Clustering

Material

https://youtu.be/kqcvqqZp3Jo?si=T1lHw0z-4wv6BvF-

- Evaluation of clustering methods

Exercises

Load the seu dataset you have created earlier today:

The method implemented in Seurat first constructs a SNN graph based on the euclidean distance in PCA space, and refine the edge weights between any two cells based on the shared overlap in their local neighborhoods (Jaccard similarity). This step is performed using the `FindNeighbors()` function, and takes as input the previously defined dimensionality of the dataset.

Code starts here:

```
seu <- Seurat::FindNeighbors(seu, dims = 1:25, reduction = "integrated.cca")
```

Code ends here

To cluster the cells, Seurat next implements modularity optimization techniques such as the Louvain algorithm (default) or SLM [SLM, Blondel et al., Journal of Statistical Mechanics], to iteratively group cells together, with the goal of optimizing the standard modularity function. The `FindClusters()` function implements this procedure, and contains a resolution parameter that sets the 'granularity' of the downstream clustering, with increased values leading to a greater number of clusters.

Code starts here:

```
seu <- Seurat::FindClusters(seu, resolution = seq(0.1, 0.8, by=0.1))
```

Code ends here

Cluster id of each cell is added to the metadata object, as a new column for each resolution tested:

Code starts here:

```
head(seu@meta.data)
```

```
                          orig.ident nCount_RNA nFeature_RNA percent.mito
PBMMC-1_AAACCTGCAGACGCAA-1    PBMMC-1       2401          909     2.540608
PBMMC-1_AAACCTGTCATCACCC-1    PBMMC-1       3532          760     5.181200
PBMMC-1_AAAGATGCATAAAGGT-1    PBMMC-1       3972         1215     4.934542
PBMMC-1_AAAGCAAAGCAGCGTA-1    PBMMC-1       3569          894     3.250210
```

```
PBMMC-1_AAAGCAACAATAACGA-1    PBMMC-1       2982          730     3.688799
PBMMC-1_AAAGCAACATCAGTCA-1    PBMMC-1      22284         3108     3.181655
                           percent.ribo percent.globin nCount_SCT nFeature_SC
T
PBMMC-1_AAACCTGCAGACGCAA-1     28.65473      0.1665973       3577          88
6
PBMMC-1_AAACCTGTCATCACCC-1     55.03964      0.1981880       3909          74
7
PBMMC-1_AAAGATGCATAAAGGT-1     30.43807      0.3776435       3964         119
2
PBMMC-1_AAAGCAAAGCAGCGTA-1     55.02942      0.3642477       3883          87
3
PBMMC-1_AAAGCAACAATAACGA-1     54.49363      0.1006036       3795          71
1
PBMMC-1_AAAGCAACATCAGTCA-1     23.40693     36.9682283       3967          95
0
                           RNA_snn_res.0.1 RNA_snn_res.0.2 RNA_snn_res.0.3
PBMMC-1_AAACCTGCAGACGCAA-1               5               7               7
PBMMC-1_AAACCTGTCATCACCC-1               0               0               0
PBMMC-1_AAAGATGCATAAAGGT-1               2               3               3
PBMMC-1_AAAGCAAAGCAGCGTA-1               0               0               0
PBMMC-1_AAAGCAACAATAACGA-1               0               0               0
PBMMC-1_AAAGCAACATCAGTCA-1               4               2               2
                           RNA_snn_res.0.4 RNA_snn_res.0.5 RNA_snn_res.0.6
PBMMC-1_AAACCTGCAGACGCAA-1               7               7               9
PBMMC-1_AAACCTGTCATCACCC-1               0               0               0
PBMMC-1_AAAGATGCATAAAGGT-1               2               3               3
PBMMC-1_AAAGCAAAGCAGCGTA-1               0               0               0
PBMMC-1_AAAGCAACAATAACGA-1               0               0               0
PBMMC-1_AAAGCAACATCAGTCA-1               5               5               5
                           RNA_snn_res.0.7 RNA_snn_res.0.8 seurat_clusters
PBMMC-1_AAACCTGCAGACGCAA-1               9               8               8
PBMMC-1_AAACCTGTCATCACCC-1               0               0               0
PBMMC-1_AAAGATGCATAAAGGT-1               2               2               2
PBMMC-1_AAAGCAAAGCAGCGTA-1               0               0               0
PBMMC-1_AAAGCAACAATAACGA-1               0               0               0
PBMMC-1_AAAGCAACATCAGTCA-1               5               5               5
```

Code ends here

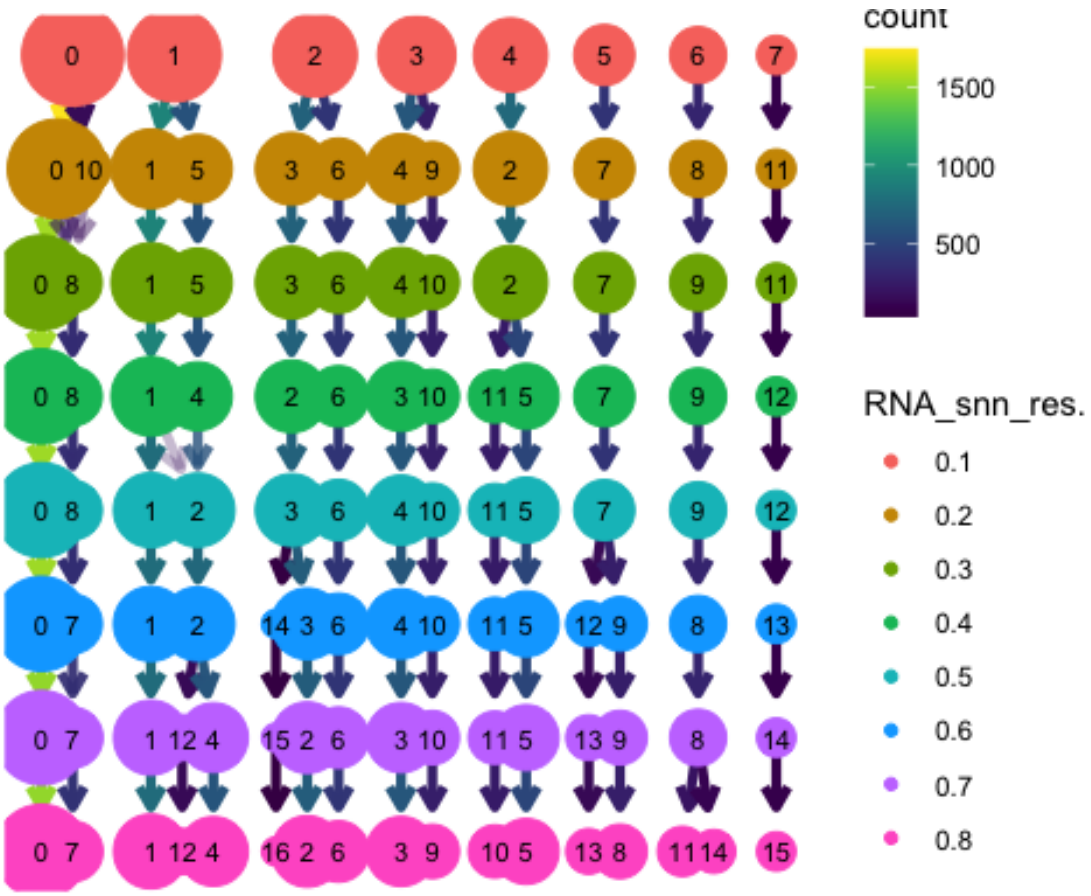
To view how clusters sub-divide at increasing resolution:

Code starts here:

```
library(clustree)

Loading required package: ggraph

Loading required package: ggplot2

Warning: package 'ggplot2' was built under R version 4.3.3
```

```
clustree::clustree(seu@meta.data[,grep("RNA_snn_res", colnames(seu@meta.data)
)],
                   prefix = "RNA_snn_res.")
```

Code ends here

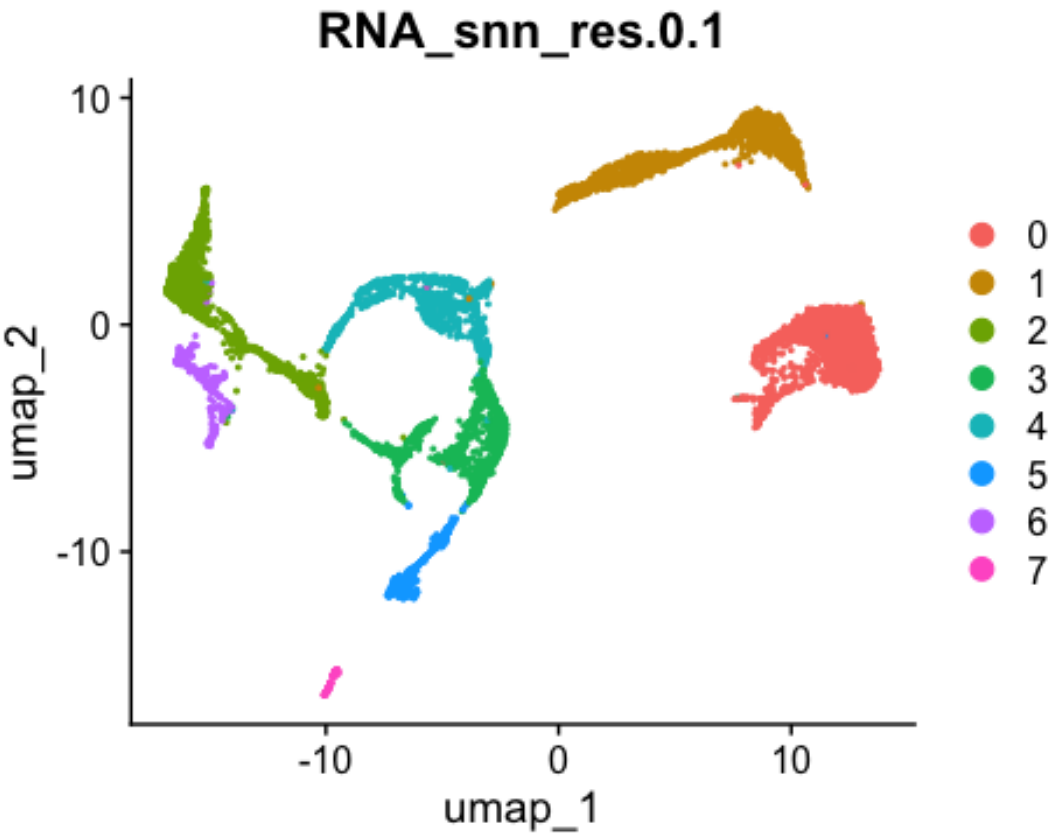


You can view the UMAP coloring each cell according to a cluster id like this:

Code starts here:

```
Seurat::DimPlot(seu, group.by = "RNA_snn_res.0.1")
```

Code ends here



Exercise

Visualise clustering based on a few more resolutions. Taking the clustering and the UMAP plots into account what do you consider as a good resolution to perform the clustering?

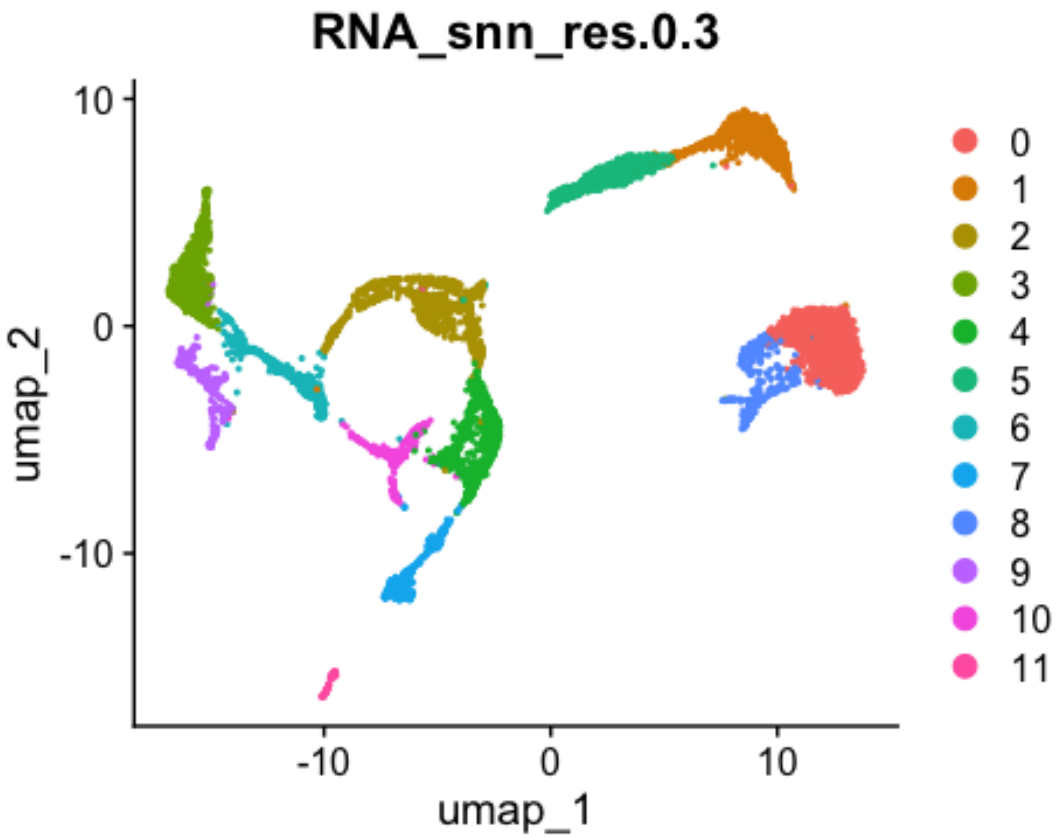
Answer

Of course, there is no 'optimal' resolution, but based on resolution of 0.3, the tree stays relatively stable for a few resolution steps, and it seems that clustering fits the UMAP well:

Code starts here:

```
Seurat::DimPlot(seu, group.by = "RNA_snn_res.0.3")
```

Code ends here



Exercise

When do the number of neighbors need to be changed? How does changing the method of clustering in `FindClusters` affect the output? Which parameter should be changed?

Answer

> As FindClusters is an unsupervised clustering method on the PCA data and UMAP is a good summary of the PCA dimension selected, clusters and UMAP plot should go along. If one has reasons to change the number of neighbors in the UMAP function, here the same parameter should be adapted.
>
> The method can be changed with algorithm = 2,3 or 4

Code startss here:

```
saveRDS(seu, "day2/seu_day2-4.rds")
```

Code ends here