Pseudotime

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R Markdown

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When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

Load library

```
library(monocle3,verbose = FALSE)
library(dplyr,verbose = FALSE)
```

Read the data

```
## Loads a sparse matrix RawCountsPseudotime
load("RawCountsPseudotime.rda")
dim(RawCountsPseudotime)
```

```
## [1] 20271  3127
```

Load cluster data

```
## Loads a factor variabele ClusterPseudotime containing cluster identities
load("ClusterPseudotime.rda")
## Change the name of variable to remind
DataClusters <- ClusterPseudotime

table(DataClusters)
```

```
## DataClusters
##        0.0        0.1        1.0        1.1       10.0       10.1       10.2
##        238        213        410         30          0          0         34
##   11.0_1_2       11.3       11.4       12.0       12.1       13.0       14.0
##          0          0          0          0          0          0          0
##       16.0       16.1       17.0       17.1       19.0       19.1        2.0
##        160         42        169         36          0          0        383
##       20.0       22.0       22.1     22.2_3       22.4       26.0       26.1
##          0          0          0          0          0          0          0
```

```
##       26.2     27.0_3       27.1       27.2      27.NA 28.0_2_3_4       28.1
##          0          0          0          0          0          0          0
##       29.0       29.1        3.0        3.1       30.0       31.0       33.0
##          0          0        253        119          0          0         90
##       34.0       35.0       35.1       36.0       37.0       37.1       38.0
##          0          0          0          0          0          0          0
##       39.0        4.0        4.1       40.0       41.0       42.0       42.1
##          0        206        142          0          0         40         27
##       43.0       44.0       45.0       46.0       48.0       49.0        5.0
##          0          0          0          0          0          0          0
##        5.1       50.0       51.0       51.1       52.0       53.0       54.0
##          0          0          0          0          0          0          0
##       55.0       56.0       57.0       59.0        6.0       60.0       61.0
##          0         43          0          0          0          0          0
##       62.0       63.0        7.0        8.0        8.1        8.2        8.3
##          0          0          0          0         77         27         15
##     10.0.0   10.0.1_4   10.0.2_3     10.0.5     10.1.0     10.1.1      8.0.0
##         45         59         57          9         33         21        103
##      8.0.1
##         46
```

```
length(DataClusters)
```

```
## [1] 3127
```

Convert DataClusters to a matrix format for input to Monocle

```
DataCluster.ID <- matrix(as.numeric(levels(DataClusters))[DataClusters], ncol = 1)
```

```
## Warning in matrix(as.numeric(levels(DataClusters))[DataClusters], ncol = 1): NAs
## introduced by coercion
```

```
rownames(DataCluster.ID) <- names(DataClusters)
colnames(DataCluster.ID) <- "Cluster.IDs"
DataCluster.ID[1:10,]
```

```
## cele-001-008.GATCAGTCAT cele-001-027.ACTCCGCCAA cele-001-042.TTCCTAGACC
##                       0                       0                       0
## cele-001-046.TTCTACGCCA cele-001-047.TTCGCTGCCT cele-001-047.ATGGAAGCAT
##                       0                       0                       0
## cele-001-064.AAGCTGACCT cele-001-065.GCCATCAACT cele-001-068.ACGGCAACCA
##                       0                       0                       0
## cele-001-071.GTCATTGCGC
##                       0
```

Generate matrix of gene short names for Monocle

```
geneNames <- matrix(rownames(RawCountsPseudotime), ncol = 1)
rownames(geneNames) <- rownames(RawCountsPseudotime)
colnames(geneNames) <- "gene_short_name"
head(geneNames)
```

```
##       gene_short_name
```

```
## aap-1 "aap-1"
## aat-1 "aat-1"
## aat-2 "aat-2"
## aat-3 "aat-3"
## aat-4 "aat-4"
## aat-5 "aat-5"
```

Initiate Monocle object

```
cds <- new_cell_data_set(expression_data = RawCountsPseudotime,
                         cell_metadata = DataCluster.ID,
                         gene_metadata = geneNames)


cds <- cds[,names(ClusterPseudotime[ClusterPseudotime %in% c("16.0","16.1")])] #previous trajectories
# cds <- cds[,names(ClusterPseudotime[ClusterPseudotime %in% c("3.0",
#                                                              "3.1",
#                                                              "4.0",
#                                                              "4.1",
#                                                              "16.0",
#                                                              "16.1",
#                                                              "17.0",
#                                                              "17.1",
#                                                              "0.0",
#                                                              "0.1",
#                                                              "1.0",
#                                                              "1.1",
#                                                              "2.0",
#                                                              "33.0",
#                                                              "42.0",
#                                                              "42.1",
#                                                              "56.0",
#                                                              "8.0.0",
#                                                              "8.0.1",
#                                                              "8.1",
#                                                              "8.2",
#                                                              "8.3",
#                                                              "10.0.0",
#                                                              "10.0.1_4",
#                                                              "10.0.2_3",
#                                                              "10.0.5",
#                                                              "10.1.0",
#                                                              "10.1.1",
#                                                              "10.2",
#                                                              "56.0")])] #all data for trajectories



colData(cds)
```

```
## DataFrame with 202 rows and 2 columns
##                          Cluster.IDs       Size_Factor
##                            <numeric>         <numeric>
```

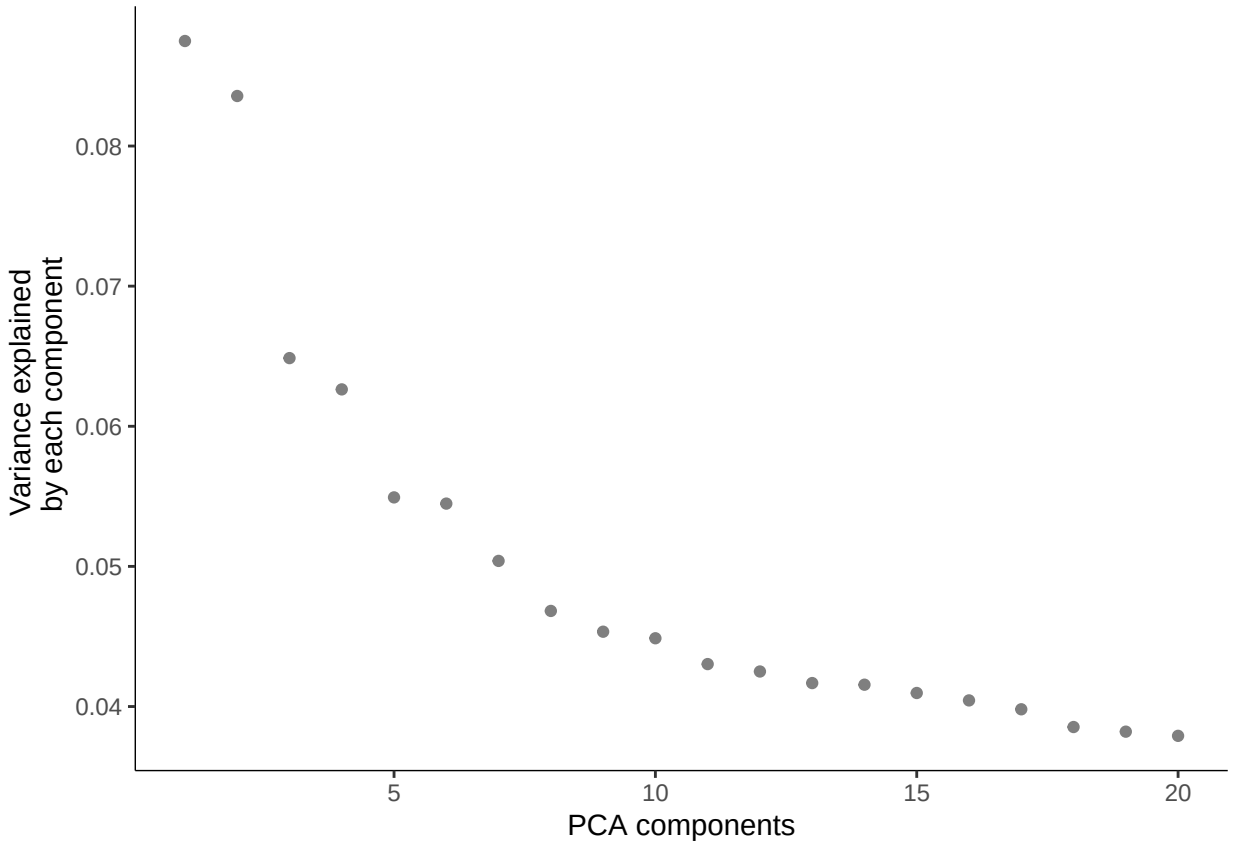
```
## cele-001-009.ATCCGTTAGC          16  1.94844648879067
## cele-001-017.CTGAAGAGAC          16 0.575984181443893
## cele-001-017.CCATCGGACC          16  2.11044203982177
## cele-001-026.TCCGGTAATC          16  2.06994315206399
## cele-001-041.TTCAAGAATC          16  1.23296613840333
## ...                             ...               ...
## cele-010-037.GCAGCGGACT        16.1  4.10838716920527
## cele-010-043.ACTCGACGCC        16.1  4.64837233930892
## cele-010-053.TGCCTAACTT        16.1  5.83633971353695
## cele-010-065.CGCATCCATC        16.1  4.34688061933438
## cele-010-065.TGCGCGATGC        16.1  5.84983934278954
```

```
## Column Cluster.IDs contains the original DataCluster IDs
colData(cds)$Cluster.IDs <- factor(colData(cds)$Cluster.IDs)
colData(cds)
```

```
## DataFrame with 202 rows and 2 columns
##                          Cluster.IDs       Size_Factor
##                             <factor>         <numeric>
## cele-001-009.ATCCGTTAGC          16  1.94844648879067
## cele-001-017.CTGAAGAGAC          16 0.575984181443893
## cele-001-017.CCATCGGACC          16  2.11044203982177
## cele-001-026.TCCGGTAATC          16  2.06994315206399
## cele-001-041.TTCAAGAATC          16  1.23296613840333
## ...                             ...               ...
## cele-010-037.GCAGCGGACT        16.1  4.10838716920527
## cele-010-043.ACTCGACGCC        16.1  4.64837233930892
## cele-010-053.TGCCTAACTT        16.1  5.83633971353695
## cele-010-065.CGCATCCATC        16.1  4.34688061933438
## cele-010-065.TGCGCGATGC        16.1  5.84983934278954
```

Step 1: Normalize and pre-process the data

```
cds <- preprocess_cds(cds, num_dim = 20)
plot_pc_variance_explained(cds)
```



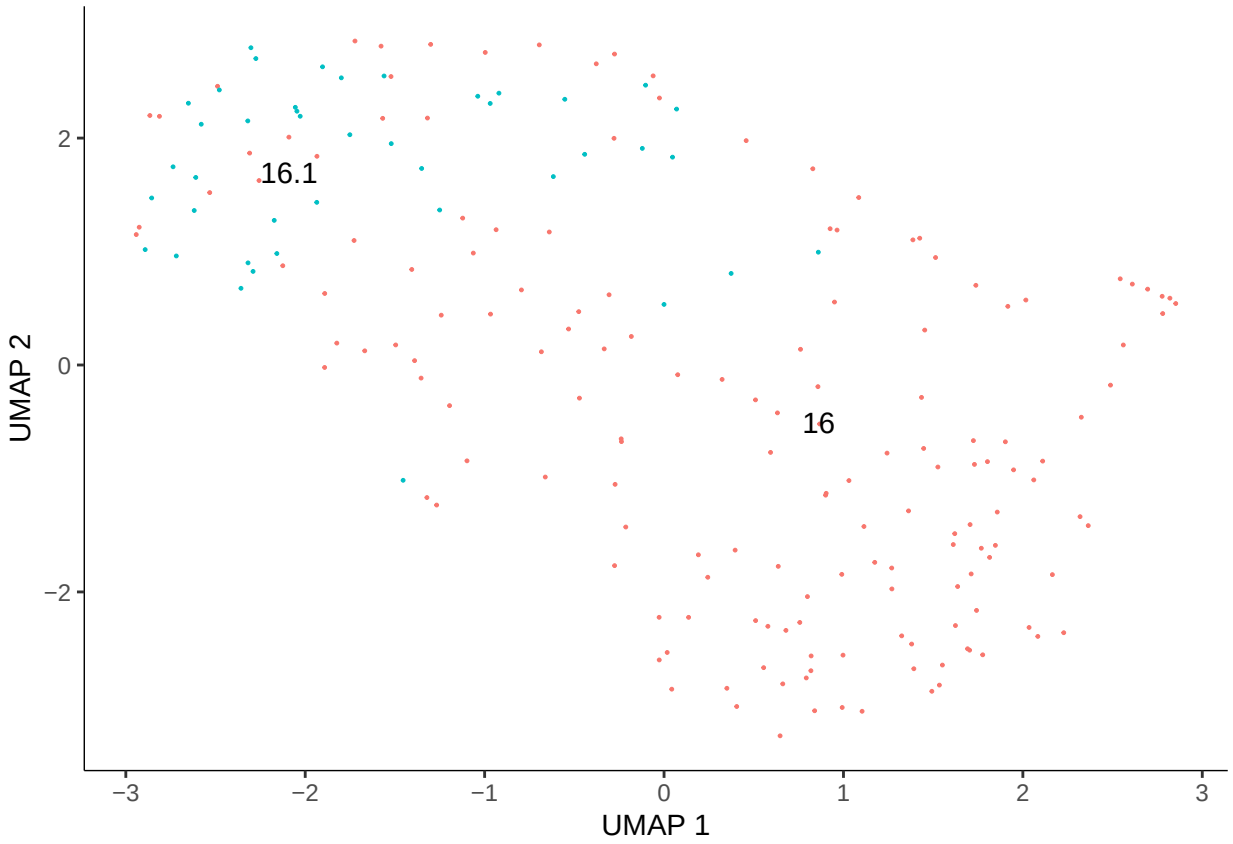
Step 2: Reduce the dimensions using UMAP

```
cds <- reduce_dimension(cds,umap.min_dist = 0.1,cores = 8)
```

```
## No preprocess_method specified, using preprocess_method = 'PCA'
## Note: reduce_dimension will produce slightly different output each time you run it unless you set 'u
```

```
plot_cells(cds, color_cells_by = "Cluster.IDs", group_label_size = 4, cell_size = 0.5)
```

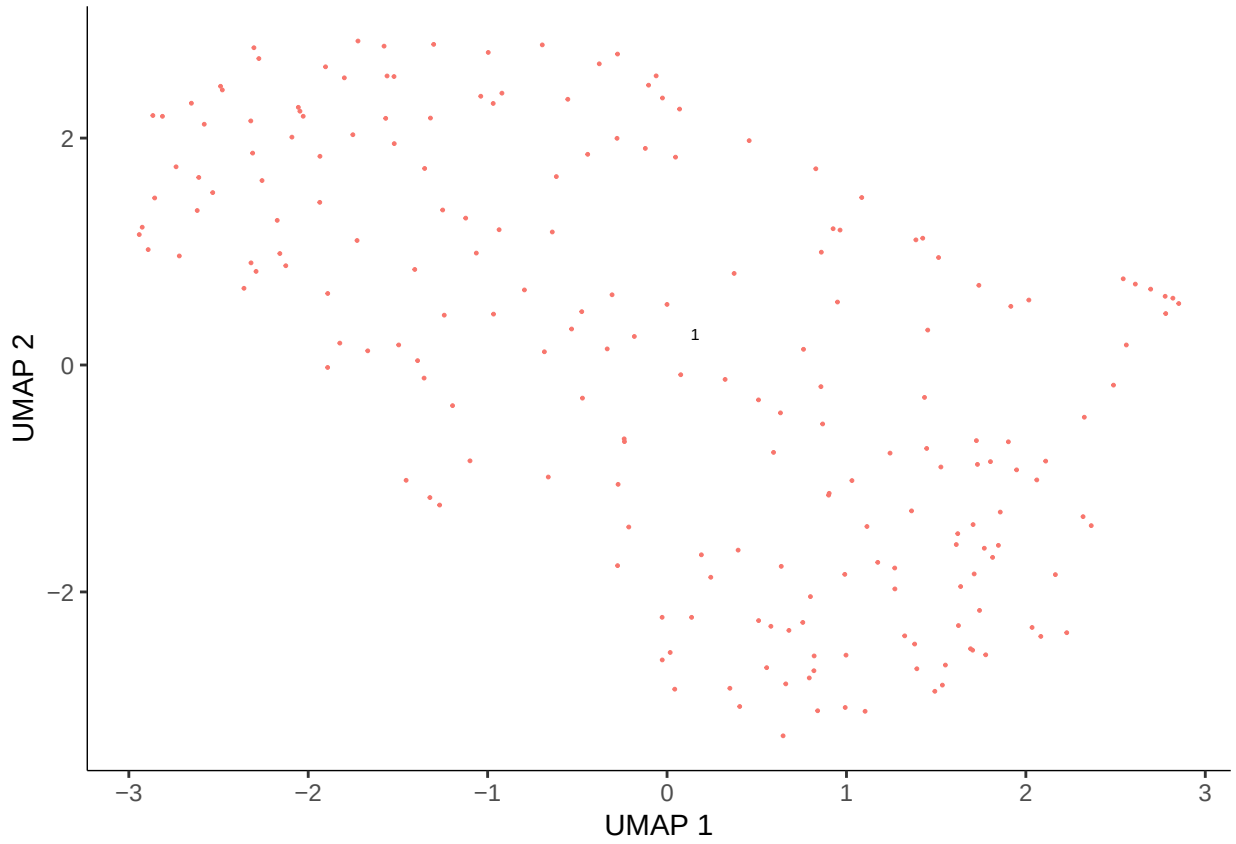
```
## No trajectory to plot. Has learn_graph() been called yet?
```



##Step 3: Cluster the cells

```
# cds = cluster_cells(cds, resolution=0.02)
cds = cluster_cells(cds, resolution=0.02)
plot_cells(cds, cell_size = 0.5)
```

```
## No trajectory to plot. Has learn_graph() been called yet?
```



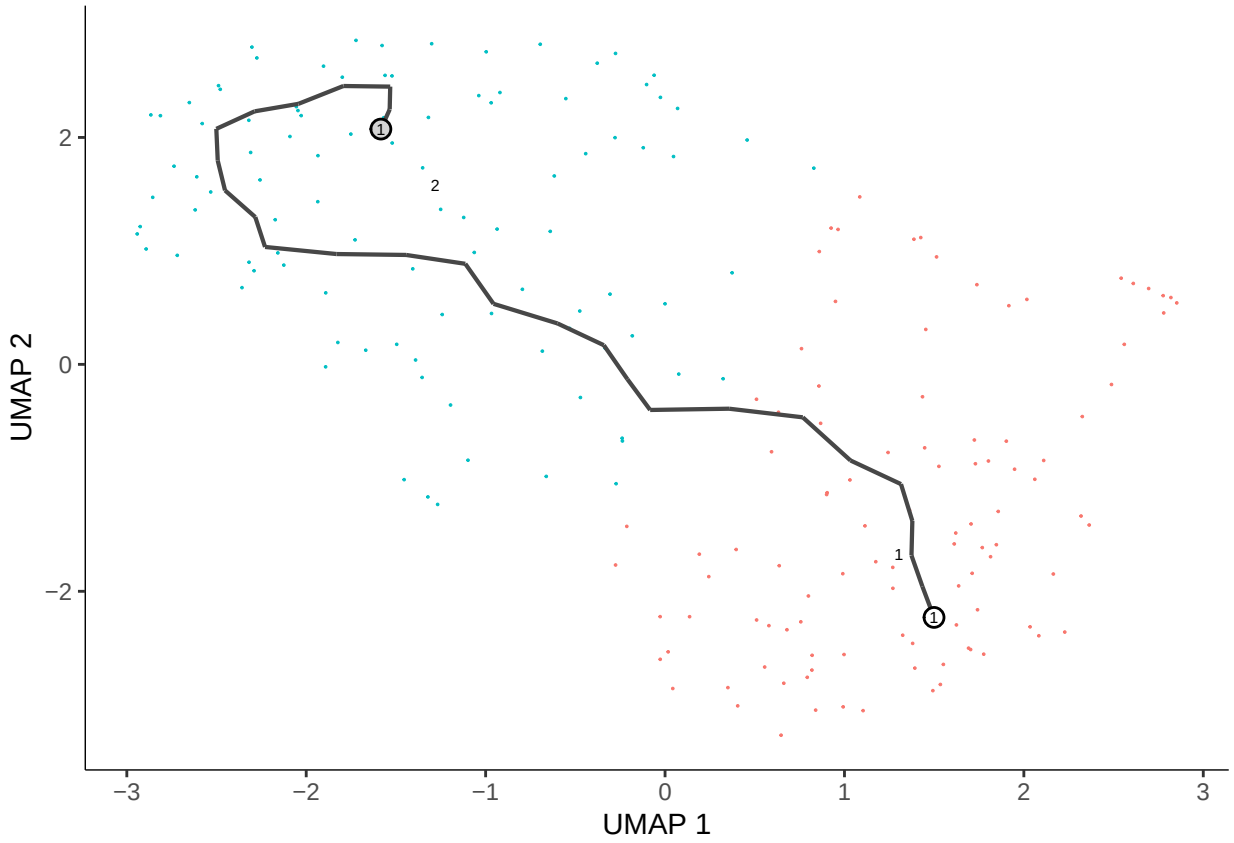
Step 4: Learn a graph and order cells

```
cds <- learn_graph(cds)
```

```
## With Shiny
# cds <- order_cells(cds)
# #
# save(file = "cdsRoots.rda", cds) #Save object state after selection of roots

## Without Shiny
load("cdsRoots.rda") #Load object

## Get info by: cds@principal_graph_aux$UMAP$root_pr_nodes

# cds <- order_cells(cds, root_pr_nodes = c("Y_8", "Y_9", "Y_12", "Y_14", "Y_15", "Y_31", "Y_34", "Y_43
# cds <- order_cells(cds, root_pr_nodes = c("Y_4",   "Y_17",  "Y_30",  "Y_33",  "Y_46",  "Y_84",  "Y_12

print(cds@principal_graph_aux$UMAP$root_pr_nodes)
```

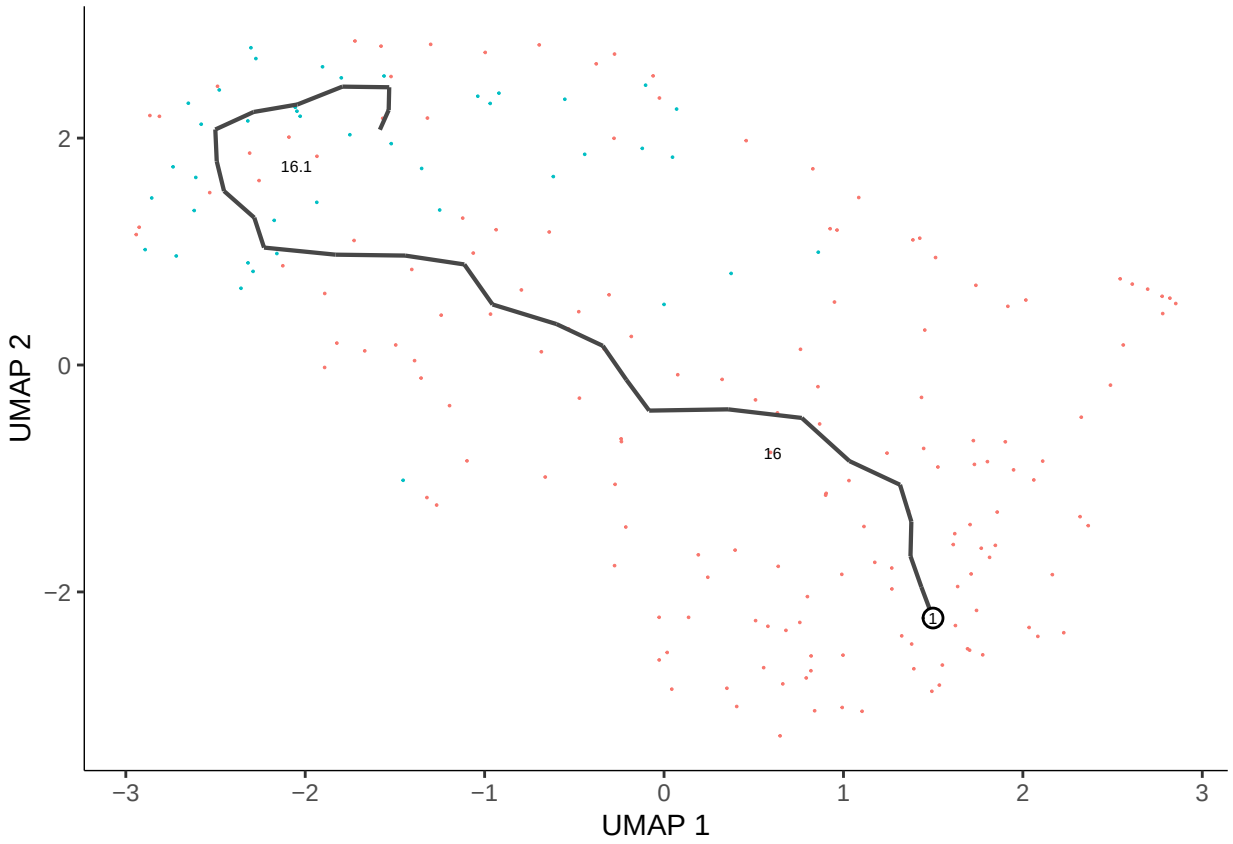
```
## [1] "Y_11"
```

```
plot_cells(cds)
```



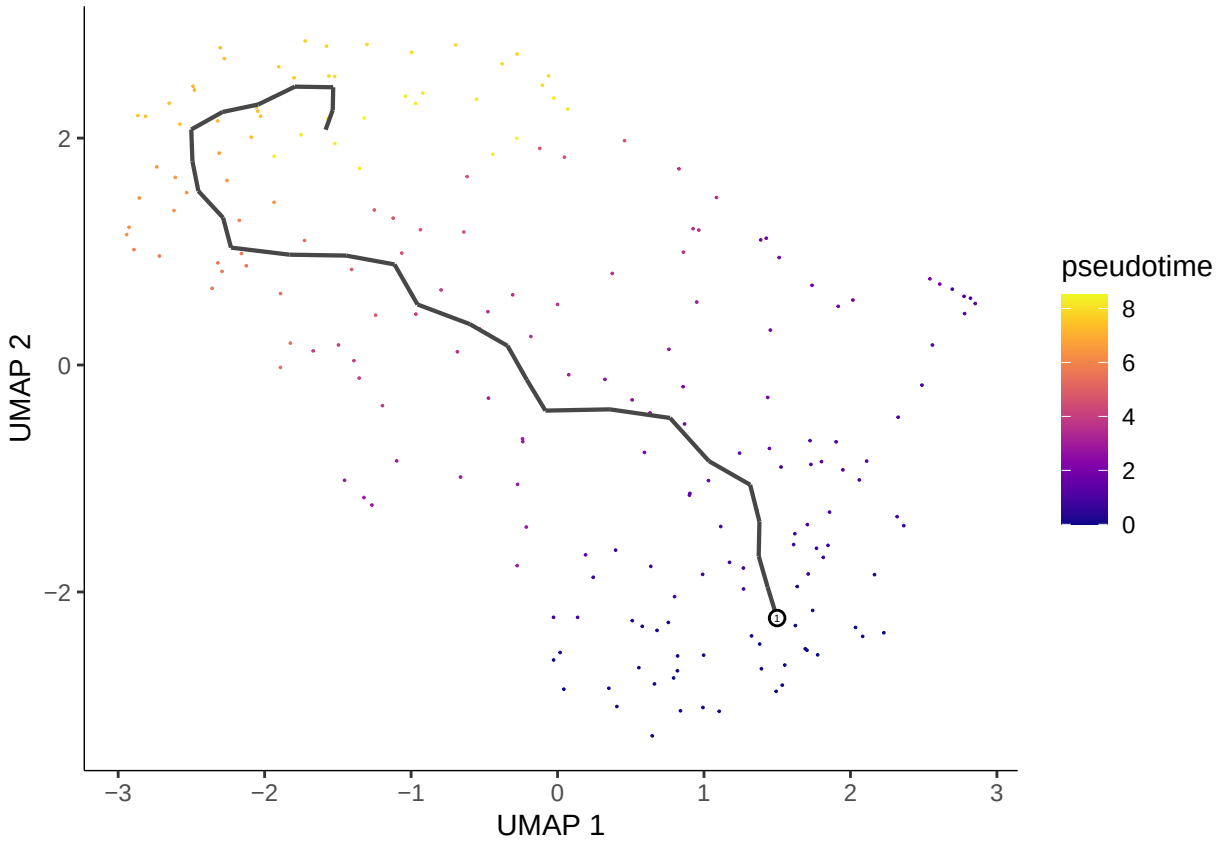
##Plot the pseudotime graph

```
plot_cells(cds,
           color_cells_by = "Cluster.IDs",
           label_groups_by_cluster=FALSE,
           label_leaves=FALSE,
           label_branch_points=FALSE)
```



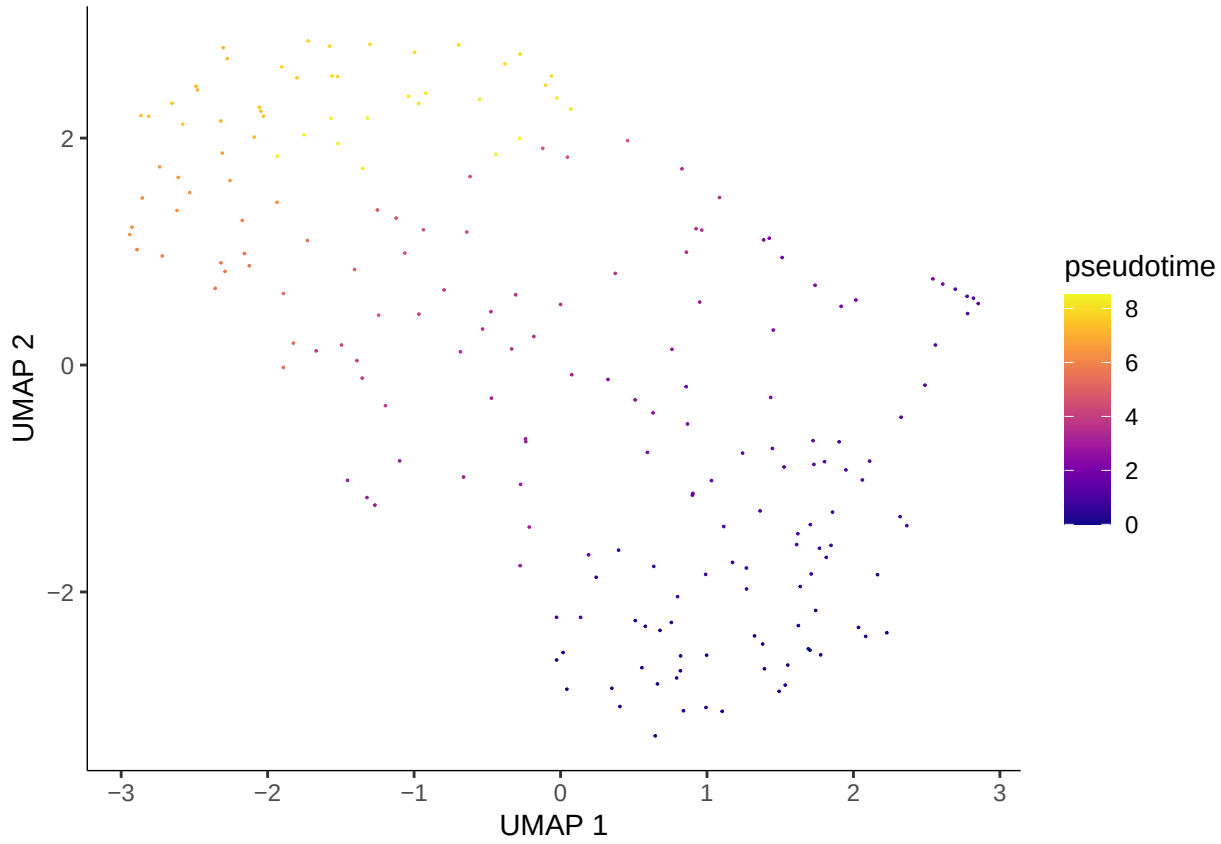
##Plot the pseudotime graph

```
plot_cells(cds,
           color_cells_by = "pseudotime",
           label_cell_groups=FALSE,
           label_leaves=FALSE,
           label_branch_points=FALSE,
           graph_label_size=1.5)
```



##Plot the pseudotime graph without trajectory

```
plot_cells(cds,
           color_cells_by = "pseudotime",
           label_cell_groups=FALSE,
           label_leaves=FALSE,
           label_branch_points=FALSE,
           graph_label_size=1.5,
           show_trajectory_graph = FALSE)
```



Correlation Analysis

Cluster 16

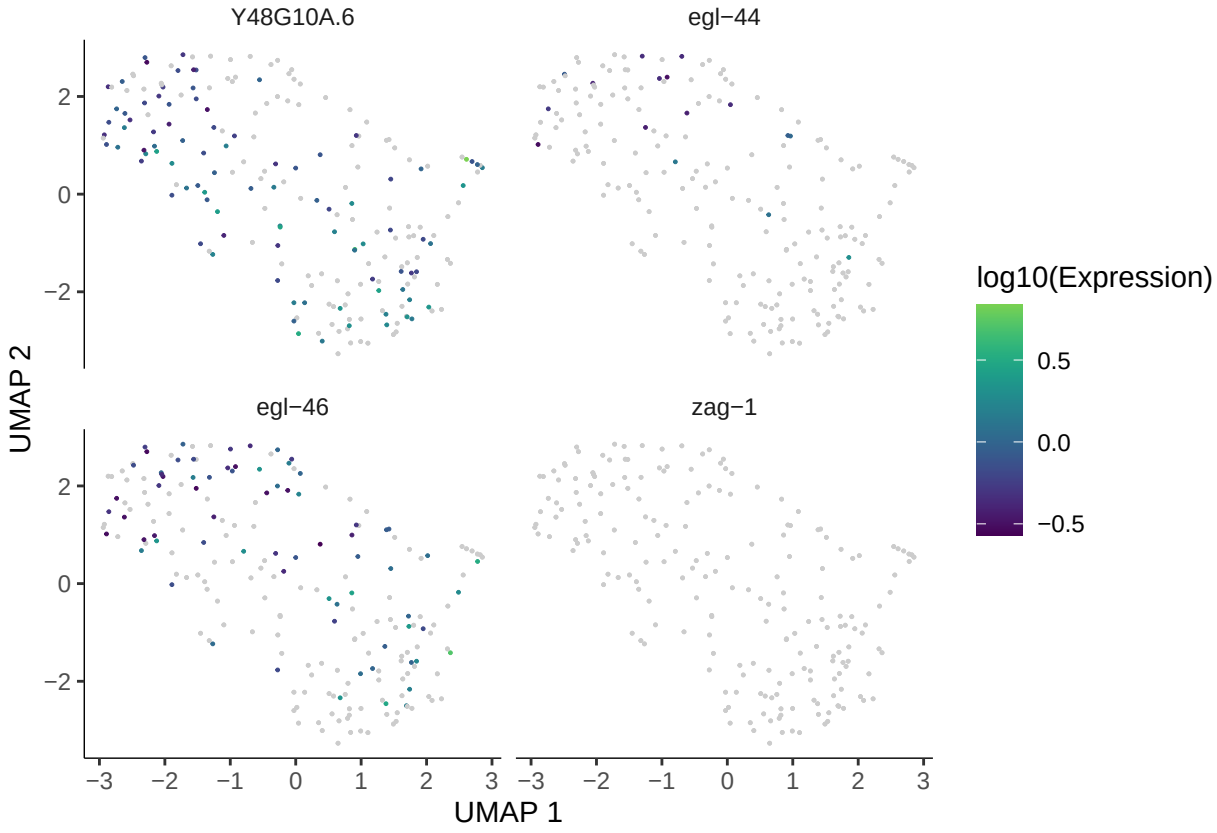
```
cluster16.cellNames <- rownames(pData(cds))[pData(cds)$Cluster.IDs %in% c(16, 16.1)]

cds_16 <- cds[,cluster16.cellNames]

cds16_pg <- graph_test(cds_16, neighbor_graph="principal_graph", cores=4, verbose = F)

cds16_genes <- cds16_pg %>%
  filter(q_value < 0.05) %>%
  arrange(desc(morans_I)) %>%
  select(gene_short_name)
```

```
cds16_genes$gene_short_name
```

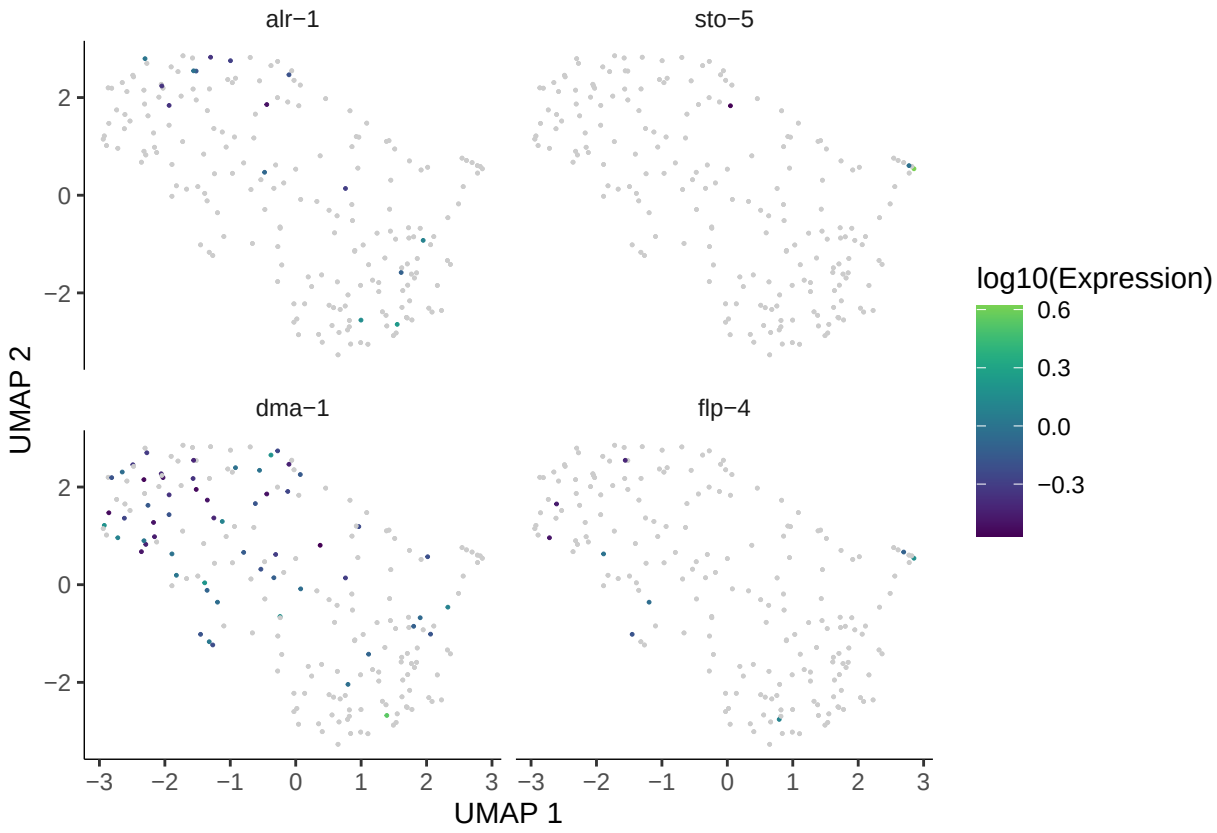
```
##   [1] apl-1       ptp-4      tba-1      cdh-3      C06B8.7    F41C3.5
##   [7] ric-4       T20F10.2   cutl-26    gpb-1      Y44A6D.2   rhgf-1
##  [13] daf-1       C04F12.1   cmd-1      tct-1      des-2      K08F4.1
##  [19] Y57G11C.43  C45G7.4    F13H10.9   Y47D3A.20  fmi-1      fat-1
##  [25] eat-6       obr-4      T01D3.3    Y46E12BL.2 chdp-1     lgc-12
##  [31] mec-7       plx-2      W05H12.2   T05A8.3    tbcb-1     ftt-2
##  [37] F26F12.3    nsf-1      hlh-11     C01G6.5    F47G9.1    rps-19
##  [43] uba-5       copd-1     gly-5      ret-1      kin-2      lin-39
```

```
##  [49] F25E5.1    zig-11     tbb-2      ceh-88     eva-1      R13A5.9
##  [55] ric-3      rpl-4      arrd-25    clh-1      qns-1      unc-115
##  [61] cdh-1      zig-1      ncx-1      vha-3      cdgs-1     ZC410.5
##  [67] unc-64     hif-1      spc-1      C43H6.4    dig-1      imp-2
##  [73] marc-5     dcap-1     aqp-2      C50E3.6    nfi-1      oig-8
##  [79] T05A12.4   hsp-3      madf-5     ZK688.5    cpsf-1     eat-3
##  [85] kcnl-4     lgc-31     Y57A10A.28 flp-14     mafr-1     sec-61
##  [91] rpl-26     mbk-2      F54G2.1    anc-1      ZC449.5    Y38C1AA.1
##  [97] frm-1      M04G7.3    gex-3      sto-2      cct-7      cct-5
## [103] ain-2      F32D8.2    lim-9      Y18D10A.2  Y97E10C.1  rap-2
## [109] gbb-2      usp-3      C39F7.1    cap-2      let-60     rps-11
## [115] unc-44     let-611    unc-43     hpo-30     gdi-1      D1022.9
## [121] eef-1A.1   emb-4      sea-2      Y105E8A.20 his-24     C03A3.1
## [127] D1007.5    rpt-3      dma-1      nhr-78     Y53F4B.9   npa-1
## [133] B0416.5    Y54E2A.4   gbb-1      rps-10     hda-2      F55A3.3
## [139] Y54G2A.26  gck-3      pes-7      eef-1A.2   glb-18     mcd-1
## [145] W03F8.4    cab-1      spe-39     lron-3     F32B4.4    acy-2
## [151] Y39H10A.6  hlh-13     dhhc-2     F40F8.11   deg-3      set-27
## [157] gst-7      gei-4      rpl-19     tbc-12     flp-1      praf-3
## [163] T03G6.3    csn-5      tbc-1      F53A2.9    F36D4.5    F53F4.14
## [169] unc-1      rom-4      dnj-28     F15A8.4    cap-1      epi-1
## [175] atl-1      cls-2      Y43F8B.2   rpm-1      ben-1      unc-86
## [181] rps-14     F55C12.5   egl-21     ZK1320.9   T26C12.1   ser-2
## [187] flp-6      npp-3      rilp-1     egl-30     col-107    gdh-1
## [193] rpl-3      F43C9.2    ets-6      T19A6.1    ddl-3      atg-16.2
## [199] C04F5.9    F56G4.4    pmr-1      Y54E10BL.3 ced-10     aakb-1
## [205] tat-1      rpl-35     Y48G10A.6  ZK524.4    C53D5.1    gly-20
## [211] rps-1      hgap-1     T05C12.11  snt-6      chp-1      C25H3.8
## [217] taf-12     R07E5.1    mrck-1     F46H5.3    cct-4      hda-3
## [223] odr-4      flap-1     cyn-5      ham-3      pyp-1      sec-22
## [229] rib-1      C03A3.2    aagr-4     ZK154.6    Y111B2A.12 hpk-1
## [235] cdh-4      sbds-1     tol-1      mkk-4      C05D10.4   dgn-1
## [241] pde-2      rod-1      mrps-12    dnj-19     cey-1      ZK1073.1
## [247] ZK154.4    F48E8.4    rps-6      gmeb-1     Y47D9A.1   K02D10.4
## [253] syd-1      cdka-1     myrf-2     rps-0      C43H6.3    unc-41
## [259] ril-1      lin-40     itsn-1     Y71F9AL.9  tlp-1      sdc-2
## [265] Y53F4B.21  hinf-1     cct-3      bath-43    rps-25     W08E3.2
## [271] kpc-1      F49E10.4   H18N23.2   pop-1      F40E10.6   rps-28
## [277] prp-6      dnj-7      sec-15     F32D1.11   Y52B11A.3  C25A1.4
## [283] dhhc-14    hgap-2     R02F2.1    msi-1      arx-3      ZK1067.4
## [289] pck-1      F49C12.12  glr-8      C26D10.6   kin-19     gem-1
## [295] F55F8.2    C53C11.2   H34C03.2   cnt-1      emc-5      sur-6
## [301] taf-4      immt-1     Y111B2A.10 T22H9.1    C17H12.10  ZC101.1
## [307] gob-1      aqp-7      C49H3.9    rbbp-5     Y37E11AM.2 F25F8.1
## [313] C10B5.3    sel-9      ceh-86     mnat-1     C24H10.2   dpy-28
## [319] M01F1.3    Y54F10BM.9 F07F6.8    K07A12.8   F56D1.2    rad-23
## [325] vps-39     ccg-1      M03F8.5    F58H7.1    Y92H12BR.7 Y53G8AL.2
## [331] fasn-1     arch-1     F08G12.3   Y48E1B.3   hsp-75     zyg-12
## [337] T16H12.3   apn-1      mtx-2      Y66H1A.8   rtel-1     snt-7
## [343] har-1      Y17G7B.3   rde-10     C04E7.4    C45B2.6    sto-5
## [349] gro-1      T08G2.2    Y18D10A.3  gtbp-1     ttr-8      Y97E10AR.4
## [355] fbxa-137   Y71H2AM.20
## 20271 Levels: 2L52.1 2RSSE.1 4R79.2 6R55.2 aagr-1 aagr-2 aagr-3 aagr-4 ... zyx-1
```

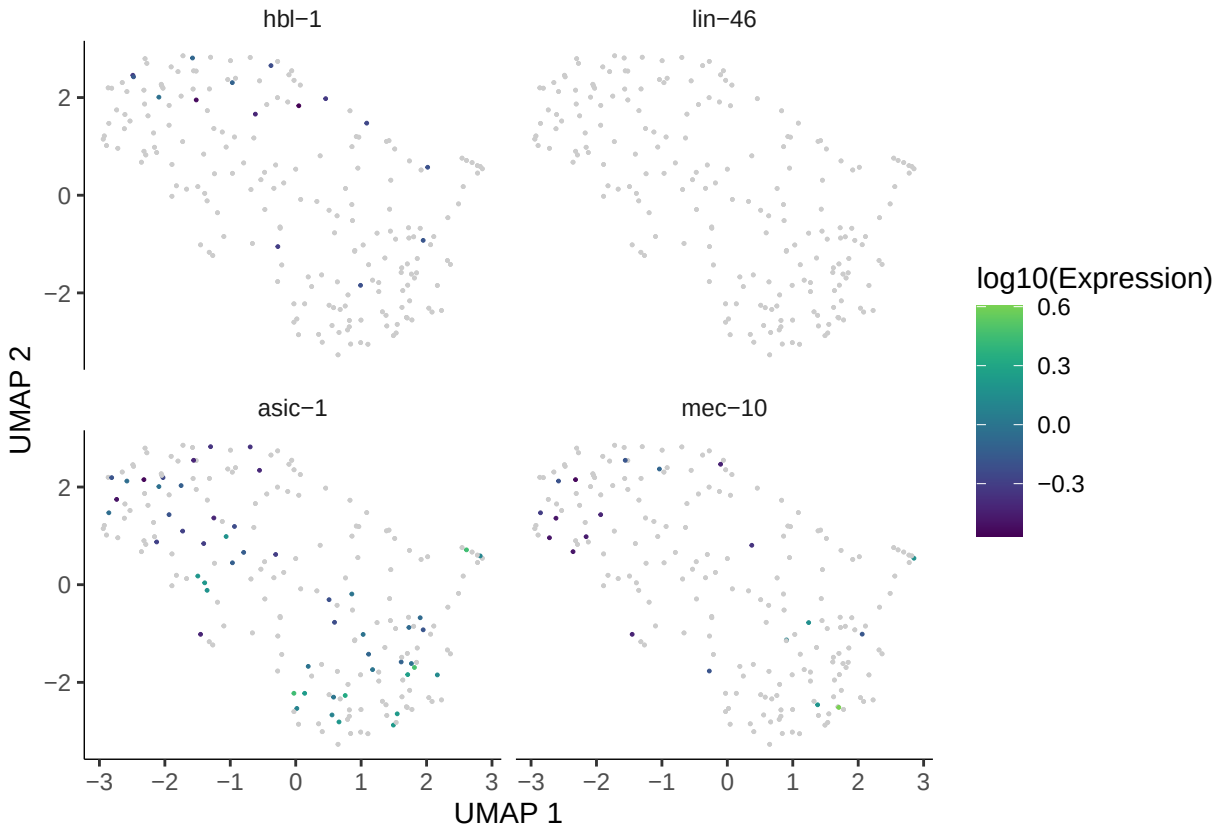
```
# Plot a few genes
plot_cells(cds_16, genes=c("Y48G10A.6", "egl-44", "egl-46", "zag-1"),
           show_trajectory_graph=FALSE,
           label_cell_groups=FALSE,
           label_leaves=FALSE,
           cell_size = 0.5)
```



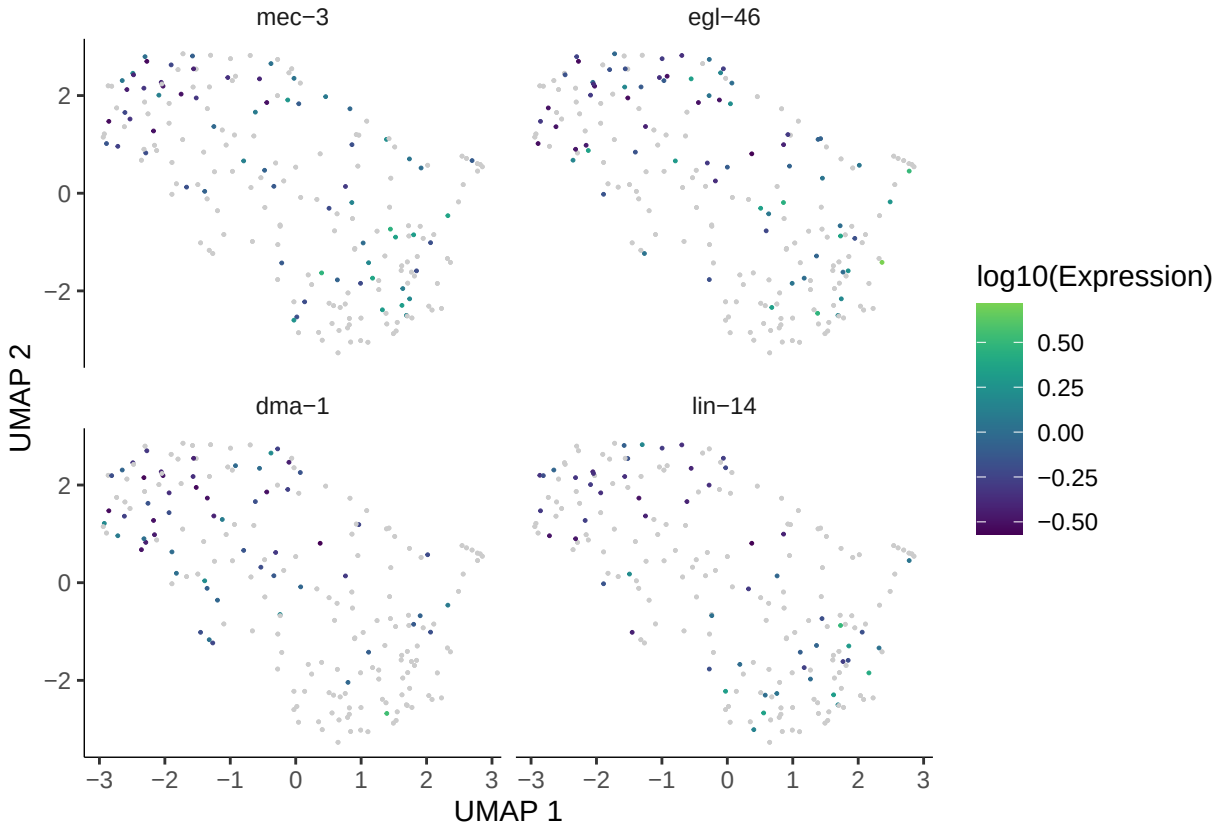

```
# Plot a few genes
plot_cells(cds_16, genes=c("alr-1", "sto-5", "dma-1", "flp-4"),
           show_trajectory_graph=FALSE,
           label_cell_groups=FALSE,
           label_leaves=FALSE,
           cell_size = 0.5)
```




```
# Plot a few genes
plot_cells(cds_16, genes=c("hbl-1", "lin-46","asic-1", "mec-10"),
           show_trajectory_graph=FALSE,
           label_cell_groups=FALSE,
           label_leaves=FALSE,
           cell_size = 0.5)
```

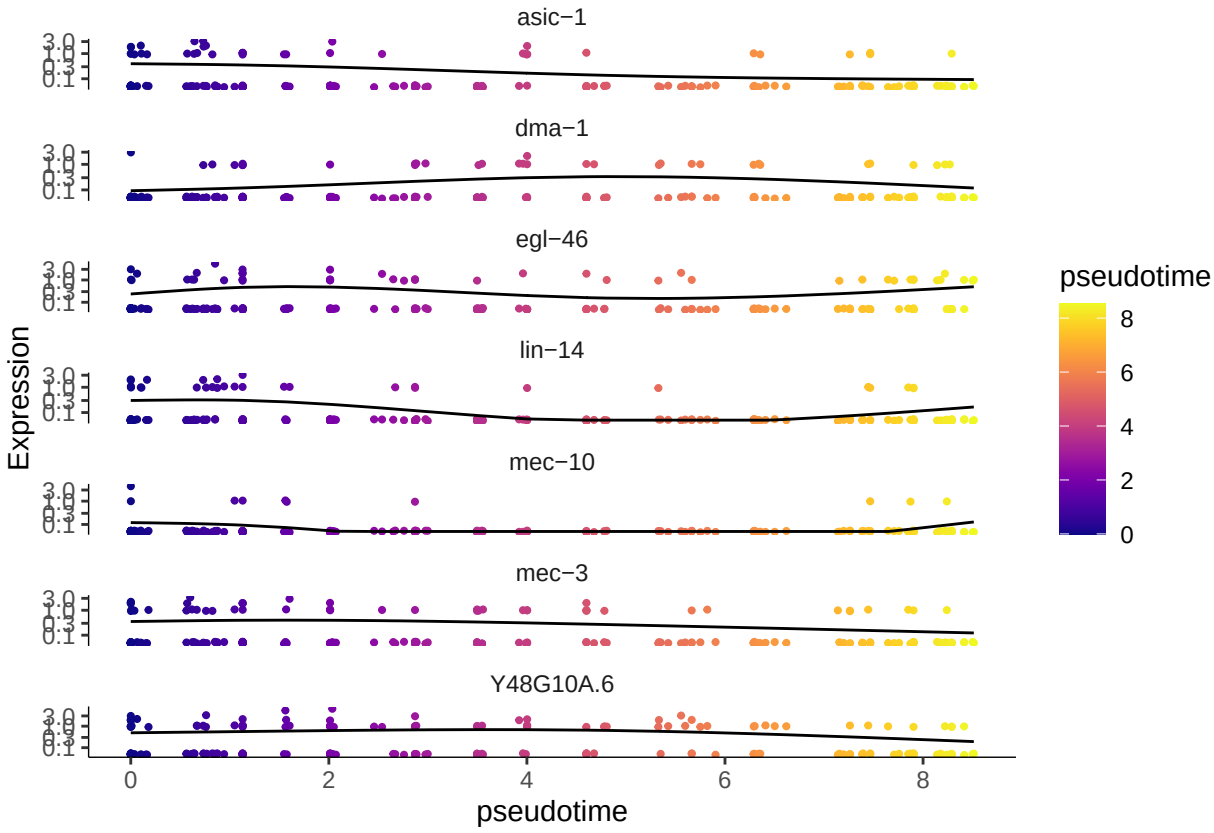



```
# Plot a few genes
plot_cells(cds_16, genes=c("mec-3", "egl-46", "dma-1", "lin-14"),
           show_trajectory_graph=FALSE,
           label_cell_groups=FALSE,
           label_leaves=FALSE,
           cell_size = 0.5)
```



#Clustering Genes by Pseudotemporal Expression Pattern

```
cds_16_lineage_cds <- cds_16[rowData(cds_16)$gene_short_name %in% c("Y48G10A.6","dma-1", "lin-14", "egl-
plot_genes_in_pseudotime(cds_16_lineage_cds,
                         # color_cells_by="embryo.time.bin",
                         min_expr=0.05)
```


asic−1
dma−1
egl−46
lin−14
mec−10
mec−3
Y48G10A.6
Expression
pseudotime
0
2
4
6
8
pseudotime