Walkthrough with Intestine dataset

Load the Data

```
addpath('./data')
load('Intestine_dataset.mat')
```

Process data and select features

Remove low-quality cells and select features:

- filter cells don't express min_exp percentage of genes
- select top #gene_selected informative genes

```
min_exp = 0.95; gene_selected = 3000;
[prodata,progene_name,procell_label] = preprocessing(data,gene_name,true_label,min_exp, ...
gene_selected);
```

Consensus clustering

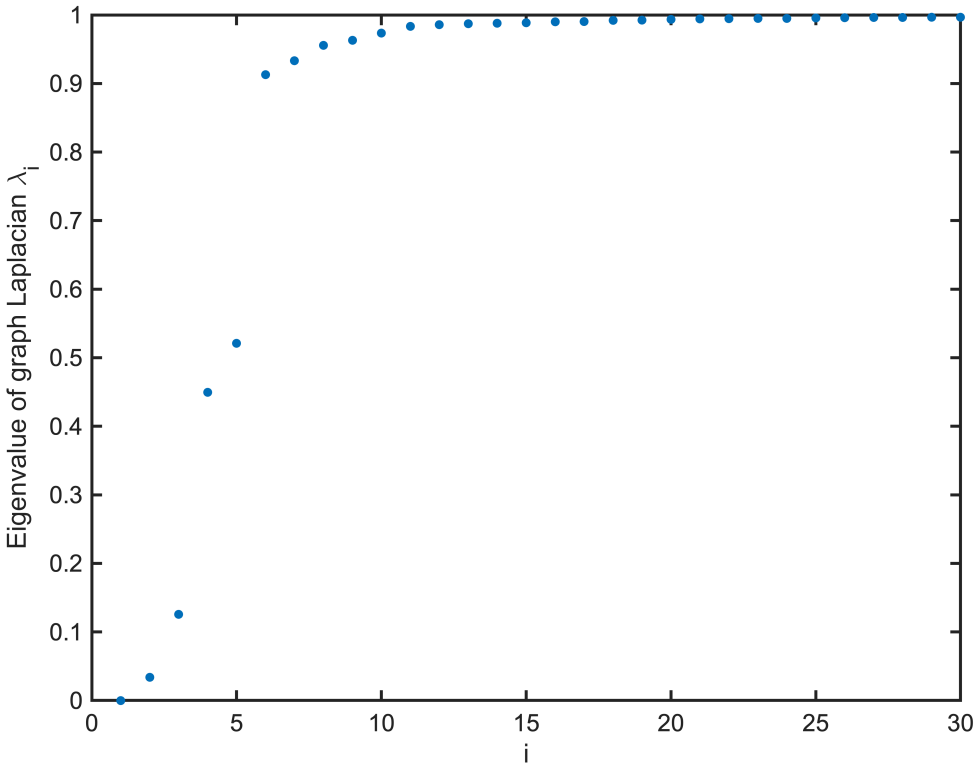
If choosing SC3 package to do the consensus clustering on the processed data, we can get the following cell-cell similarity matrix M. The example scripts of using SC3 package can be found in file SC3.R.

Number of clusters

The number of clusters is estimated by analyzing the largest gap of sorted eigenvalues of symmetric normalized graph Laplacian:

```
[eigenvalues] = plot_eigen_gap(M);
```

```
Number of cluster based on zero eigenvalues & Largest gap
     1     5
```




```
No_cluster = 3;
```

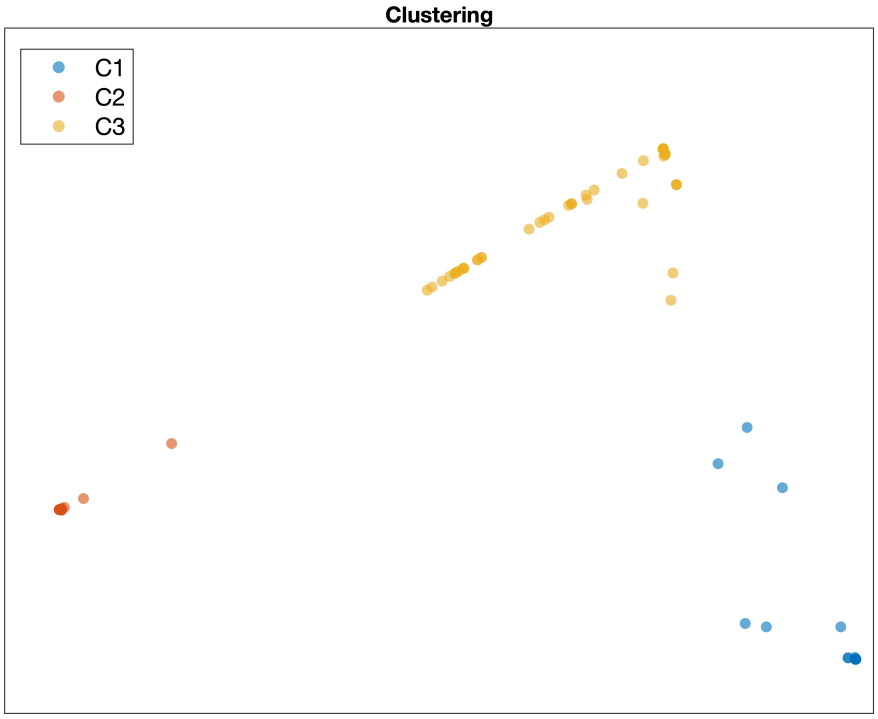
Run QuanTC

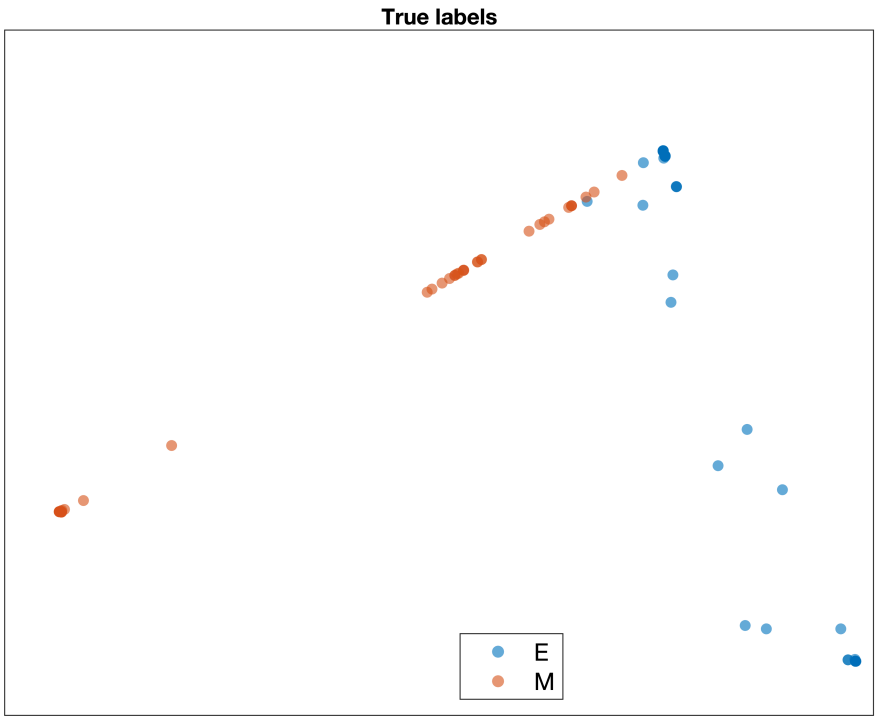
- **Soft clustering**: get the likelihoods of cells belonging to each cluster based on symmetric non-negative matrix factorization of M
- **CPI**: compute CPI value of each cell, use TC_cut to select cell with higher CPI values to be TC

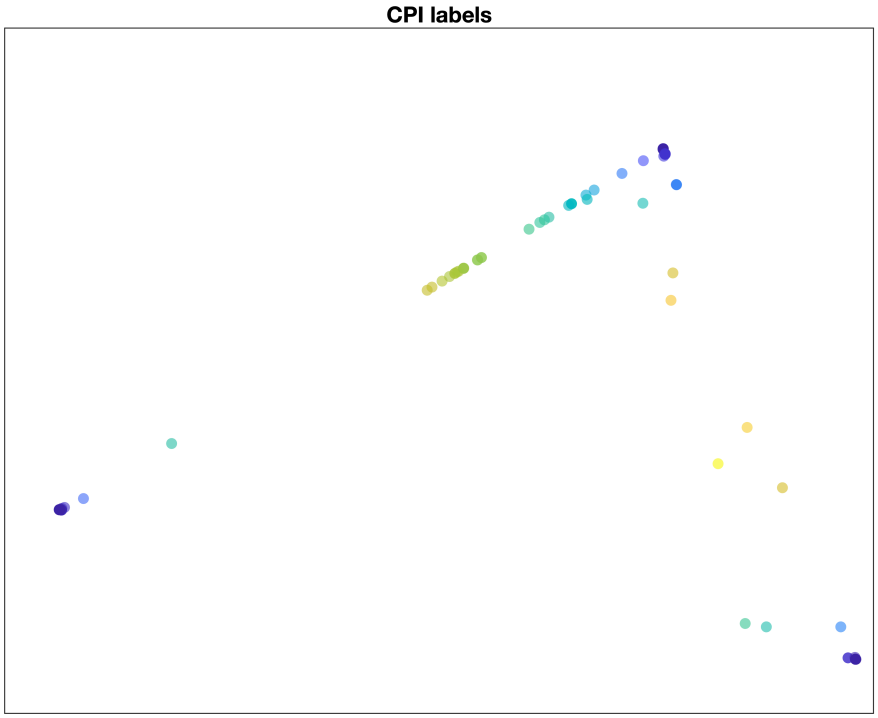
```
TC_cut = 0.34;
[result] = run_QuanTC(prodata,M,No_cluster,TC_cut);
```

- **2d-visualization**: cells are visualized through the probabilistic regularized embedding (PRE) approach

```
label_legend =  {'E','M'};
cell_visu_PRE(result,procell_label, label_legend)
```


Clustering
C1
C2
C3

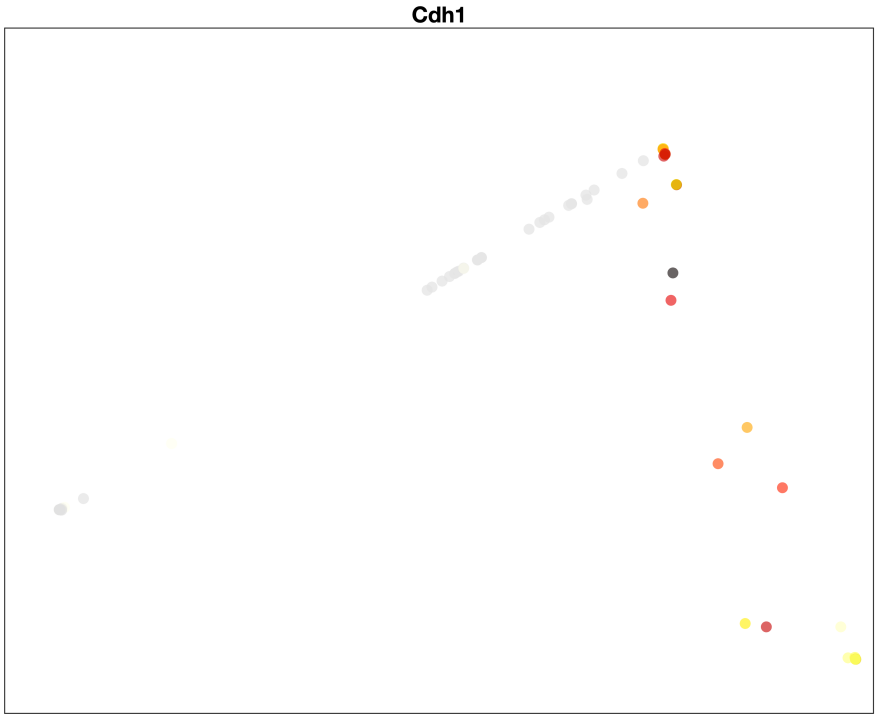

True labels
E
M



Feature plot

visualizes feature expressions on PRE

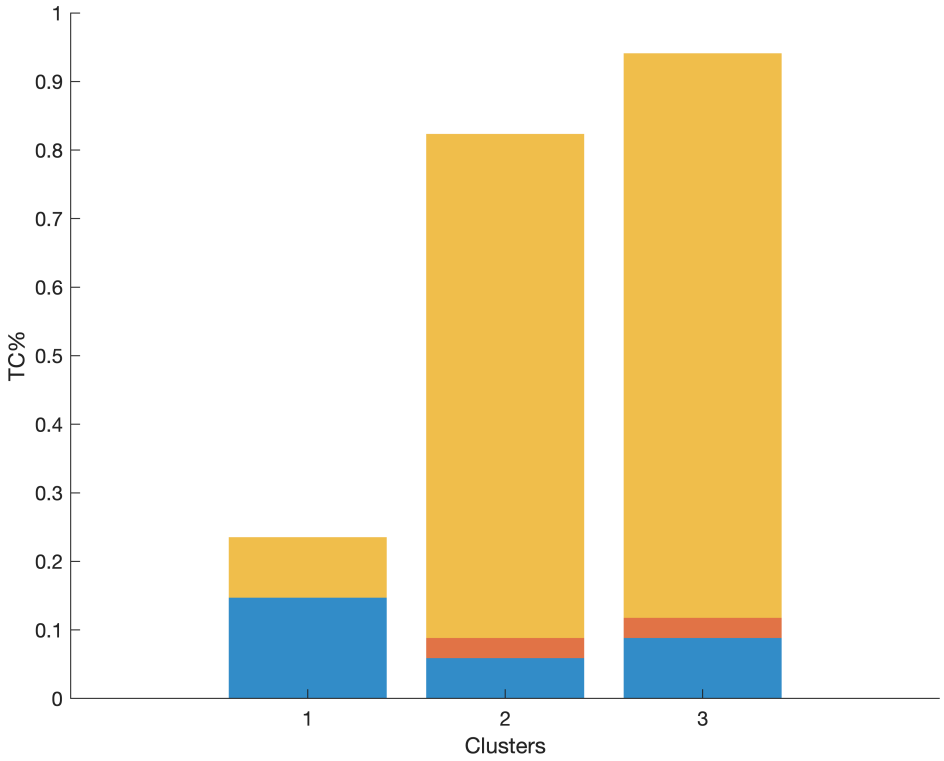
```
gene_plot = {'Cdh1'};
featureplot(result,prodata,progene_name,gene_plot)
```



Transition trajectory

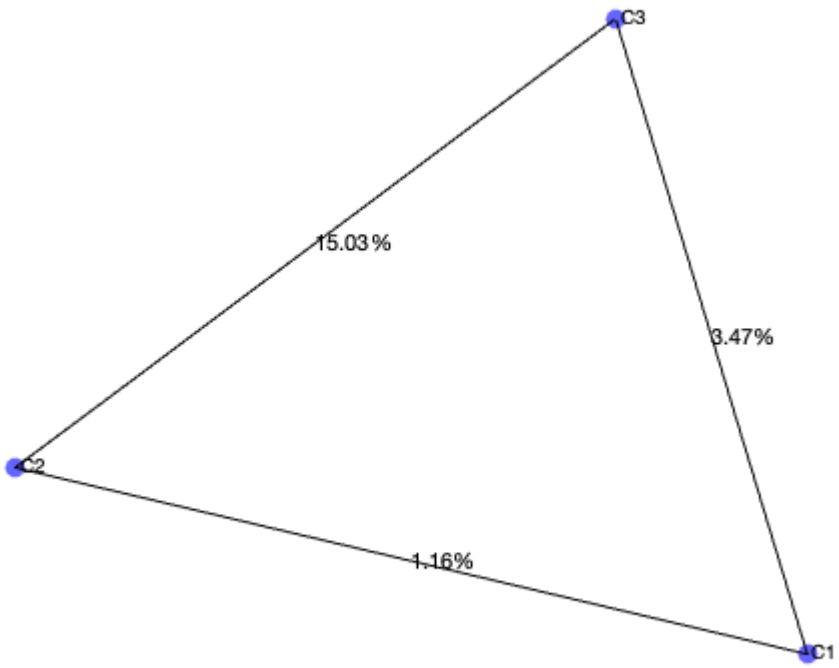
- **Start/ end cluster**: choose non-ICS based on the percentage of TC associated with each cluster relative to the total number of TC

```
pTC(result,No_cluster)
```



- **Potential transition trajectories**: list the percetage of cells over whole cell population to choose the potential transition trajectories

```
start_cluster = 1; %choose starting cluster based on the above graph
%plot TC% between clusters among all the cells
[path,ordered_cell] = traj(result,start_cluster,No_cluster);
```




```
trajectory: 1  2, percentage of cells involved: 0.55491
trajectory: 1  3  2, percentage of cells involved: 0.87283
```

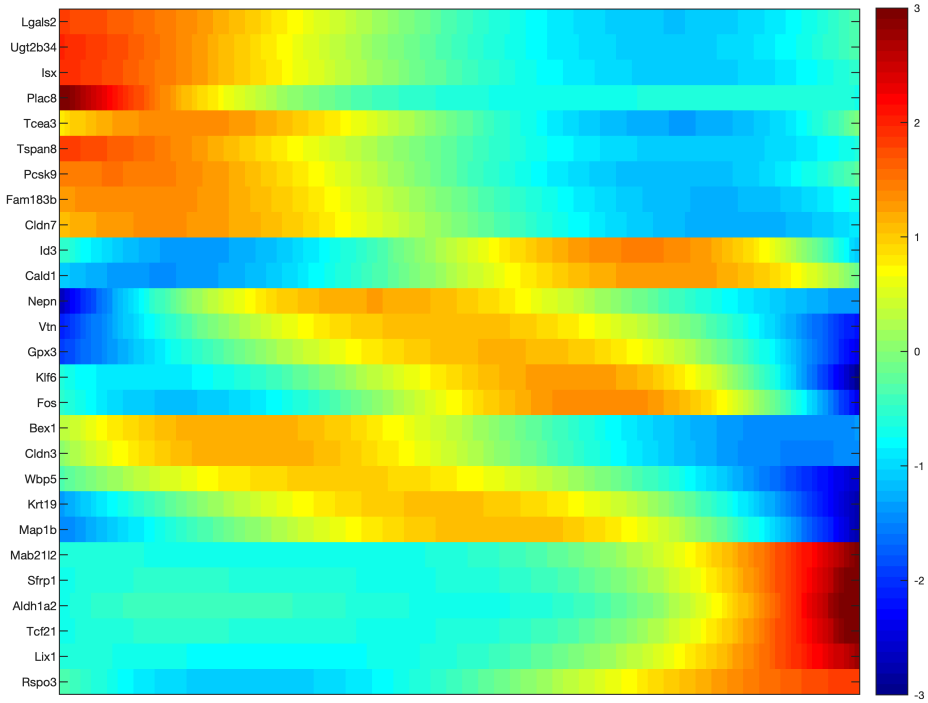
Finding cluster marker genes and the transition genes that mark transition from one trajectory

```
lam2 = 10; path_id =2;
[marker_gene,transition_gene] = markers(result,prodata,M,path{path_id},ordered_cell{path_id},la
```

```
ans = 1.0000
Consistency of clustering: 1
marker_gene = 3×1 cell array
    {520×1 double}
    {815×1 double}
    {917×1 double}
transition_gene = 2×2 cell array
    {10×1 double}    {16×1 double}
    {22×1 double}    {43×1 double}
```

Heatmap

```
a = 5; b = 3; %plot top a marker genes and top 2b transition genes
[gene_plot,gene_cluster] = heatmap(prodata,marker_gene,transition_gene,path{path_id}, ...
ordered_cell{path_id},progene_name,a,b);
```



Plot transition genes along the transiton trajectory

```
trans_gene_plot = transition_gene{1,1}(1);%E-blue, M-purple
tran_gene_plot(result,prodata,trans_gene_plot,path{path_id},ordered_cell{path_id},progene_name
```