1.1.0                 yaml_2.2.1
#>  [89] goftest_1.2-3                 knitr_1.30
#>  [91] fs_1.5.0                      fitdistrplus_1.1-6
#>  [93] purrr_0.3.4                   RANN_2.6.1
#>  [95] pbapply_1.5-0                 future_1.23.0
#>  [97] nlme_3.1-149                  mime_0.12
#>  [99] xml2_1.3.2                    compiler_4.0.3
#> [101] plotly_4.10.0                 png_0.1-7
#> [103] spatstat.utils_2.2-0          reprex_0.3.0
#> [105] tweenr_1.0.1                  tibble_3.1.6
#> [107] statmod_1.4.35                stringi_1.7.5
#> [109] forcats_0.5.0                 lattice_0.20-41
#> [111] Matrix_1.3-4                  nloptr_1.2.2.2
#> [113] vctrs_0.3.8                   pillar_1.6.4
#> [115] lifecycle_1.0.1               lmtest_0.9-39
#> [117] GlobalOptions_0.1.2           RcppAnnoy_0.0.19
#> [119] bitops_1.0-7                  data.table_1.14.2
#> [121] cowplot_1.1.1                 irlba_2.3.3
#> [123] GenomicRanges_1.40.0          httpuv_1.6.3
#> [125] patchwork_1.1.1               R6_2.5.1
#> [127] promises_1.2.0.1              KernSmooth_2.23-17
#> [129] gridExtra_2.3                 IRanges_2.22.2
#> [131] parallelly_1.28.1             codetools_0.2-16
#> [133] boot_1.3-25                   MASS_7.3-53
#> [135] assertthat_0.2.1              SummarizedExperiment_1.18.2
#> [137] MAST_1.14.0                   rjson_0.2.20
#> [139] SeuratObject_4.0.2            sctransform_0.3.2
#> [141] GenomeInfoDbData_1.2.3        S4Vectors_0.26.1
#> [143] mgcv_1.8-33                   parallel_4.0.3
#> [145] hms_0.5.3                     rpart_4.1-15
#> [147] tidyverse_1.3.0              tidyr_1.1.4
#> [149] minqa_1.2.4                   rmarkdown_2.5
#> [151] Rtsne_0.15                    ggforce_0.3.2
#> [153] Biobase_2.48.0                shiny_1.7.1
#> [155] lubridate_1.7.9
```