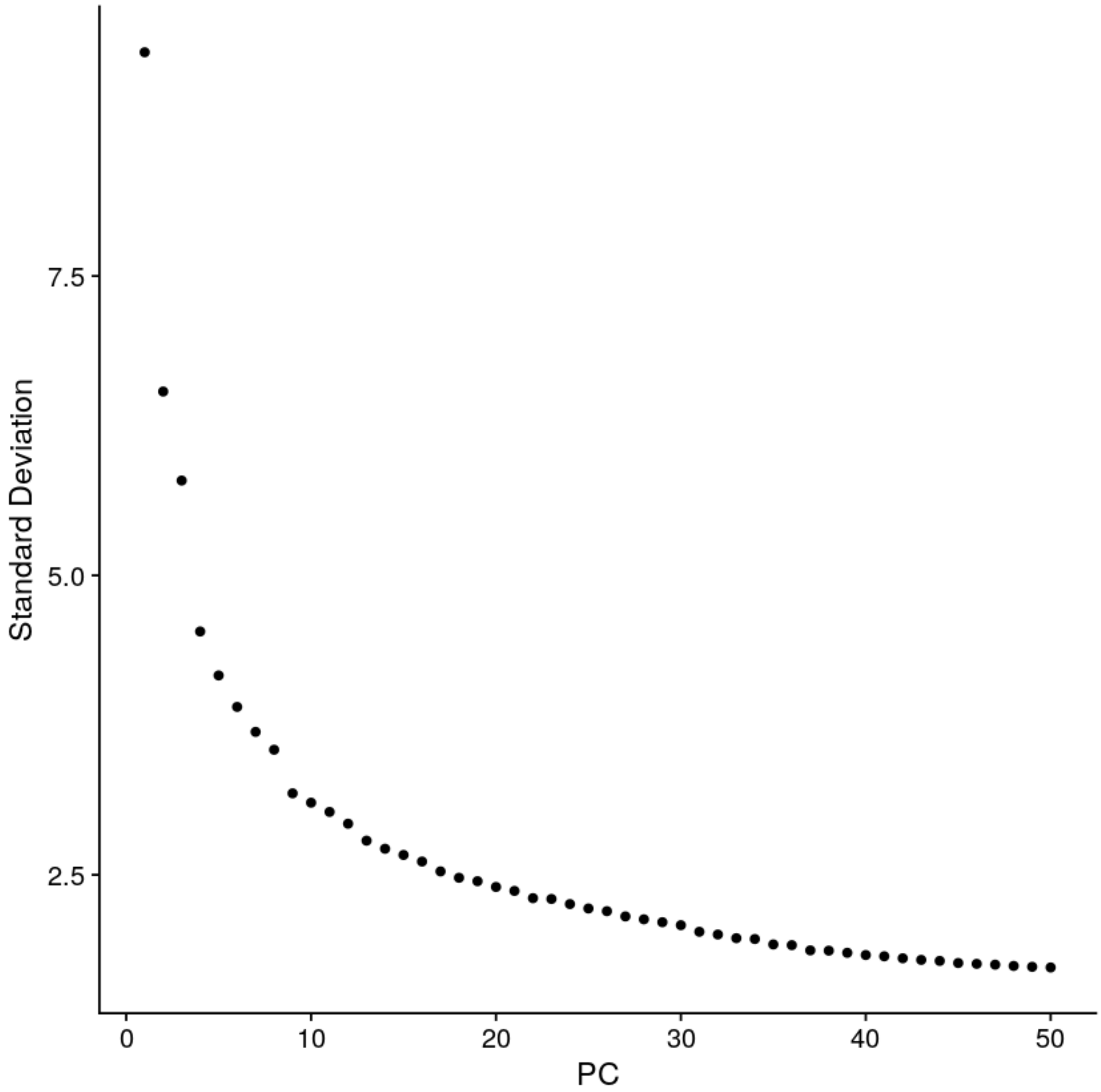
[22]:
```
DefaultAssay(cluster_subset) <- "integrated"
cluster_subset <- ScaleData(cluster_subset, verbose = FALSE)
cluster_subset <- RunPCA(cluster_subset, npcs = 50, verbose = FALSE)
ElbowPlot(cluster_subset, ndims = 50)
#these codes scale the newly created subsetted Seurat object, runs a PCA test␣
 ↪and displays the corresponding elblwo plot.
```



[23]:
```
cluster_subset <- RunUMAP(cluster_subset, reduction = "pca", dims = 1:43)
cluster_subset <- FindNeighbors(cluster_subset, reduction = "pca", dims = 1:43)
cluster_subset <- FindClusters(cluster_subset, resolution = 0.45)
#the FindNeighbors and FindClusters commands is used to cluster cells based on␣
 ↪the nearest neighbours using graphs and those cells are then clustered based␣
 ↪on their groups.
```

```
13:27:41 UMAP embedding parameters a = 0.9922 b = 1.112

13:27:41 Read 2180 rows and found 43 numeric columns

13:27:41 Using Annoy for neighbor search, n_neighbors = 30

13:27:41 Building Annoy index with metric = cosine, n_trees = 50

0%   10   20   30   40   50   60   70   80   90   100%

[----|----|----|----|----|----|----|----|----|----|

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13:27:41 Writing NN index file to temp file /tmp/RtmpZaelGq/filef381c8687c5

13:27:41 Searching Annoy index using 1 thread, search_k = 3000

13:27:42 Annoy recall = 100%

13:27:42 Commencing smooth kNN distance calibration using 1 thread

13:27:43 Initializing from normalized Laplacian + noise

13:27:43 Commencing optimization for 500 epochs, with 93036 positive edges

13:27:45 Optimization finished

Computing nearest neighbor graph

Computing SNN


Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 2180
Number of edges: 91441

Running Louvain algorithm…
Maximum modularity in 10 random starts: 0.8532
Number of communities: 6
Elapsed time: 0 seconds
```
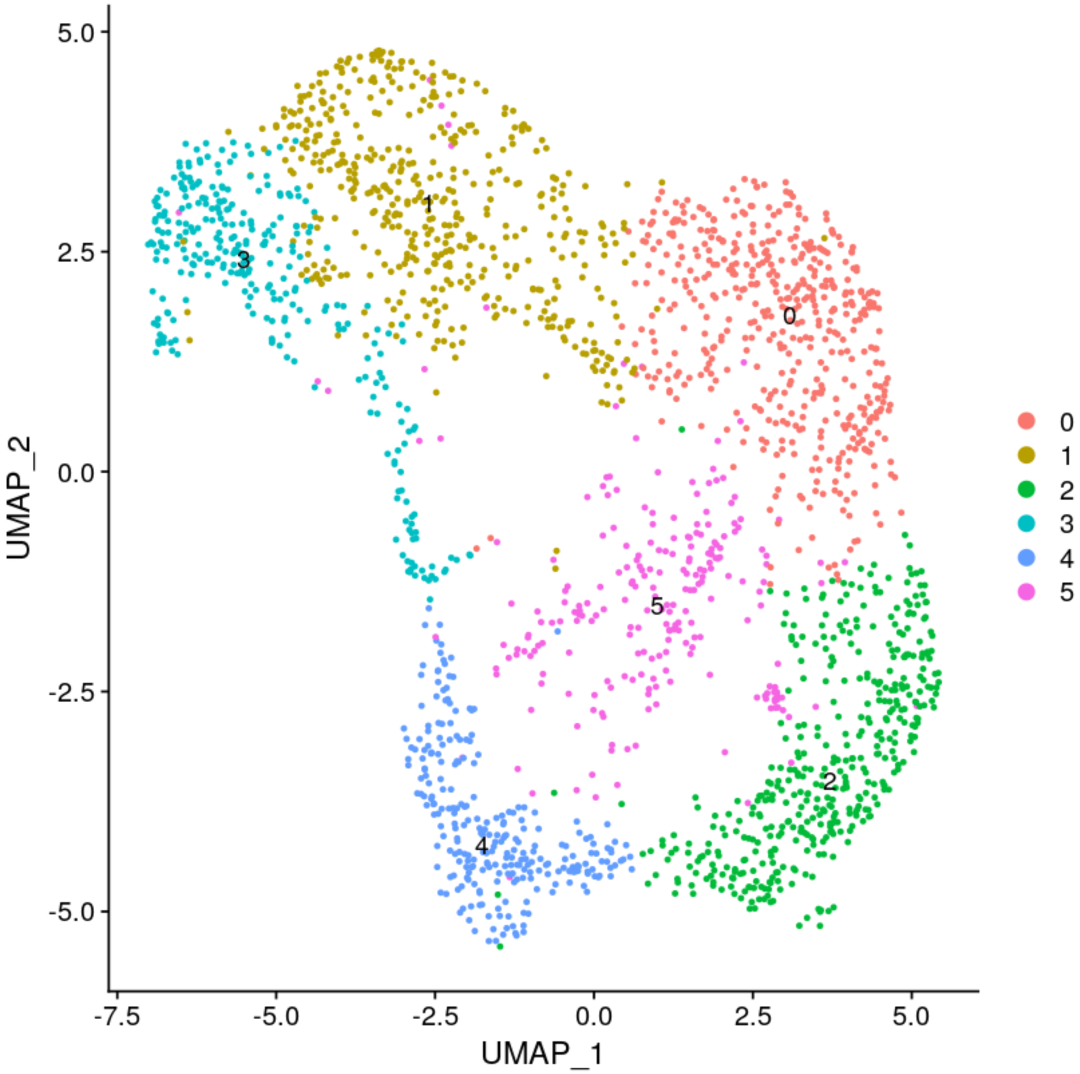

[25]:

```
DimPlot(cluster_subset, reduction = "umap", label=TRUE)
cluster_subset.ids <- c("0", "1", "2","3","4", "5")
names(cluster_subset.ids) <- levels(cluster_subset)
```

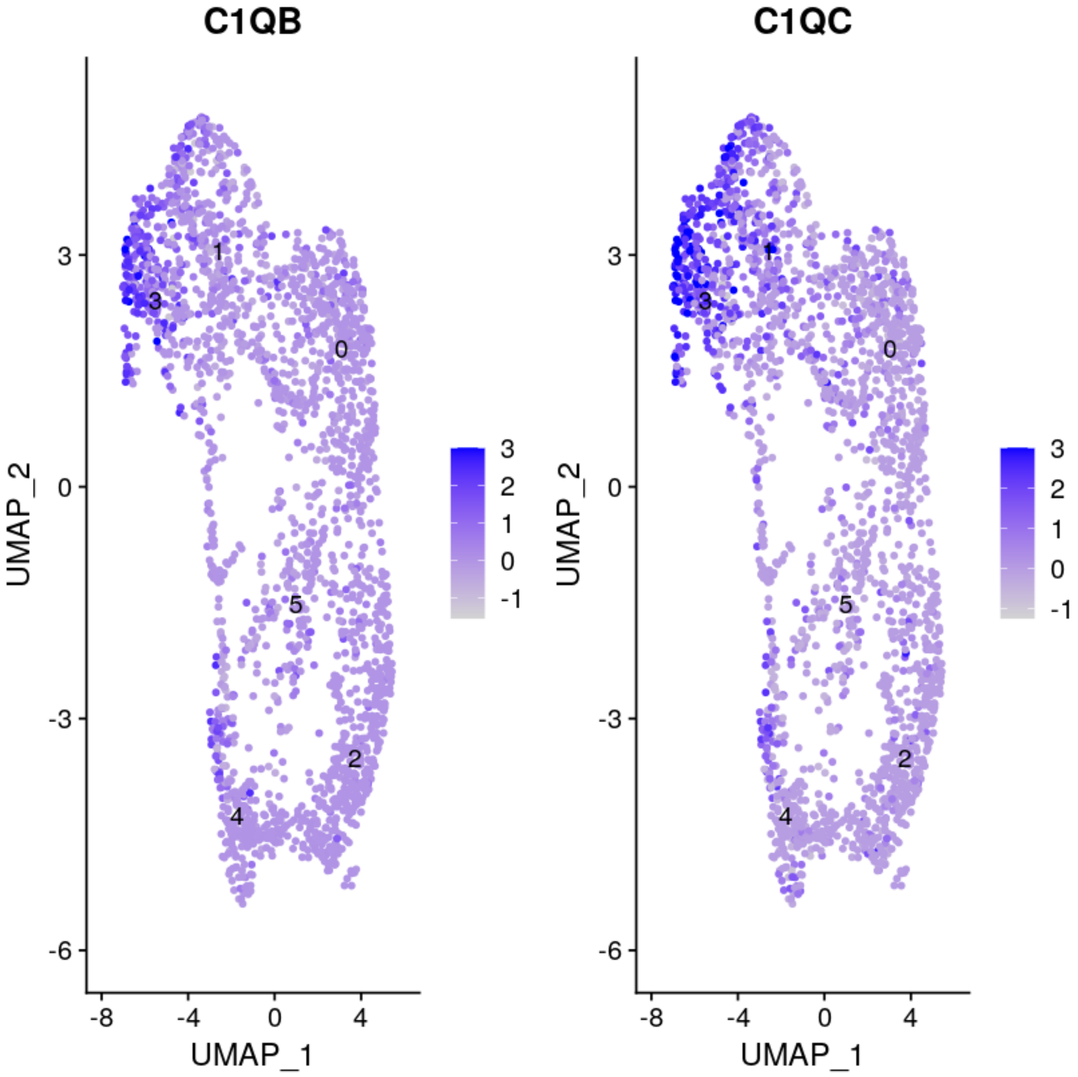
```
cluster_subset <- RenameIdents(cluster_subset, cluster_subset.ids)
#these codes are used for assigning names to the clusters. first a list is made␣
 ↪which contains the names each cluster has to be given, then the exisiting␣
 ↪values are renamed by the assigned names and an UMAP with the names cluster␣
 ↪is displayyed.
```

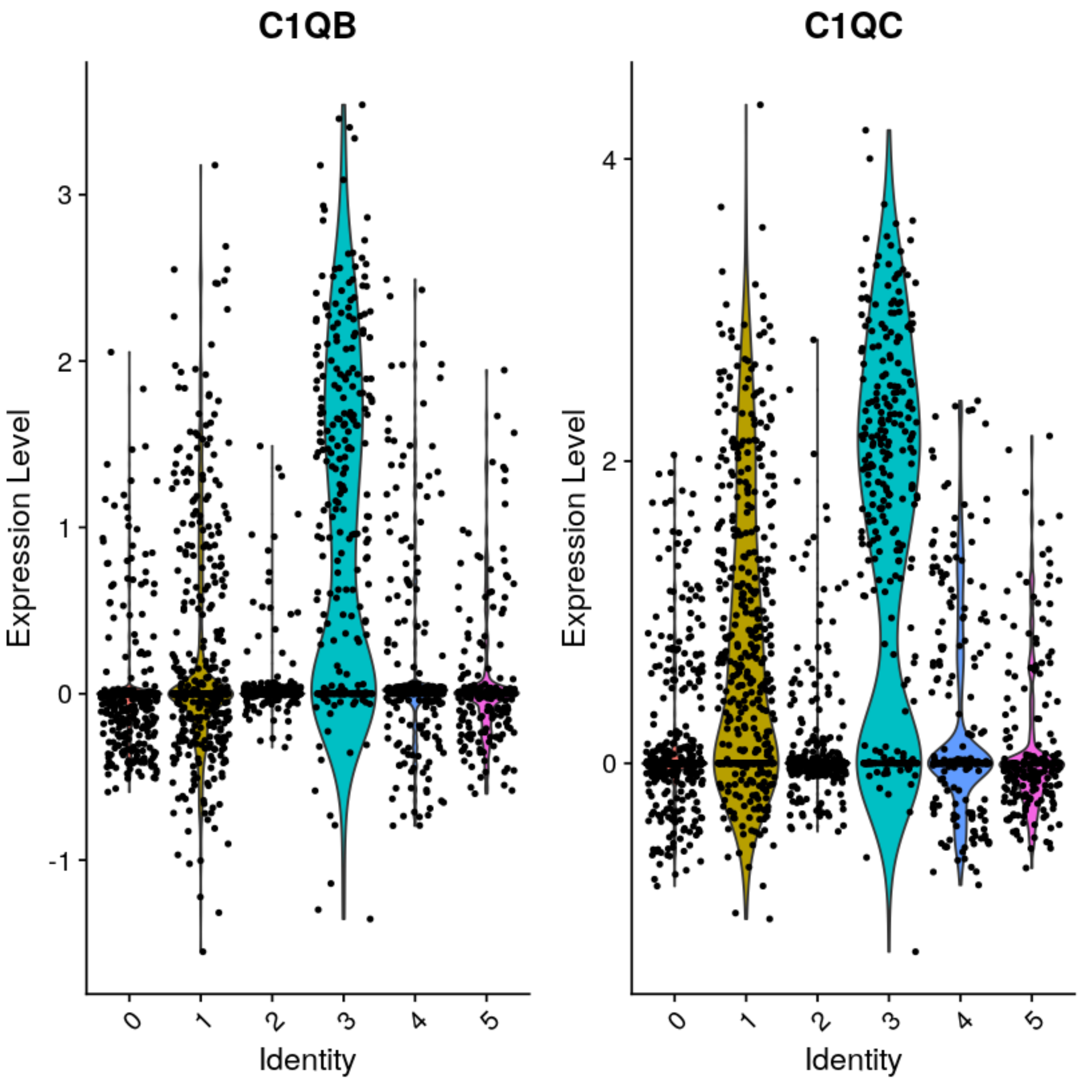


[42]:
```
saveRDS(cluster_subset,"Annotated.cluster.subset.rds")
#this is used to save the object as a RDS file
```

[26]:
```
FeaturePlot(cluster_subset, features = c("C1QB", "C1QC"), max.cutoff = 3,␣
 ↪label=TRUE, pt.size=1)
VlnPlot(cluster_subset, features = c("C1QB", "C1QC"))
```

```
#these codes display feature and violin plots comparing the markers C1QB and C1QC to view their expression levels in the subsetted(Macrophage) cluster.
```





[27]:
```
DefaultAssay(cluster_subset) <- "RNA"
cluster_subset@meta.data
#this code dsiplays all the data or information present in the object.
```

A data.frame: 2180 × 12

	orig.ident <chr>	nCount_RNA <dbl>	nFeature_R <int>
ERR5263321-GAACGGAAGTACGTTC-	DISEASE1	2467	1102
ERR5263319-GTCAAGTGTGTGAATA-	DISEASE1	5843	1427
ERR5263322-GGGCACTTCGGAGCAA-	DISEASE1	1538	908
ERR5263321-AGGCCACCAGTTCATG-	DISEASE1	2770	969
ERR5263321-AAGTCTGCATTACGAC-	DISEASE1	2359	811
ERR5263315-AAAGATGTCACGGTTA-	DISEASE1	2075	902
ERR5263321-TCCCGATTCGTGGACC-	DISEASE1	11979	1759
ERR5263321-TCATTTGAGTAGCGGT-	DISEASE1	6238	1256
ERR5263321-TGGGAAGGTCAAAGAT-	DISEASE1	1682	616
ERR5263319-GAATGAAGTCCAGTAT-	DISEASE1	3483	943
ERR5263321-CCCATACGTCTAGTGT-	DISEASE1	3769	1180
ERR5263315-GGTGTTATCTAAGCCA-	DISEASE1	2048	919
ERR5263321-ACCTTTAGTGCCTTGG-	DISEASE1	4030	1447
ERR5263319-GTATTCTAGCGTGTCC-	DISEASE1	8178	2280
ERR5263322-CGATTGAGTGGCTCCA-	DISEASE1	2340	710
ERR5263321-CGTTGGGGTCGAATCT-	DISEASE1	4973	1361
ERR5263321-CCTTTCTCATAGTAAG-	DISEASE1	4638	1200
ERR5263321-GTTAAGCAGCGTCTAT-	DISEASE1	2017	955
ERR5263321-GCTCCTAGTGGTCTCG-	DISEASE1	4857	1164
ERR5263321-CAGTAACCACGGACAA-	DISEASE1	4485	1147
ERR5263321-AACCATGAGAAGAAGC-	DISEASE1	5897	1182
ERR5263321-CACAAACAGTCTTGCA-	DISEASE1	9290	1984
ERR5263321-CCTAGCTTCAGAGACG-	DISEASE1	3153	1220
ERR5263319-ACTGTCCCAGTGGAGT-	DISEASE1	5037	1604
ERR5263321-GTGAAGGAGTAGCCGA-	DISEASE1	6379	1504
ERR5263321-TTCGAAGCAATGACCT-	DISEASE1	2651	966
ERR5263321-TCCACACGTGTTTGTG-	DISEASE1	7223	1876
ERR5263321-CAGATCATCGACGGAA-	DISEASE1	4671	1064
ERR5263319-GGGTCTGGTCACCCAG-	DISEASE1	8645	2000
ERR5263321-GTGCGGTAGATGTAAC-	DISEASE1	6245	1030
ERR5263385-CCACTACTCTGTTTGT-	HC2	7154	2355
ERR5263382-CTACCCAAGTCAATAG-	HC2	27410	3436
ERR5263385-TACAGTGTCGCAAGCC-	HC2	14069	2715
ERR5263385-CTCTGGTAGGAGCGAG-	HC2	11987	3006
ERR5263385-AACTCCCCAGTAAGAT-	HC2	5133	1932
ERR5263382-TCAGGATAGTAGCCGA-	HC2	13865	2862
ERR5263385-TAAACCGTCCAGTATG-	HC2	17015	2980
ERR5263385-GGTGAAGTCTGTGCAA-	HC2	17169	3276
ERR5263385-ATAACGCCATGAACCT-	HC2	4730	1543
ERR5263385-CAGGTGCAGCGTGAAC-	HC2	12062	2843
ERR5263385-CACAAACAGATATGGT-	HC2	5165	1827
ERR5263385-CCCAATCAGCGTAGTG-	HC2	9602	2154
ERR5263385-CTGTTTATCGCCAGCA-	HC2	13139	2898
ERR5263382-GTAGGCCTCTTTACAC-	HC2	10429	1837
ERR5263382-GTTCTCGCATCGACGC-	HC2	23236	1842
ERR5263382-CAGCATAGTCAATACC-	HC2	14581	2968
ERR5263382-TTCTTAGCAGTACACT-	HC2	14338	2840
ERR5263385-TGACAACAGTGGACGT-	HC2	9857	2436
ERR5263382-TGGTTCCAGTCTCCTC-	HC2	1984	629
ERR5263385-AGGGTGACAAGTCTAC-	HC2	5556	1956

[28]:

```
cluster_subset$Status.1 <- paste(Idents(cluster_subset), cluster_subset$Status, sep= "_")
#this code adds a column called Status.1 and pastes the values of the cluster identities along with the status(Condition) of the cells.
```

[29]:

```
Idents(cluster_subset) <- "Status.1"
cluster_subset@meta.data
```

A data.frame: 2180 × 13

	orig.ident <chr>	nCount_RNA <dbl>	nFeature_R <int>
ERR5263321-GAACGGAAGTACGTTC-	DISEASE1	2467	1102
ERR5263319-GTCAAGTGTGTGAATA-	DISEASE1	5843	1427
ERR5263322-GGGCACTTCGGAGCAA-	DISEASE1	1538	908
ERR5263321-AGGCCACCAGTTCATG-	DISEASE1	2770	969
ERR5263321-AAGTCTGCATTACGAC-	DISEASE1	2359	811
ERR5263315-AAAGATGTCACGGTTA-	DISEASE1	2075	902
ERR5263321-TCCCGATTCGTGGACC-	DISEASE1	11979	1759
ERR5263321-TCATTTGAGTAGCGGT-	DISEASE1	6238	1256
ERR5263321-TGGGAAGGTCAAAGAT-	DISEASE1	1682	616
ERR5263319-GAATGAAGTCCAGTAT-	DISEASE1	3483	943
ERR5263321-CCCATACGTCTAGTGT-	DISEASE1	3769	1180
ERR5263315-GGTGTTATCTAAGCCA-	DISEASE1	2048	919
ERR5263321-ACCTTTAGTGCCTTGG-	DISEASE1	4030	1447
ERR5263319-GTATTCTAGCGTGTCC-	DISEASE1	8178	2280
ERR5263322-CGATTGAGTGGCTCCA-	DISEASE1	2340	710
ERR5263321-CGTTGGGGTCGAATCT-	DISEASE1	4973	1361
ERR5263321-CCTTTCTCATAGTAAG-	DISEASE1	4638	1200
ERR5263321-GTTAAGCAGCGTCTAT-	DISEASE1	2017	955
ERR5263321-GCTCCTAGTGGTCTCG-	DISEASE1	4857	1164
ERR5263321-CAGTAACCACGGACAA-	DISEASE1	4485	1147
ERR5263321-AACCATGAGAAGAAGC-	DISEASE1	5897	1182
ERR5263321-CACAAACAGTCTTGCA-	DISEASE1	9290	1984
ERR5263321-CCTAGCTTCAGAGACG-	DISEASE1	3153	1220
ERR5263319-ACTGTCCCAGTGGAGT-	DISEASE1	5037	1604
ERR5263321-GTGAAGGAGTAGCCGA-	DISEASE1	6379	1504
ERR5263321-TTCGAAGCAATGACCT-	DISEASE1	2651	966
ERR5263321-TCCACACGTGTTTGTG-	DISEASE1	7223	1876
ERR5263321-CAGATCATCGACGGAA-	DISEASE1	4671	1064
ERR5263319-GGGTCTGGTCACCCAG-	DISEASE1	8645	2000
ERR5263321-GTGCGGTAGATGTAAC-	DISEASE1	6245	1030
ERR5263385-CCACTACTCTGTTTGT-	HC2	7154	2355
ERR5263382-CTACCCAAGTCAATAG-	HC2	27410	3436
ERR5263385-TACAGTGTCGCAAGCC-	HC2	14069	2715
ERR5263385-CTCTGGTAGGAGCGAG-	HC2	11987	3006
ERR5263385-AACTCCCCAGTAAGAT-	HC2	5133	1932
ERR5263382-TCAGGATAGTAGCCGA-	HC2	13865	2862
ERR5263385-TAAACCGTCCAGTATG-	HC2	17015	2980
ERR5263385-GGTGAAGTCTGTGCAA-	HC2	17169	3276
ERR5263385-ATAACGCCATGAACCT-	HC2	4730	1543
ERR5263385-CAGGTGCAGCGTGAAC-	HC2	12062	2843
ERR5263385-CACAAACAGATATGGT-	HC2	5165	1827
ERR5263385-CCCAATCAGCGTAGTG-	HC2	9602	2154
ERR5263385-CTGTTTATCGCCAGCA-	HC2	13139	2898
ERR5263382-GTAGGCCTCTTTACAC-	HC2	10429	1837
ERR5263382-GTTCTCGCATCGACGC-	HC2	23236	1842
ERR5263382-CAGCATAGTCAATACC-	HC2	14581	2968
ERR5263382-TTCTTAGCAGTACACT-	HC2	14338	2840
ERR5263385-TGACAACAGTGGACGT-	HC2	9857	2436
ERR5263382-TGGTTCCAGTCTCCTC-	HC2	1984	629
ERR5263385-AGGGTGACAAGTCTAC-	HC2	5556	1956

[30]:

```r
mark3_6<- FindMarkers(cluster_subset, ident.1 = "3_DISEASE", ident.2= "3_HC", verbose= FALSE)
head(mark3_6, n=15)
#this code gives the names of top 15 genes which are differentially expressed in The cluster between Diseased and Healthy Cnditions.
```

A data.frame: 15 × 5

	p_val <dbl>	avg_log2FC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>
HLA-DRB5	1.256407e-48	3.736725	0.798	0.000	3.647727e-44
HLA-B.5	2.091992e-44	3.711853	0.798	0.033	6.073682e-40
HLA-DRB1	4.069076e-35	3.787733	0.857	0.192	1.181375e-30
HLA-DPB1.4	6.811437e-34	3.880340	0.583	0.000	1.977565e-29
HLA-DRA.5	1.756299e-26	4.040695	0.619	0.075	5.099062e-22
HLA-A.5	5.416049e-25	1.846551	0.440	0.000	1.572442e-20
RPS27	1.883732e-22	1.594719	0.964	0.832	5.469039e-18
HLA-DRA	4.400714e-22	2.841697	0.595	0.084	1.277659e-17
RPL37	6.963920e-22	1.774316	0.964	0.808	2.021835e-17
HLA-C	4.422085e-21	1.644738	0.524	0.051	1.283864e-16
RPS29	8.460485e-21	2.297213	0.845	0.523	2.456333e-16
RPS4Y1	4.132592e-20	1.270535	0.357	0.000	1.199815e-15
HSPA1B.3	2.220476e-19	4.675379	0.679	0.173	6.446708e-15
RPS18.1	9.510846e-19	1.706475	0.940	0.813	2.761284e-14
HSPA6	1.092857e-18	2.943946	0.583	0.112	3.172893e-14

[31]:

```r
markers3_6<- subset(mark3_6,  mark3_6$p_val_adj<0.01 & mark3_6$avg_log2FC > 0.5, sep="\t")
#this code is used to subset the dataframe "mark3_6" into markers3_6 by filtering the genes that have their adjusted p values less than 0.01 and average log2 fold change greater than 0.5

write.table(markers3_6, "ma3.txt")
nrow(read.table("ma3.txt"))
read.table("ma3.txt")
#this code writes the data in markers3_6 as a text file and reads the table along with displaying the number of rows for calculating the number of upregulated genes.
```

```
103
```

A data.frame: 103 × 5

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
HLA-DRB5	1.256407e-48	3.736725	0.798	0.000	3.647727e-44
HLA-B.5	2.091992e-44	3.711853	0.798	0.033	6.073682e-40
HLA-DRB1	4.069076e-35	3.787733	0.857	0.192	1.181375e-30
HLA-DPB1.4	6.811437e-34	3.880340	0.583	0.000	1.977565e-29
HLA-DRA.5	1.756299e-26	4.040695	0.619	0.075	5.099062e-22
HLA-A.5	5.416049e-25	1.846551	0.440	0.000	1.572442e-20
RPS27	1.883732e-22	1.594719	0.964	0.832	5.469039e-18
HLA-DRA	4.400714e-22	2.841697	0.595	0.084	1.277659e-17
RPL37	6.963920e-22	1.774316	0.964	0.808	2.021835e-17
HLA-C	4.422085e-21	1.644738	0.524	0.051	1.283864e-16
RPS29	8.460485e-21	2.297213	0.845	0.523	2.456333e-16
RPS4Y1	4.132592e-20	1.270535	0.357	0.000	1.199815e-15
HSPA1B.3	2.220476e-19	4.675379	0.679	0.173	6.446708e-15
RPS18.1	9.510846e-19	1.706475	0.940	0.813	2.761284e-14
HSPA6	1.092857e-18	2.943946	0.583	0.112	3.172893e-14
RPL39	1.820971e-18	1.302455	0.952	0.888	5.286826e-14
ENSG00000255823	2.123177e-18	2.105805	0.988	0.846	6.164220e-14
RPL26	2.183219e-18	1.503636	0.940	0.860	6.338541e-14
HSPA1A.2	7.593692e-17	4.561221	0.750	0.276	2.204676e-12
CRIP1	1.006219e-16	2.258140	0.798	0.411	2.921357e-12
RPL35A	2.438163e-16	1.305648	0.940	0.850	7.078718e-12
RPL34	2.726739e-16	1.595162	0.929	0.855	7.916541e-12
RPL30	6.066402e-16	1.149546	0.952	0.944	1.761259e-11
RPS15A	9.898638e-16	1.219732	0.952	0.855	2.873872e-11
RPS21	1.597231e-15	1.453460	0.881	0.762	4.637240e-11
RPL22	2.079838e-15	1.324975	0.893	0.710	6.038393e-11
RPL36A	2.100680e-15	1.511693	0.869	0.636	6.098904e-11
RPS28	8.198890e-15	1.021432	0.952	0.921	2.380384e-10
RBM3	1.076171e-14	1.221256	0.607	0.173	3.124447e-10
RPL12	1.504163e-14	1.040708	0.976	0.902	4.367036e-10
RPS2	1.224239e-09	1.1510704	0.833	0.682	3.554332e-05
PTMS	1.466983e-09	1.0088060	0.417	0.117	4.259092e-05
CD52	1.601156e-09	0.9461139	0.238	0.023	4.648637e-05
RPS12	1.961004e-09	0.7190907	0.940	0.925	5.693383e-05
RPS5	2.040141e-09	0.9451714	0.893	0.724	5.923142e-05
RPS9.4	2.361833e-09	1.1326006	0.881	0.813	6.857111e-05
HSPA8	2.767333e-09	1.0906411	0.833	0.523	8.034398e-05
NME2	2.994733e-09	1.1536034	0.714	0.458	8.694610e-05
TPT1	3.782704e-09	0.8020822	0.964	0.949	1.098232e-04
GADD45B	3.904325e-09	1.6084506	0.821	0.467	1.133543e-04
RPLP1	4.398067e-09	0.6845817	0.988	0.981	1.276891e-04
RPS15	4.913834e-09	0.8120104	0.917	0.935	1.426633e-04
RPL35	9.370796e-09	0.8473703	0.857	0.785	2.720623e-04
HLA-E.2	9.806756e-09	0.7848106	0.607	0.276	2.847195e-04
RPS6	1.149616e-08	0.9508497	0.905	0.738	3.337682e-04
RPL31	1.319417e-08	1.0838110	0.774	0.579	3.830662e-04
VIM	2.390553e-08	0.9176227	0.952	0.921	6.940493e-04
JAML	2.404369e-08	0.8221210	0.190	0.014	6.980604e-04
RPL5	2.898639e-08	1.0505510	0.833	0.706	8.415619e-04
RPL19	3.455662e-08	0.7227152	0.881	0.916	1.003282e-03