

# Liver E13.5 Clustering Report

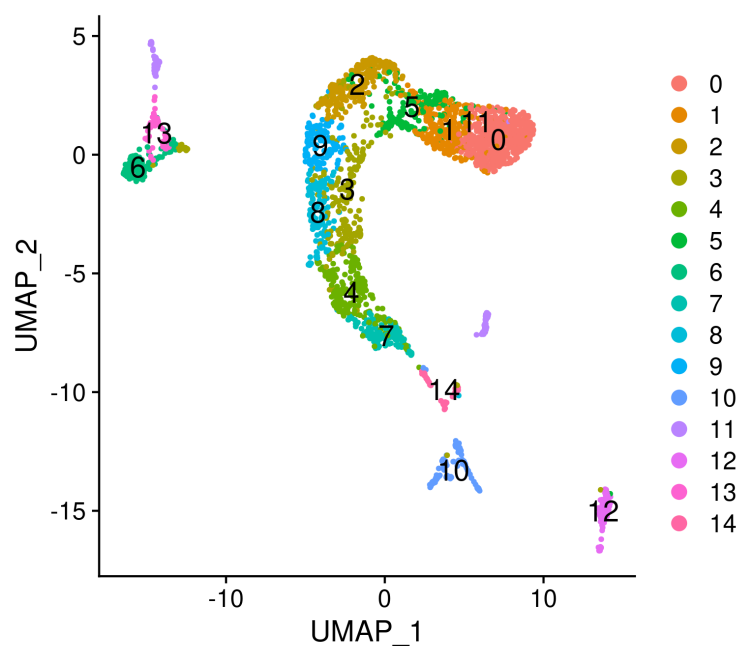
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## 1 Seurat Codes and Parameters

## 2 Clustering and Annotation

### 2.1 Clustering 1



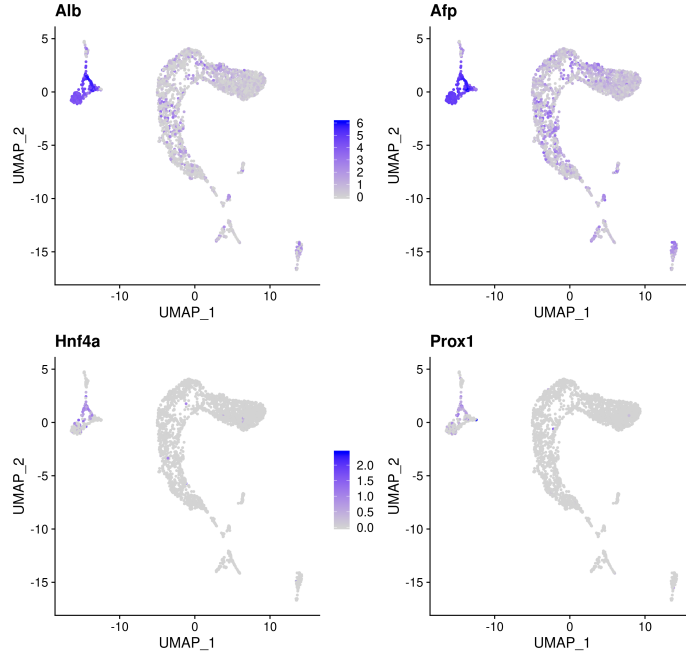
**Figure 1:** Unlabeled Clusters

**Figure 1** is clustering result using 0.8 resolution. RDS file is available in `/p200/liujiang_group/yinyao/Dataset/Seurat/Liver.rds`. After extracting markers, some of these clusters can be annotated, but others need further clustering, like C11 (cluster 11, purple), which consists of two parts away from each other.

### 2.2 Annotation 1

*Afp*, *Alb* are reported as the markers of hepatocyte or hepatoblast (Gordillo et al., 2015; Chaudhari et al., 2016; Su et al., 2017; Han et al., 2018). Moreover, besides *Afp* and *Alb*, hepatocyte also expresses *Hnf4α* and

*Prox1*(Gordillo et al., 2015). Therefore, C6 and C13 are annotated as hepatocyte and hepatoblast, respectively, for their high expression of *Afp* and *Alb*, and for expression of *Hnf4α* and *Prox1* in C13 (**Figure 2**).



**Figure 2:** Markers of C6/C13

*Cd68*, *Marco* are reported as the markers of macrophage(Su et al., 2017; Han et al., 2018), so C12 is annotated as macrophage. *Pbp*, *Itga2b* are reported as the markers of megakaryocyte(Su et al., 2017), so C14 is annotated as megakaryocyte (**Figure 3**).

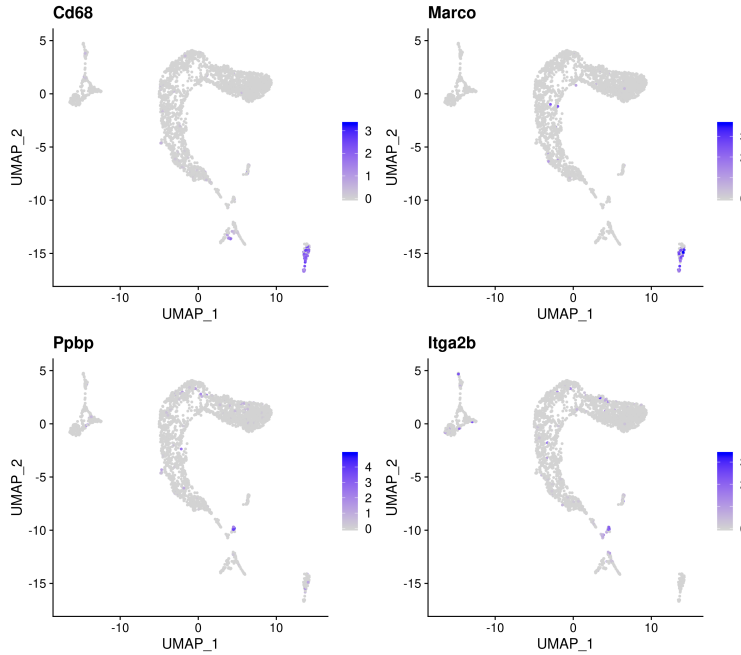
However, in C10, there are two kinds of cell markers, stem cells' and neutrophils'. Similarly, in C11, there are endothelial cells' and mesenchymal cells'. So they need further clustering.

## 2.3 Clustering 2

In order to simply divide one cluster into fewer parts, the resolution used in this part is 0.6.

**Cluster 10** C10 is divided into 3 parts (**Figure ??**), saved as /p200/liujiang\_group/yinyao/Dataset/Seurat/Liver\_C10.rds. Stem cell marker *Cd34*, *Cmtm7*(Han et al., 2018) are highly expressed in subcluster 0 of C10. Neutrophil marker *S100a9*, *S100a8*(Han et al., 2018) are highly expressed in subcluster 2 of C10. The annotation of subcluster 1 is not defined yet.

**Cluster 11** C11 is divided into 2 parts as expected, one of which is annotated as endothelial cell and the other is annotated as mesenchymal cell, due to the endothelial marker *Lyve1*, *Kdr*(Gordillo et al., 2015) in subcluster 0, and the mesenchymal marker *Pdgfra*, *Col1a2*(Han et al., 2018) in subcluster 1.()



**Figure 3:** Markers of C12/C14

**Figure 4:** Subclusters of C10

**Figure 5:** Stem and Neutrophil Markers in C10

## References

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**Figure 6:** Endothelial and Mesenchymal Markers in C11 **Figure 7:** Endothelial and Mesenchymal Markers in C11