3.4 Annotating clusters

Cell clustering allows unsupervised definition of cell types.

We need to be able to annotate the identified cell clusters.

This is non-trivial and often requires an expert in the tissue being studied.

Computational tools can leverage large volumes of existing single-cell data to accelerate the process of cell type annotation.

3.4.1 Different approaches to cell type annotation

Benchmarking study Abdelaal et al. 2019 https://doi.org/10.1186/s13059-019-1795-z
Review Pasquini et al. 2021 https://doi.org/10.1016/j.csbj.2021.01.015

One can annotate the cell types of individual cells or of clusters of cells.

Briefly, there are three main computational approaches to annotate cell types (see Figure below)

A. **Marker-gene-database-based** approaches use sets of marker genes from the literature and previous single-cell studies. The sets of marker genes can distinguish different cell types. Single cells or clusters are scored using these marker gene sets estimate the overall expression levels of these genes. See the Seurat AddModuleScore function (https://www.waltermuskovic.com/2021/04/15/seurat-s-addmodulescore-function/) Some heuristics and scoring criteria are then applied to assign the cell type.

B. **Correlation-based** approaches apply multiple correlation measures to estimate the similarity between the single cells / clusters in the input dataset against some reference data e.g. single-cell atlases or bulk RNA-seq databases such as GTeX / FANTOM.

C. **Supervised-classification-based** approaches use supervised learning to predict cell type labels. The learning model is first trained on some single-cell reference atlas before being applied onto the input dataset to compute the probability that a single cell / cluster belongs to a particular cell type.

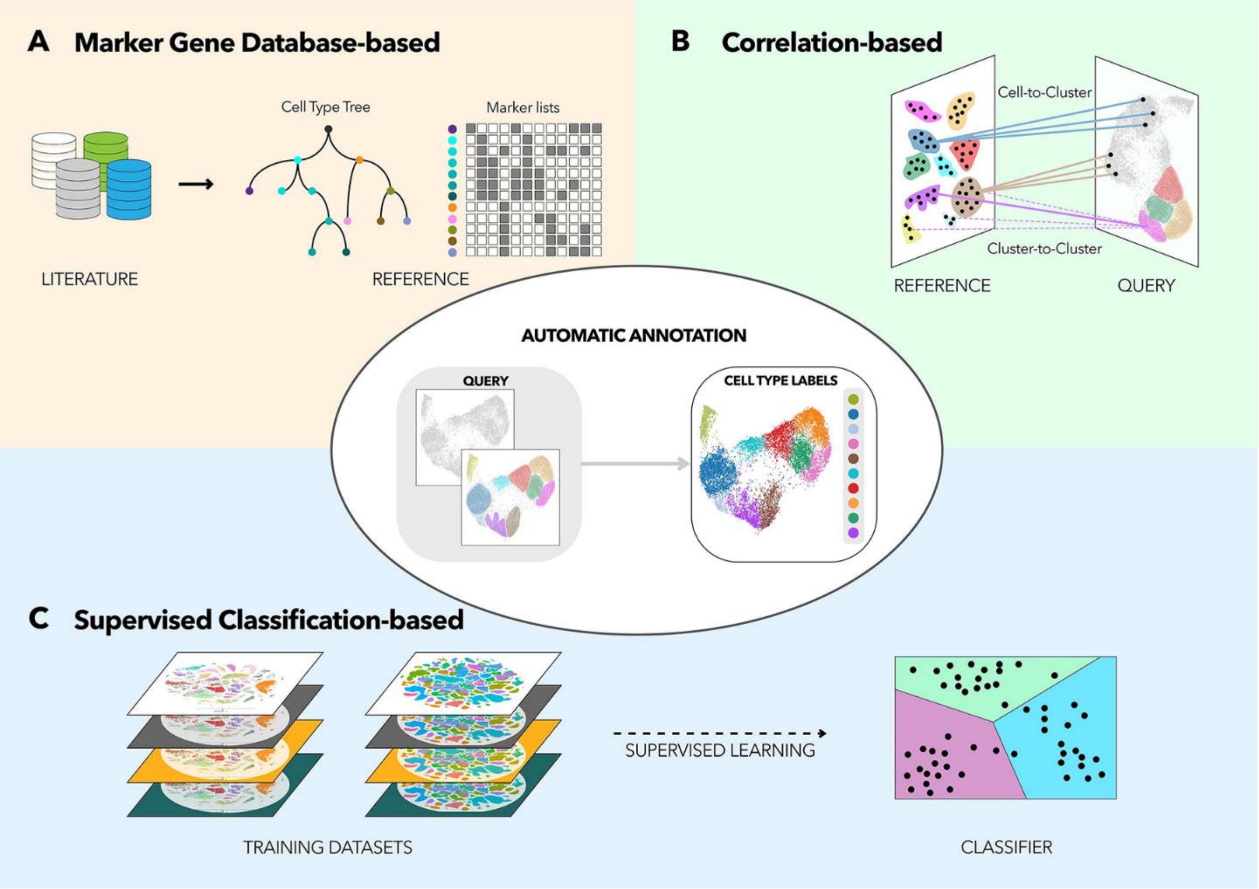


Figure 3.14: There are three main approaches to cell type annotation in single-cell studies, namely marker gene approaches, correlation approaches and supervised classification approaches. Image taken from Pasquini et al. https://doi.org/10.1016/j.csbj.2021.01.015

In all three approaches, the choice of reference data is crucial for the success of the cell type annotation.

Ideally, the reference data should encompass the cell types that are expected in the data to be annotated. For example, do not annotate a bone marrow dataset using a brain atlas reference.

The cell type labels in the reference data will determine the “resolution” of the predicted cell type annotation. Using a reference that only has coarse cell type labels (e.g. a general T-cell label as opposed to CD4+ Naive, CD8+ Naïve, etc. labels) can only generate coarse annotations.

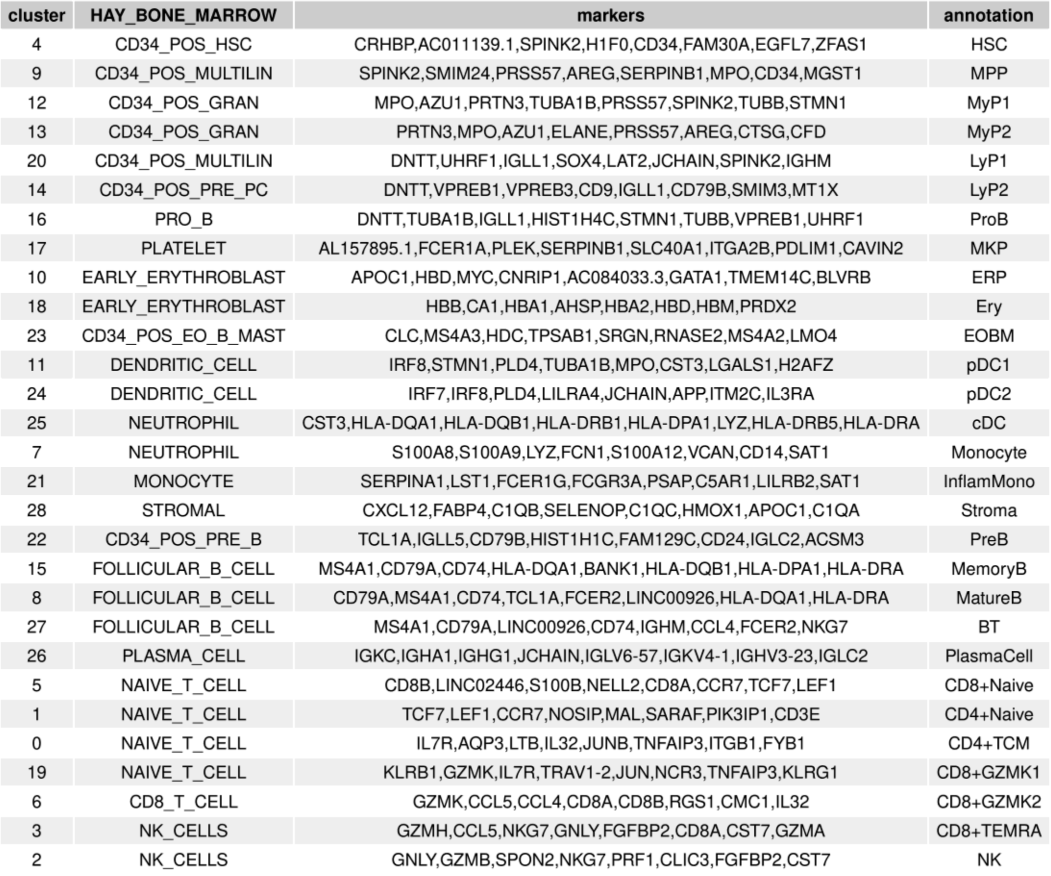
It is important to have the option of having unassigned cells. This happens when a cell (or cluster) has a low score across all the cell types in the reference. The cell could be of a novel, rare type that is not annotated in the reference data or could represent stressed or dying cells that have “lost” their cell identity.

Furthermore, a cell’s type can be plastic, and cells can trans-differentiate between two types. In this case, a cell (or cluster) might have high scores for two different cell types.

3.4.2 Annotation via marker genes and functional analysis

We will annotate the clusters we found previously based on marker genes and the HAY_BONE_MARROW gene signature overrepresentation analysis that we did earlier. The final annotations will be assigned manually.

The major cell states: HSC / progenitors / erythroid / dendritic cell / monocyte / B cell / T cell / NK cell can be determined from the HAY_BONE_MARROW signatures. Some cellular substates can be resolved using marker genes.



cluster	HAY_BONE_MARROW	markers	annotation
4	CD34_POS_HSC	CRHBP,AC011139.1,SPINK2,H1F0,CD34,FAM30A,EGFL7,ZFAS1	HSC
9	CD34_POS_MULTILIN	SPINK2,SMIM24,PRSS57,AREG,SERPINB1,MPO,CD34,MGST1	MPP
12	CD34_POS_GRAN	MPO,AZU1,PRTN3,TUBA1B,PRSS57,SPINK2,TUBB,STMN1	MyP1
13	CD34_POS_GRAN	PRTN3,MPO,AZU1,ELANE,PRSS57,AREG,CTSG,CFD	MyP2
20	CD34_POS_MULTILIN	DNTT,UHRF1,IGLL1,SOX4,LAT2,JCHAIN,SPINK2,IGHM	LyP1
14	CD34_POS_PRE_PC	DNTT,VPREB1,VPREB3,CD9,IGLL1,CD79B,SMIM3,MT1X	LyP2
16	PRO_B	DNTT,TUBA1B,IGLL1,HIST1H4C,STMN1,TUBB,VPREB1,UHRF1	ProB
17	PLATELET	AL157895.1,FCER1A,PLEK,SERPINB1,SLC40A1,ITGA2B,PDLIM1,CAVIN2	MKP
10	EARLY_ERYTHROBLAST	APOC1,HBD,MYC,CNRIP1,AC084033.3,GATA1,TMEM14C,BLVRB	ERP
18	EARLY_ERYTHROBLAST	HBB,CA1,HBA1,AHSP,HBA2,HBD,HBM,PRDX2	Ery
23	CD34_POS_EO_B_MAST	CLC,MS4A3,HDC,TPSAB1,SRGN,RNASE2,MS4A2,LMO4	EOBM
11	DENDRITIC_CELL	IRF8,STMN1,PLD4,TUBA1B,MPO,CST3,LGALS1,H2AFZ	pDC1
24	DENDRITIC_CELL	IRF7,IRF8,PLD4,LILRA4,JCHAIN,APP,ITM2C,IL3RA	pDC2
25	NEUTROPHIL	CST3,HLA-DQA1,HLA-DQB1,HLA-DRB1,HLA-DPA1,LYZ,HLA-DRB5,HLA-DRA	cDC
7	NEUTROPHIL	S100A8,S100A9,LYZ,FCN1,S100A12,VCAN,CD14,SAT1	Monocyte
21	MONOCYTE	SERPINA1,LST1,FCER1G,FCGR3A,PSAP,C5AR1,LILRB2,SAT1	InflamMono
28	STROMAL	CXCL12,FABP4,C1QB,SELENOP,C1QC,HMOX1,APOC1,C1QA	Stroma
22	CD34_POS_PRE_B	TCL1A,IGLL5,CD79B,HIST1H1C,FAM129C,CD24,IGLC2,ACSM3	PreB
15	FOLLICULAR_B_CELL	MS4A1,CD79A,CD74,HLA-DQA1,BANK1,HLA-DQB1,HLA-DPA1,HLA-DRA	MemoryB
8	FOLLICULAR_B_CELL	CD79A,MS4A1,CD74,TCL1A,FCER2,LINC00926,HLA-DQA1,HLA-DRA	MatureB
27	FOLLICULAR_B_CELL	MS4A1,CD79A,LINC00926,CD74,IGHM,CCL4,FCER2,NKG7	BT
26	PLASMA_CELL	IGKC,IGHA1,IGHG1,JCHAIN,IGLV6-57,IGKV4-1,IGHV3-23,IGLC2	PlasmaCell
5	NAIVE_T_CELL	CD8B,LINC02446,S100B,NELL2,CD8A,CCR7,TCF7,LEF1	CD8+Naive
1	NAIVE_T_CELL	TCF7,LEF1,CCR7,NOSIP,MAL,SARAF,PIK3IP1,CD3E	CD4+Naive
0	NAIVE_T_CELL	IL7R,AQP3,LTB,IL32,JUNB,TNFAIP3,ITGB1,FYB1	CD4+TCM
19	NAIVE_T_CELL	KLRB1,GZMK,IL7R,TRAV1-2,JUN,NCR3,TNFAIP3,KLRG1	CD8+GZMK1
6	CD8_T_CELL	GZMK,CCL5,CCL4,CD8A,CD8B,RGS1,CMC1,IL32	CD8+GZMK2
3	NK_CELLS	GZMH,CCL5,NKG7,GNLY,FGFBP2,CD8A,CST7,GZMA	CD8+TEMRA
2	NK_CELLS	GNLY,GZMB,SPON2,NKG7,PRF1,CLIC3,FGFBP2,CST7	NK

Figure 3.15: Annotation of the 29 clusters in the bone marrow dataset via marker genes and HAY_BONE_MARROW gene signature analysis.

Code for the table above:

This code extracts the most significant HAY_BONE_MARROW signature and top 8 marker genes by log-fold-change for each cluster and generates a table of annotations of the clusters.

```
library(gridExtra)
library(Seurat)
library(data.table)
oupAnnot = data.table(cluster = reorderCluster)
# Add in top 5 marker genes
ggData <- oupMarker[, head(.SD, 8), by = "cluster"]
ggData <- ggData[, paste0(gene, collapse = ","), by = "cluster"]
colnames(ggData)[2] <- "markers"
oupAnnot <- ggData[oupAnnot, on = "cluster"]

# Add in the BONE_MARROW enrichment from the results of enrichment
# analysis calculated previously (contained in oupMarkerFunc)
ggData <- oupMarkerFunc[grep("BONE_MARROW", ID)][, head(.SD, 1), by = "cluster"]
ggData <- ggData[, c("cluster", "ID")]
ggData$ID <- gsub("HAY_BONE_MARROW_", "", ggData$ID)
colnames(ggData)[2] <- "HAY_BONE_MARROW"
oupAnnot <- ggData[oupAnnot, on = "cluster"]
# Add in annotation
oupAnnot$annotation <- c("HSC","MPP","MyP1","MyP2","LyP1","LyP2","ProB",
                         "ERP","Ery","MKP","EOBM",
                         "pDC1","pDC2","cDC","Monocyte","InflamMono","Stroma",
                         "PreB","MemoryB","MatureB","BT","PlasmaCell",
                         "CD8+Naive","CD4+Naive","CD4+TCM",
                         "CD8+GZMK1","CD8+GZMK2","CD8+TEMRA","NK")
# Output table
png("images/clustReAnnotTable.png",
    width = 12, height = 9, units = "in", res = 300)
p1 <- tableGrob(oupAnnot, rows = NULL)
grid.arrange(p1)
dev.off()

saveRDS(seu, file = "bmSeu.rds")
```

3.4.3 Visualizing annotated clusters

The final step is to relabel the cell clusters into the annotation labels. The resulting annotated cell types can then be visualized in t-SNE and UMAP projections (Figure @ref{clust-annotTsum}).

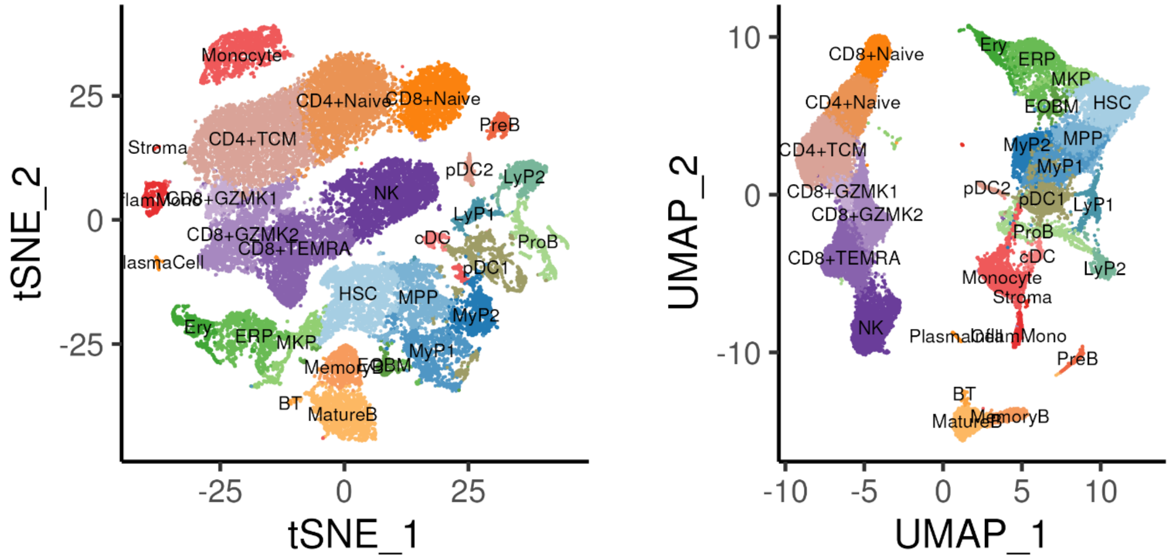


Figure 3.16: t-SNE and UMAP projections coloure by annotated cell types.

Code for the plots above:

We create a new metadata called `celltype` in the Seurat object seu by mapping the cluster labels.

```
# Add new annotation into seurat and replot
tmpMap <- oupAnnot$annotation
names(tmpMap) <- oupAnnot$cluster
seu$celltype <- tmpMap[as.character(seu$cluster)]
seu$celltype <- factor(seu$celltype, levels = tmpMap)
Idents(seu) <- seu$celltype  # Set seurat to use celltype

# Plot ideal resolution on tSNE and UMAP
p1 <- DimPlot(seu, reduction = "tsne", pt.size = 0.1, label = TRUE,
              label.size = 3, cols = colCls) + plotTheme + coord_fixed()
p2 <- DimPlot(seu, reduction = "umap", pt.size = 0.1, label = TRUE,
              label.size = 3, cols = colCls) + plotTheme + coord_fixed()
ggsave(p1 + p2 & theme(legend.position = "none"),
       width = 8, height = 4, filename = "images/clustReAnnotTsUm.png")

# Save Seurat Object at end of each section
saveRDS(seu, file = "bmSeu.rds")
```