**Домашнее задание №3**
**Анализ single-cell RNA-seq в R**

0. Создаем новый проект в RStudio.
Скачиваем файл из папки R_single-cell_RNA-seq > hw > pbmc_hw_sub.RData
Файл содержит матрицы каунтов scRNA-seq для образца PBMC, человек.
Вам понадобится скрипт R_scRNA-seq.R.

1. Загружаем данные в проект, это уже готовый seurat object для образца.
pbmc <- readRDS("~/path_to/pbmc_hw_sub.RData")

2. Фильтрация.
Тщательно фильтруем данные, аккуратно выбираем фильтры на mt-контент, nFeature; рисуем картинки до-после фильтрации. Сколько клеток было, сколько стало после фильтрации?

*Количество клеток: 2700*

```
> dim(meta) # shows number of cells and number of metadata columns for cells
[1] 2700    3

> head(meta)
                orig.ident nCount_RNA nFeature_RNA
AAACATACAACCAC-1     pbmc3k       2419          779
AAACATTGAGCTAC-1     pbmc3k       4903         1352
AAACATTGATCAGC-1     pbmc3k       3147         1129
AAACCGTGCTTCCG-1     pbmc3k       2639          960
AAACCGTGTATGCG-1     pbmc3k        980          521
AAACGCACTGGTAC-1     pbmc3k       2163          781
> summary(meta$nCount_RNA)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
    546    1756    2196    2365    2762   15818
> summary(meta$nFeature_RNA)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  212.0   690.0   816.0   845.5   952.0  3400.0
> head(pbmc[[]])
                orig.ident nCount_RNA nFeature_RNA percent.mt
AAACATACAACCAC-1     pbmc3k       2419          779  3.0177759
AAACATTGAGCTAC-1     pbmc3k       4903         1352  3.7935958
AAACATTGATCAGC-1     pbmc3k       3147         1129  0.8897363
AAACCGTGCTTCCG-1     pbmc3k       2639          960  1.7430845
AAACCGTGTATGCG-1     pbmc3k        980          521  1.2244898
AAACGCACTGGTAC-1     pbmc3k       2163          781  1.6643551
                percent.rb
AAACATACAACCAC-1   43.69574
AAACATTGAGCTAC-1   42.40261
AAACATTGATCAGC-1   31.68097
AAACCGTGCTTCCG-1   24.25161
AAACCGTGTATGCG-1   14.89796
AAACGCACTGGTAC-1   36.19972
```

*Количество клеток до фильтрации:*

```
> #Number of cells before filtration
> dim(pbmc)[2]
```

*Количество клеток после фильтрации:*

```
[1] 2700
> #Number of cells after filtration
> dim(pbmc)[2]
[1] 2635
```

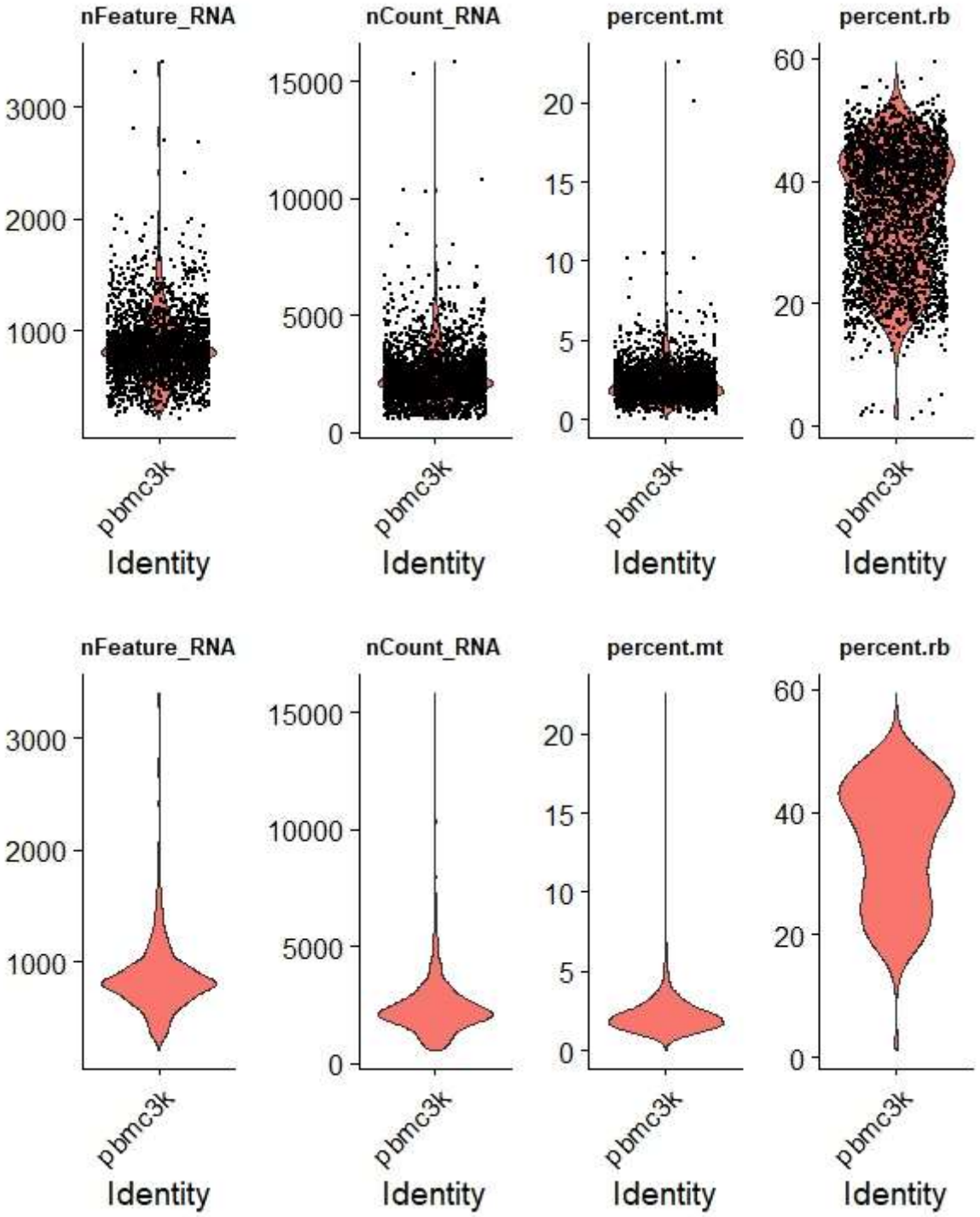

nFeature_RNA
nCount_RNA
percent.mt
percent.rb
3000
2000
1000
15000
10000
5000
0
20
15
10
5
0
60
40
20
0
pbmc3k
Identity

График корреляции между числом каунтов и процентом mt-контента и каунтов и числом генов в образце:

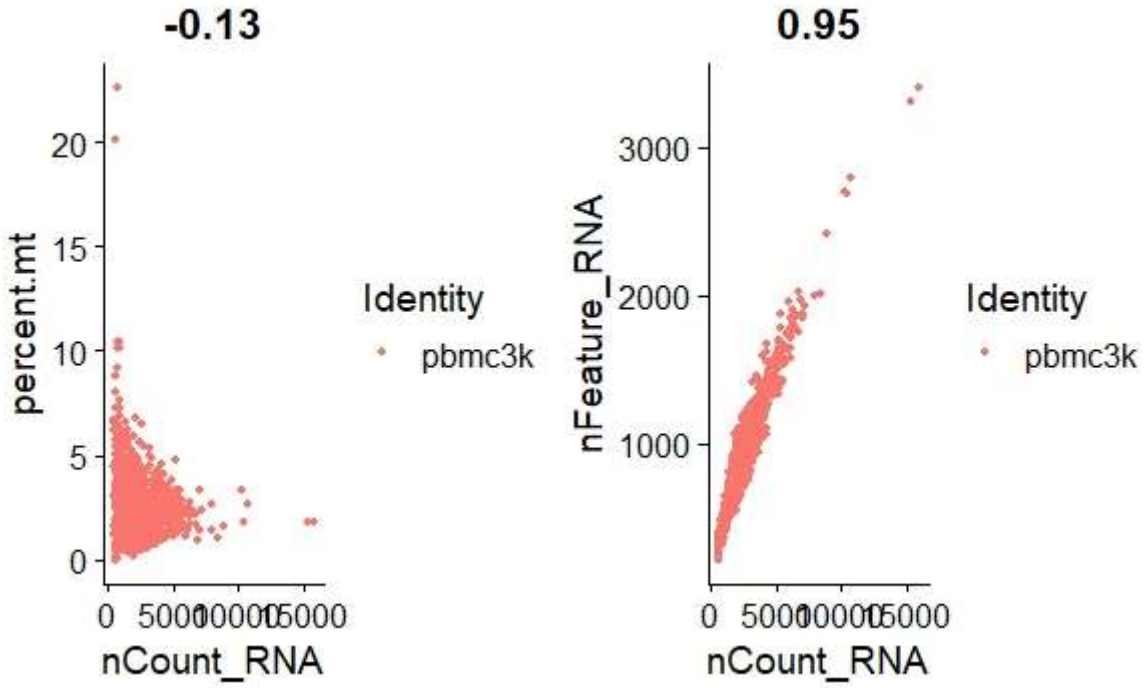



График корреляции между числом каунтов и процентом rb-контента:

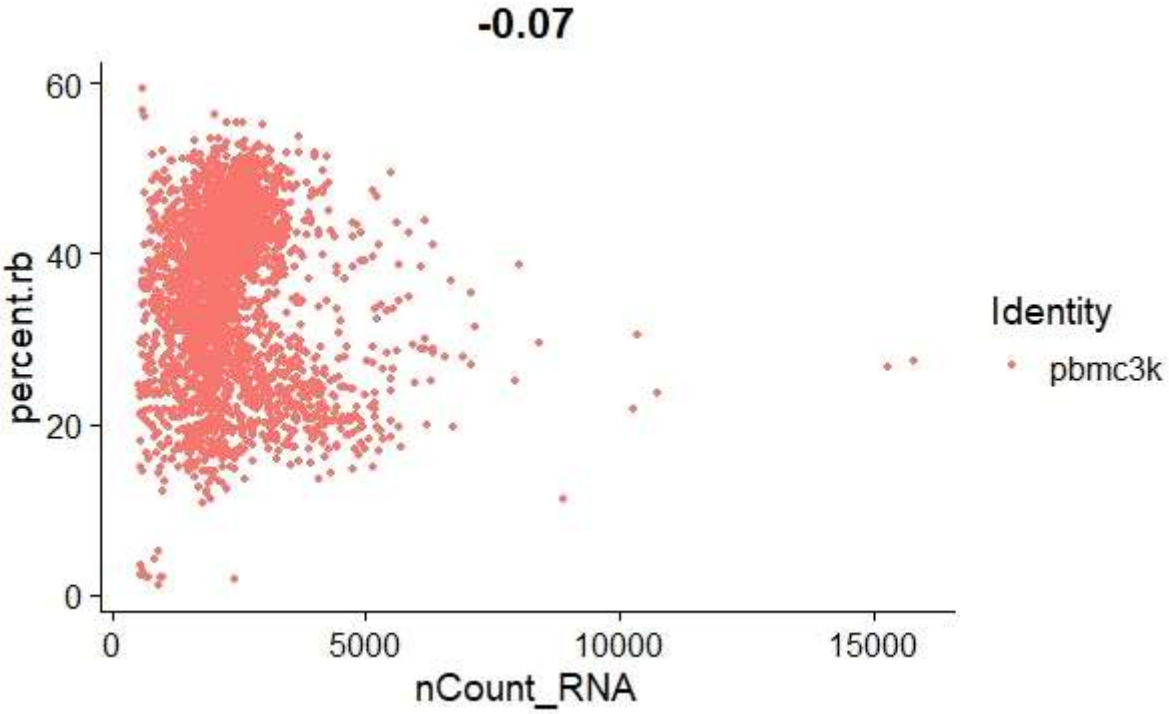



График корреляции между процентом rb-контента и процентом mt-контента:

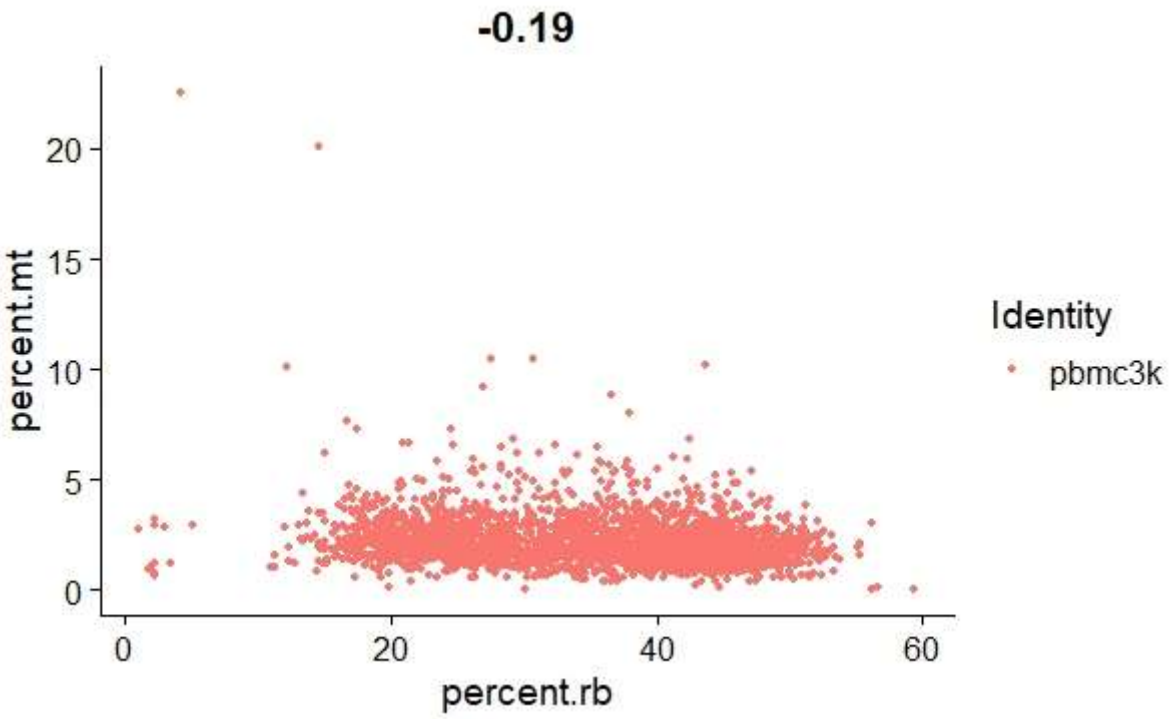
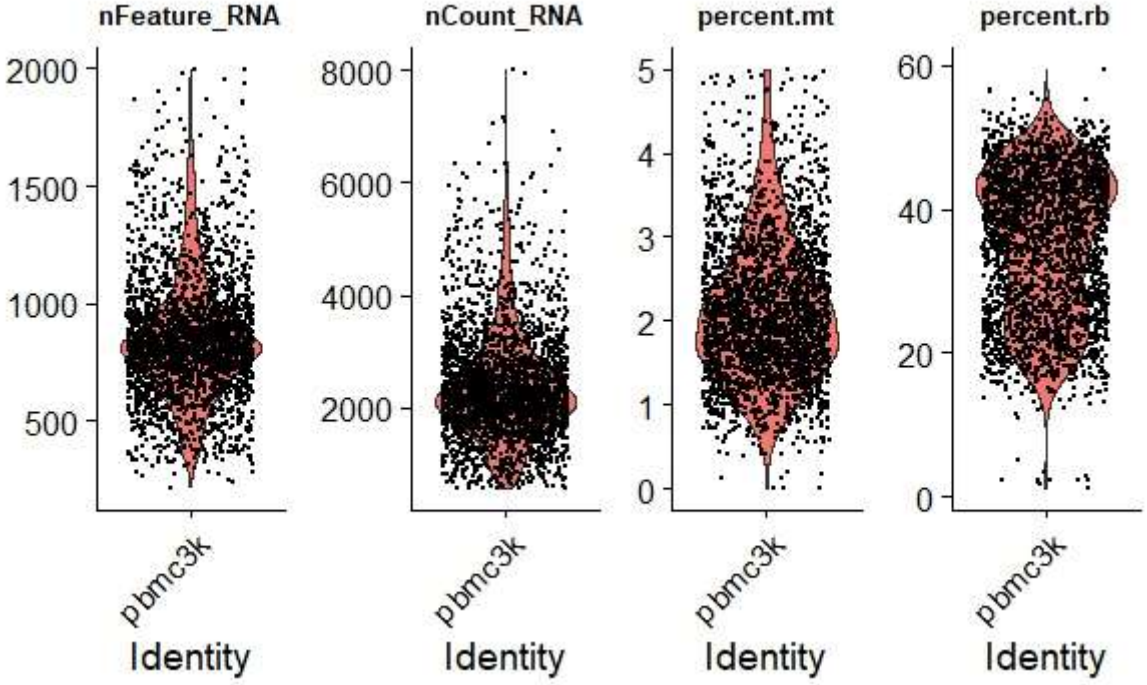
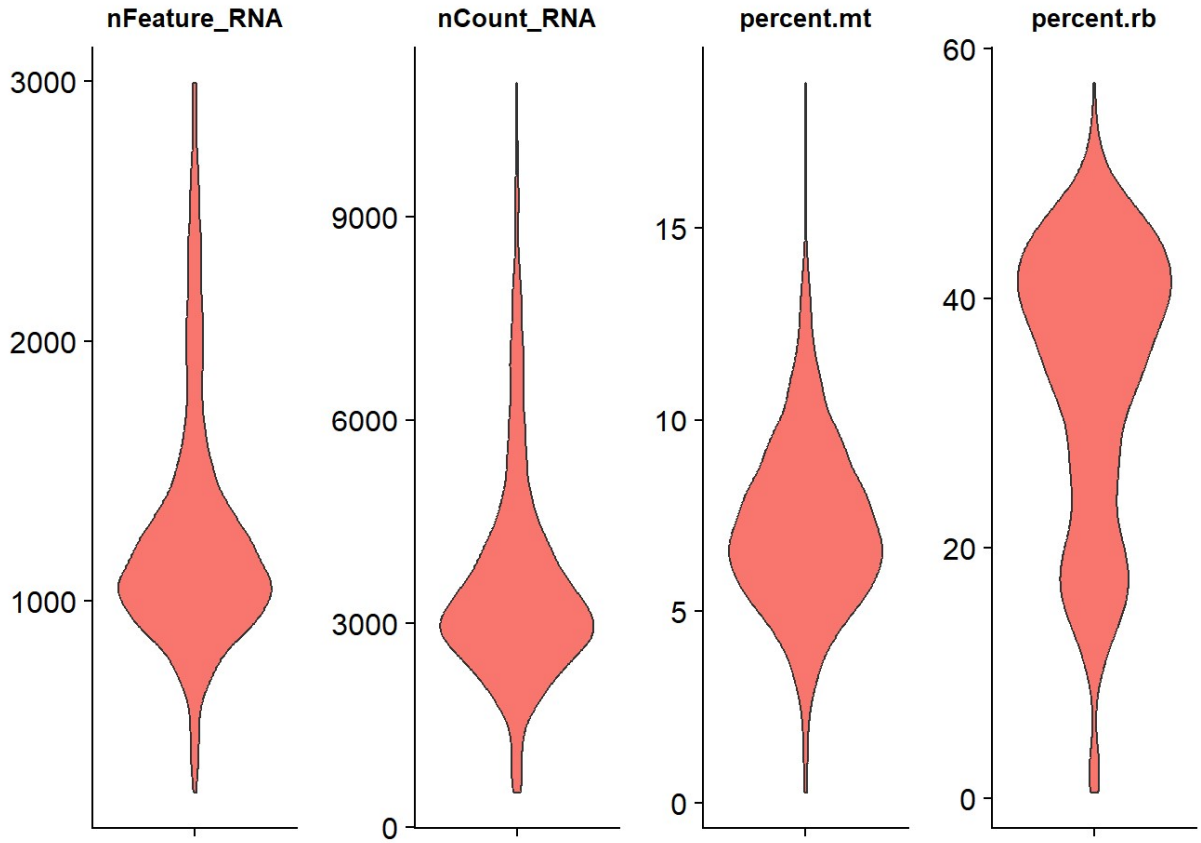



График отфильтрованных данных:






3. Делаем нормализацию, variable features, scale, RunPCA, рисуем ElbowPlot. Сколько PC (главных компонент) описывает разницу? 10 или больше, меньше? Выбираем количество PC для следующего шага

```
> pbmc <- NormalizeData(pbmc) #log normalization
Normalizing layer: counts
Performing log-normalization
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
> #Next step discovers the most variable features (genes) - these are usually
most interesting for downstream analysis.
> pbmc <- FindVariableFeatures(pbmc, selection.method = "vst", nfeatures = 20
00)
Finding variable features for layer counts
Calculating gene variances
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
Calculating feature variances of standardized and clipped values
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
> top10 <- head(VariableFeatures(pbmc), 10)
> top10
 [1] "PPBP"   "LYZ"    "S100A9" "IGLL5"  "GNLY"   "FTL"    "PF4"
 [8] "FTH1"   "S100A8" "FCER1A"
# ScaleData converts normalized gene expression to Z-score (values centered a
t 0 and with variance of 1).
> # It's stored in pbmc[['RNA']]@scale.data and used in following PCA. Defaul
t is to run scaling only on variable genes.
> all.genes <- rownames(pbmc)
> pbmc <- ScaleData(pbmc, features = all.genes) # optionally you can add here
vars.to.regress = "percent.mt"
Centering and scaling data matrix
  |============================================================| 100%
```

```
> # Run PCA
> pbmc <- RunPCA(pbmc, features = VariableFeatures(object = pbmc))
PC_ 1
Positive:  CST3, TYROBP, LST1, AIF1, FTL, LYZ, FCN1, FTH1, S100A9, TYMP
           FCER1G, CFD, LGALS1, S100A8, LGALS2, CTSS, SERPINA1, IFITM3, SPI1,
CFP
           PSAP, IFI30, SAT1, COTL1, S100A11, NPC2, GRN, LGALS3, GSTP1, PYCAR
D
Negative:  MALAT1, LTB, IL32, IL7R, CD2, B2M, ACAP1, CD27, STK17A, CTSW
           CD247, GIMAP5, AQP3, CCL5, SELL, GZMA, TRAF3IP3, CST7, MAL, ITM2A
           HOPX, GIMAP7, MYC, BEX2, LDLRAP1, GZMK, ZAP70, ETS1, TNFAIP8, RIC3
PC_ 2
Positive:  CD79A, MS4A1, TCL1A, HLA-DQA1, HLA-DQB1, HLA-DRA, LINC00926, CD79B
, HLA-DRB1, CD74
           HLA-DPB1, HLA-DMA, HLA-DQA2, CD37, HLA-DRB5, HLA-DPA1, HLA-DMB, LT
B, FCRLA, HVCN1
           BLNK, P2RX5, IGLL5, IRF8, SWAP70, ARHGAP24, FCGR2B, SMIM14, PPP1R1
4A, C16orf74
Negative:  NKG7, PRF1, CST7, GZMB, GZMA, FGFBP2, CTSW, GNLY, B2M, SPON2
           CCL4, GZMH, FCGR3A, CCL5, CD247, XCL2, CLIC3, AKR1C3, SRGN, HOPX
           TTC38, APMAP, CTSC, S100A4, IGFBP7, ID2, ANXA1, IL32, XCL1, TPST2
PC_ 3
Positive:  HLA-DQA1, CD79A, CD79B, HLA-DQB1, MS4A1, CD74, HLA-DPB1, HLA-DPA1,
HLA-DRB1, TCL1A
           HLA-DQA2, HLA-DRA, HLA-DRB5, LINC00926, HLA-DMA, HLA-DMB, HVCN1, F
CRLA, CD37, GZMB
           PLAC8, IRF8, BLNK, FGFBP2, FCGR3A, IGLL5, SWAP70, SMIM14, P2RX5, P
RF1
Negative:  IL7R, TMSB4X, VIM, IL32, S100A8, S100A6, FYB, GIMAP7, S100A4, MAL
           AQP3, S100A9, CD2, S100A10, CD14, GIMAP4, LDLRAP1, RBP7, CD27, ANX
A1
           LGALS2, S100A12, PPBP, GIMAP5, NDFIP1, NRGN, FOLR3, LYZ, SPARC, GP
X1
PC_ 4
Positive:  PPBP, PF4, SDPR, SPARC, GNG11, HIST1H2AC, NRGN, GP9, CLU, CD9
           AP001189.4, TUBB1, ITGA2B, PTCRA, CA2, TMEM40, TREML1, MYL9, ACRBP
, MMD
           F13A1, SEPT5, MPP1, TSC22D1, CMTM5, RP11-367G6.3, GP1BA, LY6G6F, C
LEC1B, MAP3K7CL
Negative:  MALAT1, VIM, LTB, IL7R, GIMAP7, IL32, EIF3H, S100A10, AQP3, MAL
           CD2, CD27, GIMAP4, TRAF3IP3, PPA1, S100A6, S100A4, GIMAP5, S100A11
, ANXA1
           CCDC109B, CYTIP, KLF6, TRADD, ATP5H, UBXN1, ANXA5, RBM3, TRABD2A,
PTGES3
PC_ 5
Positive:  LTB, CKB, MS4A7, IL7R, SIGLEC10, RP11-290F20.3, CYTIP, AQP3, HMOX1
, VIM
           MPP1, LILRB2, SDPR, HN1, GDI2, CTD-2006K23.1, PF4, PTGES3, CORO1B,
TIMP1
           VMO1, HIST1H2AC, ATP1A1, ANXA5, GNG11, CA2, CLU, WARS, IFITM2, SPA
RC
Negative:  S100A8, FGFBP2, NKG7, GZMB, GNLY, CCL4, CST7, LGALS2, S100A9, GZMA
           PRF1, SPON2, CD14, CCL3, S100A12, RBP7, GZMH, MS4A6A, FOLR3, CTSW
           GSTP1, XCL2, CLIC3, TYROBP, IGFBP7, TTC38, XCL1, AKR1C3, ASGR1, LY
Z
```

*Наиболее дифференциально экспрессируемые гены:*

```
> # Some ways to investigate PCA results and explore the heterogeneity of the
data
> print(pbmc[["pca"]], dims = 1:5, nfeatures = 5) # Top 5 genes explaining th
e difference
PC_ 1
Positive:  CST3, TYROBP, LST1, AIF1, FTL
Negative:  MALAT1, LTB, IL32, IL7R, CD2
PC_ 2
Positive:  CD79A, MS4A1, TCL1A, HLA-DQA1, HLA-DQB1
Negative:  NKG7, PRF1, CST7, GZMB, GZMA
PC_ 3
Positive:  HLA-DQA1, CD79A, CD79B, HLA-DQB1, MS4A1
Negative:  IL7R, TMSB4X, VIM, IL32, S100A8
PC_ 4
Positive:  PPBP, PF4, SDPR, SPARC, GNG11
```

```
Negative:  MALAT1, VIM, LTB, IL7R, GIMAP7
PC_ 5
Positive:  LTB, CKB, MS4A7, IL7R, SIGLEC10
Negative:  S100A8, FGFBP2, NKG7, GZMB, GNLY
```

*топ 2000 генов с дифференциальной экспрессией:*

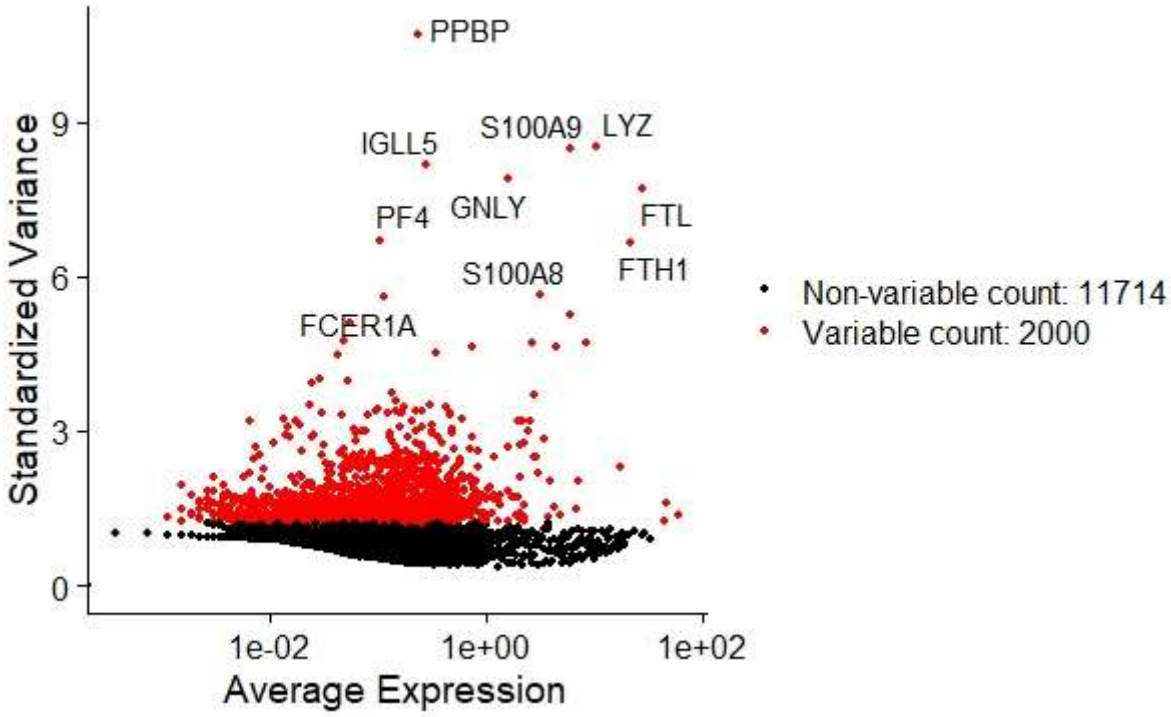
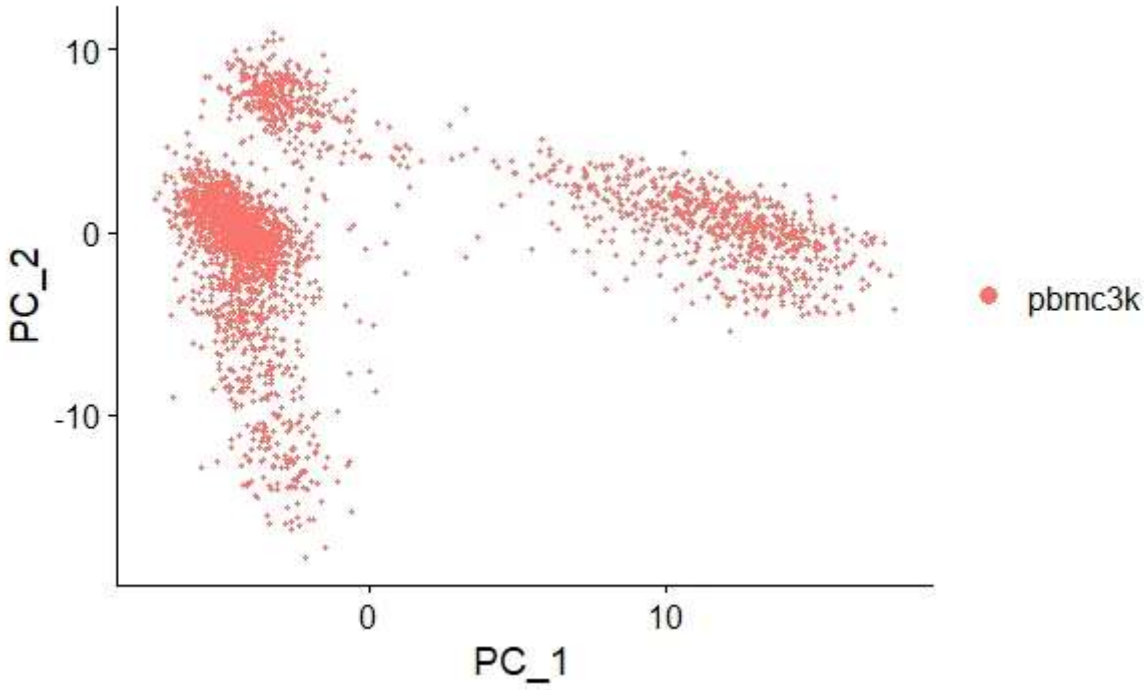
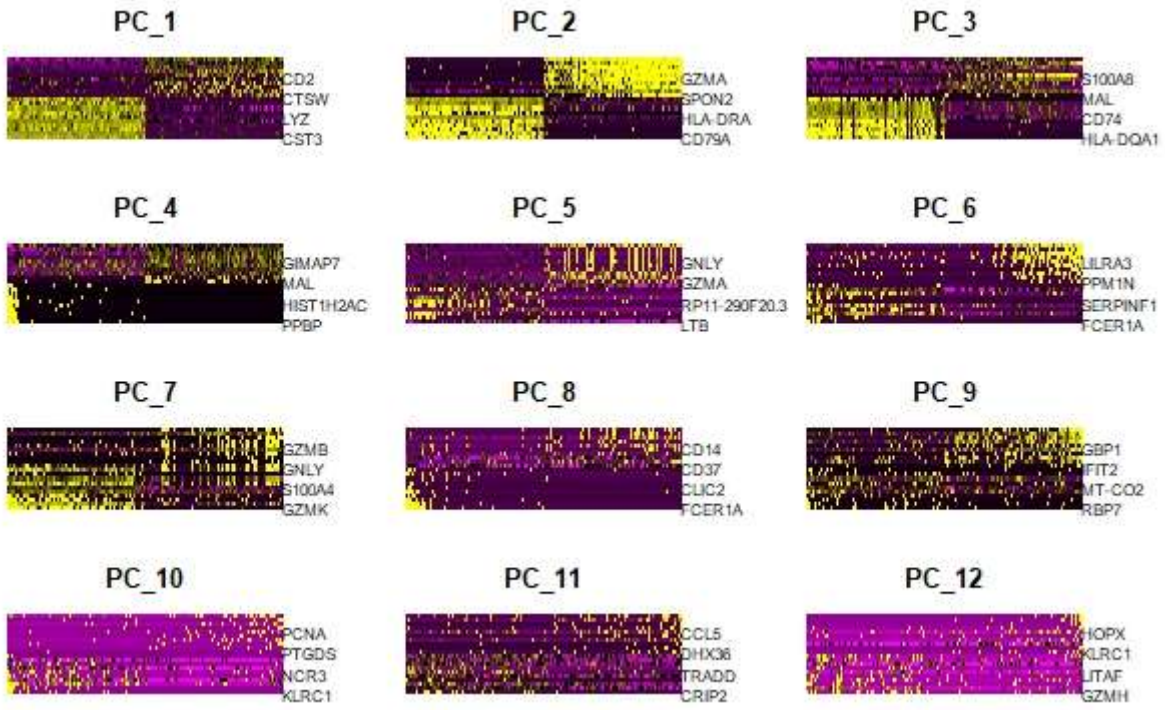


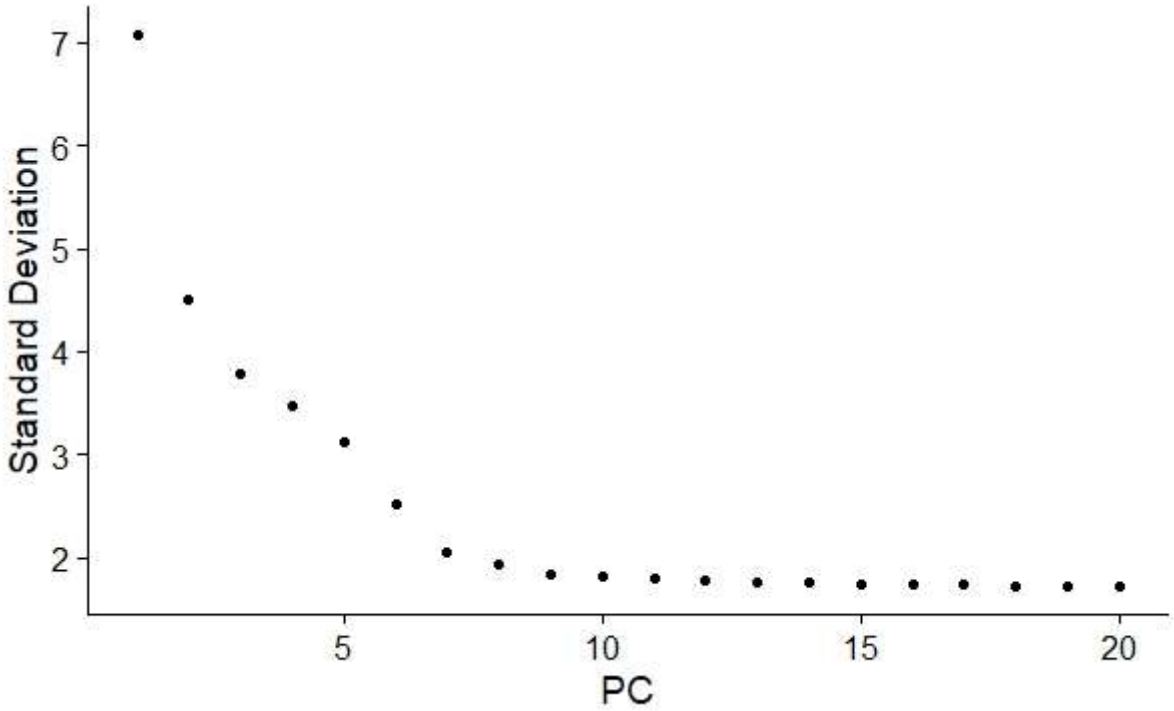

*График PCA:*




*Хитмап дифференциально экспрессируемых генов:*




*ElbowPlot:*




4. UMAP и кластеризация.

Подставляем в функцию RunUMAP параметр dims = 1:n_PC

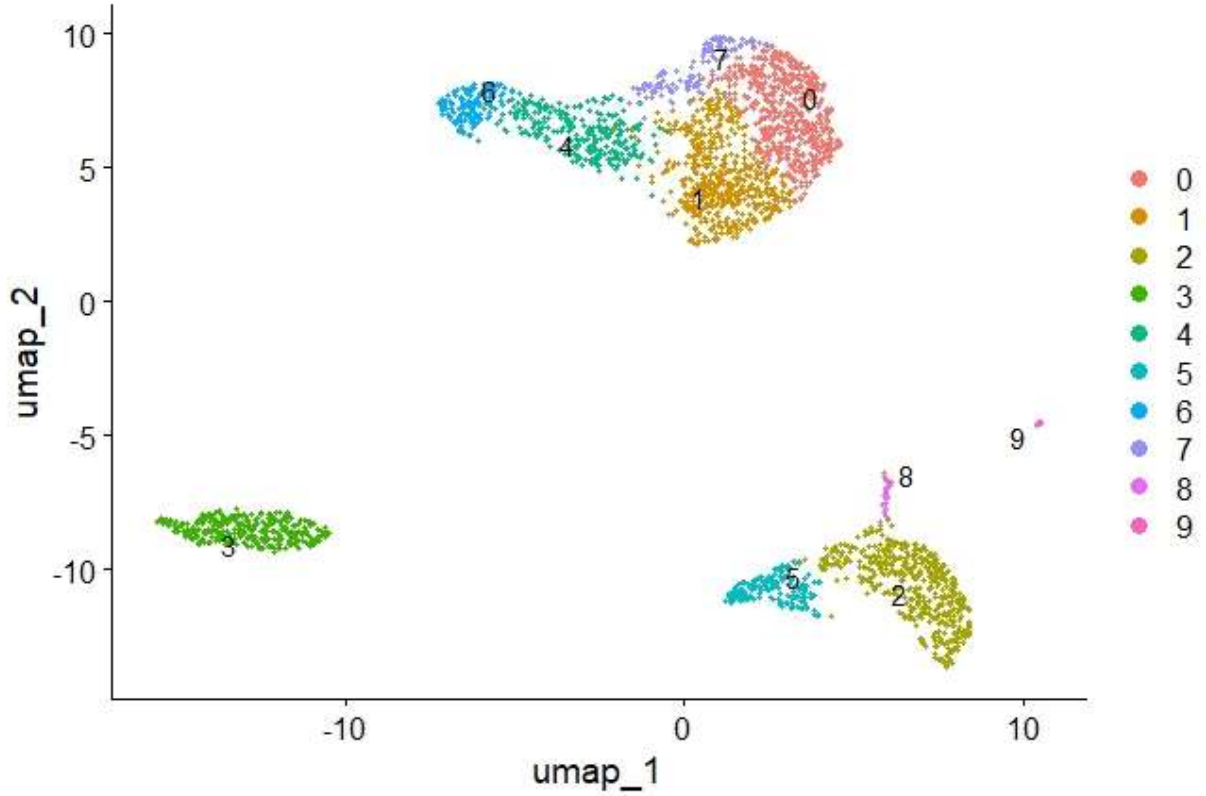
Как выглядит UMAP? Сколько получилось кластеров? Какой resolution выбрали (0.3, 0.4, 0.5, 0.6)?

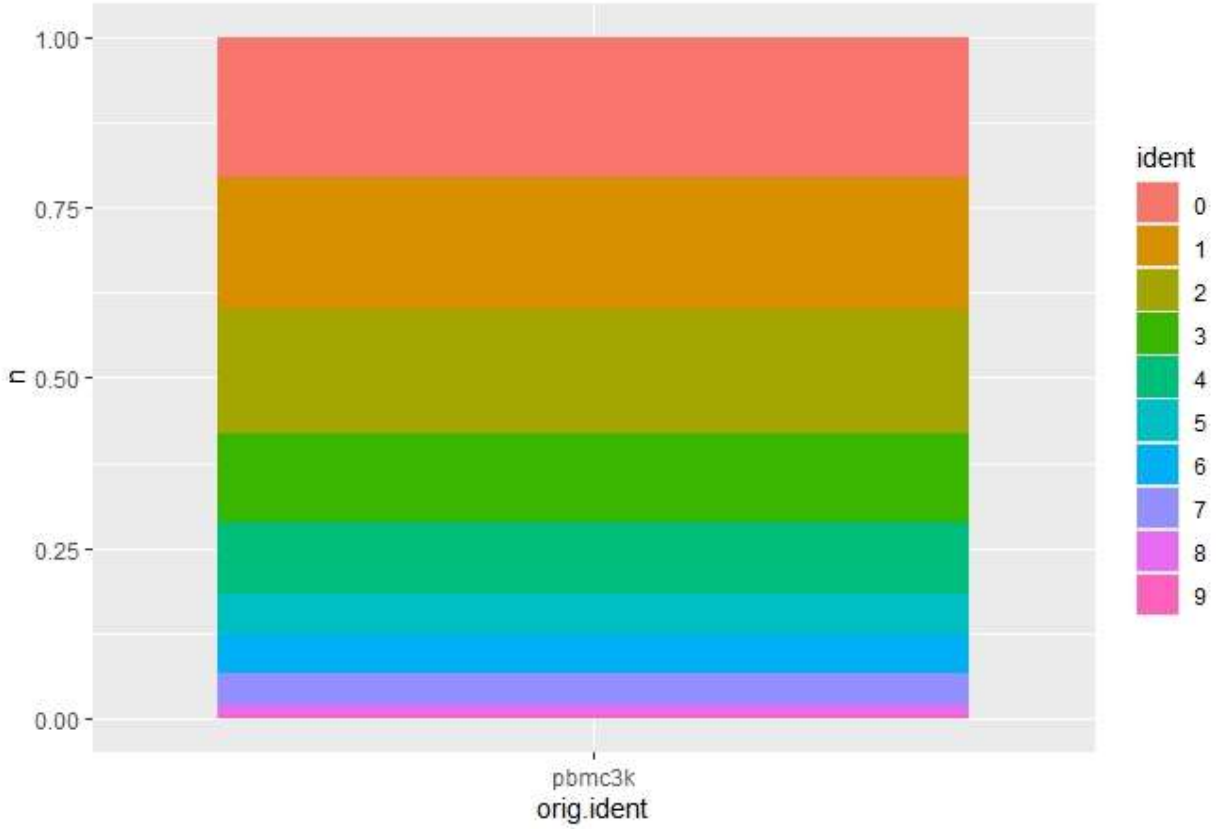
```
> # Let's run UMAP
> pbmc <- RunUMAP(pbmc, dims = 1:8, verbose = F)
> # Now let's make clustering
> pbmc <- FindNeighbors(pbmc, dims = 1:8) #1:10)
Computing nearest neighbor graph
```

```
Computing SNN
> pbmc <- FindClusters(pbmc, resolution = 0.5) # Resolution may vary ~0.4-1.2
, depending on how well (biologically) it describes clusters
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 2635
Number of edges: 91573

Running Louvain algorithm...
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
Maximum modularity in 10 random starts: 0.8789
Number of communities: 10
Elapsed time: 0 seconds
> # Look at cluster IDs of the first 5 cells
> head(Idents(pbmc), 5)
AAACATACAACCAC-1 AAACATTGAGCTAC-1 AAACATTGATCAGC-1 AAACCGTGCTTCCG-1
               1                3                1                5
AAACCGTGTATGCG-1
               6
Levels: 0 1 2 3 4 5 6 7 8 9
> # Table of the clusters composition
> table(pbmc@meta.data$seurat_clusters)

  0   1   2   3   4   5   6   7   8   9
543 512 475 344 277 164 146 127  35  12
```

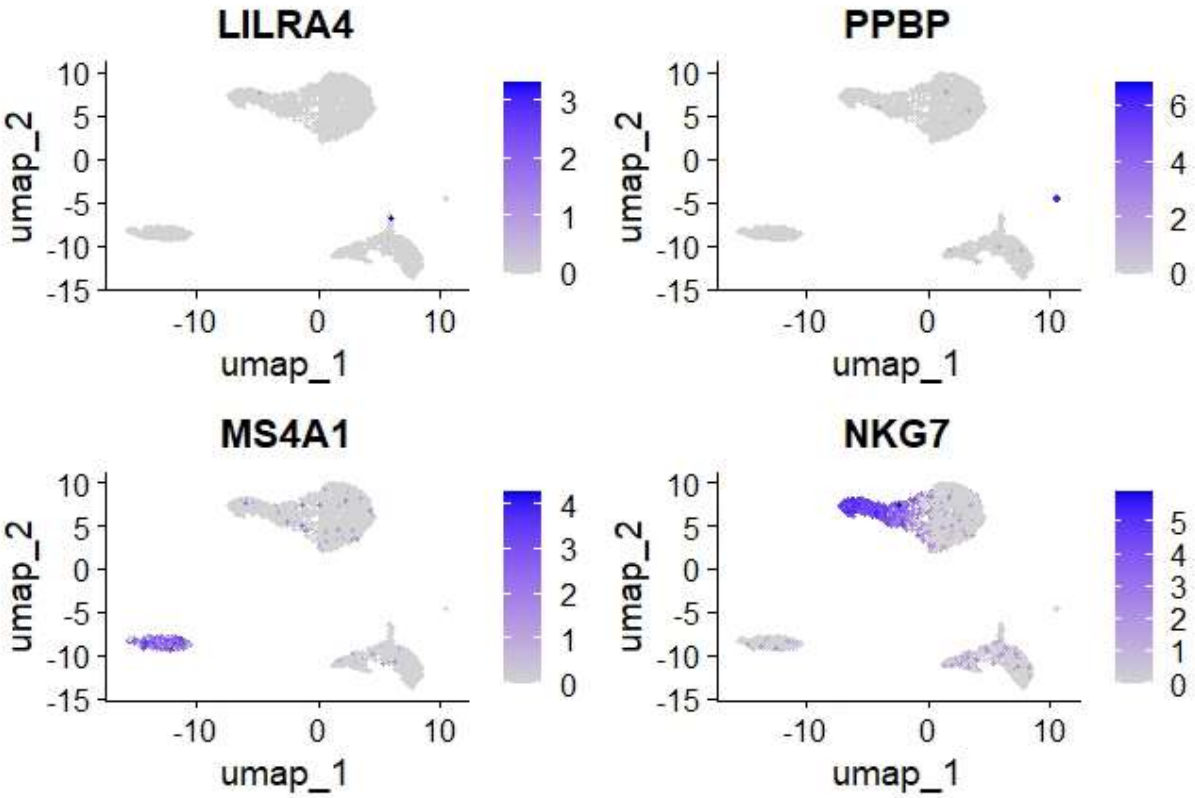
```
> cc.genes.updated.2019
$s.genes
 [1] "MCM5"     "PCNA"     "TYMS"     "FEN1"     "MCM7"
 [6] "MCM4"     "RRM1"     "UNG"      "GINS2"    "MCM6"
[11] "CDCA7"    "DTL"      "PRIM1"    "UHRF1"    "CENPU"
[16] "HELLS"    "RFC2"     "POLR1B"   "NASP"     "RAD51AP1"
[21] "GMNN"     "WDR76"    "SLBP"     "CCNE2"    "UBR7"
[26] "POLD3"    "MSH2"     "ATAD2"    "RAD51"    "RRM2"
[31] "CDC45"    "CDC6"     "EXO1"     "TIPIN"    "DSCC1"
[36] "BLM"      "CASP8AP2" "USP1"     "CLSPN"    "POLA1"
[41] "CHAF1B"   "MRPL36"   "E2F8"

$g2m.genes
 [1] "HMGB2"   "CDK1"    "NUSAP1"  "UBE2C"   "BIRC5"   "TPX2"
 [7] "TOP2A"   "NDC80"   "CKS2"    "NUF2"    "CKS1B"   "MKI67"
[13] "TMPO"    "CENPF"   "TACC3"   "PIMREG"  "SMC4"    "CCNB2"
[19] "CKAP2L"  "CKAP2"   "AURKB"   "BUB1"    "KIF11"   "ANP32E"
[25] "TUBB4B"  "GTSE1"   "KIF20B"  "HJURP"   "CDCA3"   "JPT1"
[31] "CDC20"   "TTK"     "CDC25C"  "KIF2C"   "RANGAP1" "NCAPD2"
[37] "DLGAP5"  "CDCA2"   "CDCA8"   "ECT2"    "KIF23"   "HMMR"
[43] "AURKA"   "PSRC1"   "ANLN"    "LBR"     "CKAP5"   "CENPE"
[49] "CTCF"    "NEK2"    "G2E3"    "GAS2L3"  "CBX5"    "CENPA"
> table(pbmc[[]]$Phase)

  G1  G2M    S
1165  408 1062
```

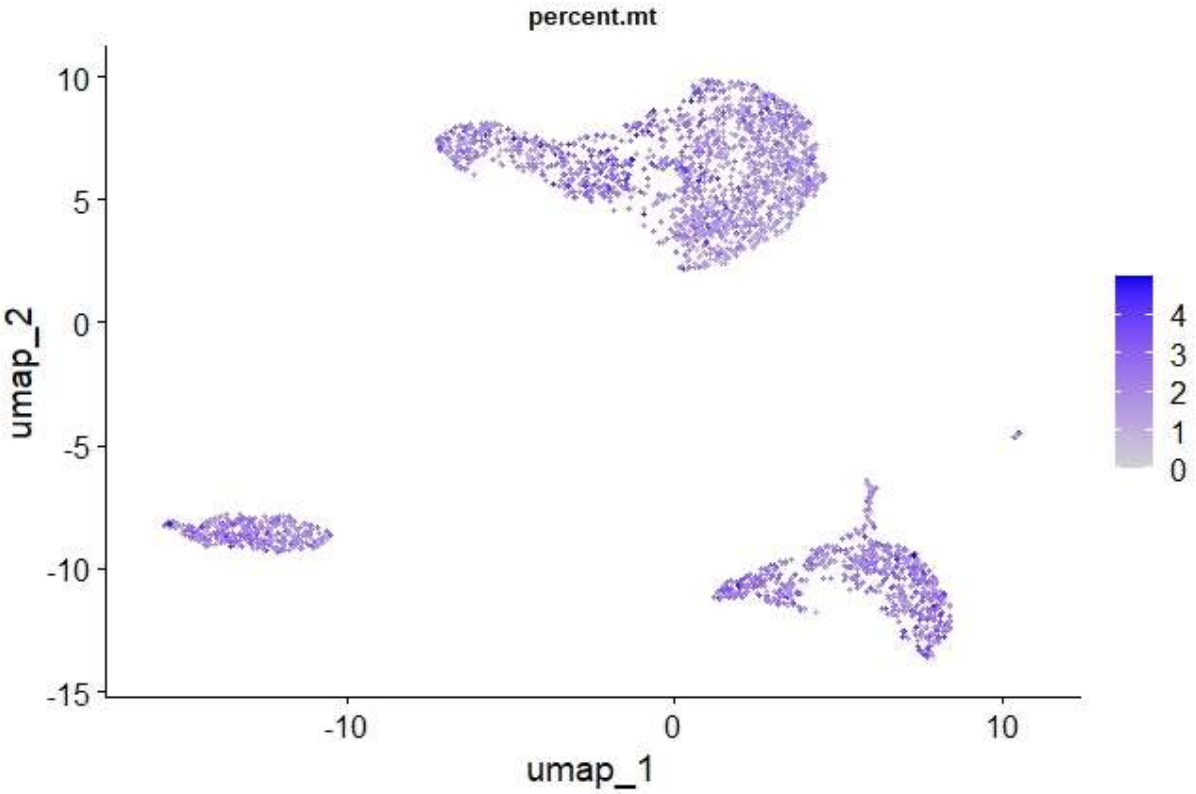
*UMAP-график (получилось 10 кластеров клеток):*

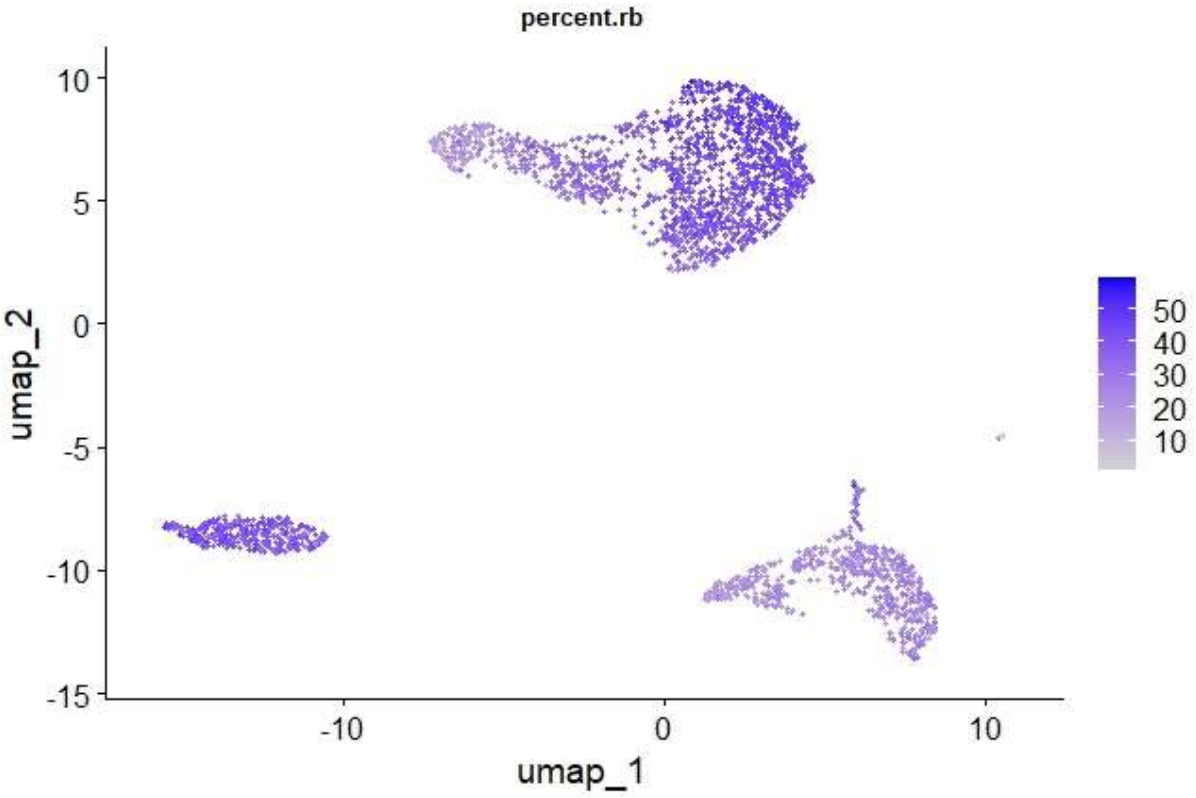

umap_2
umap_1
10
5
0
-5
-10
-10
0
10
0
1
2
3
4
5
6
7
8
9

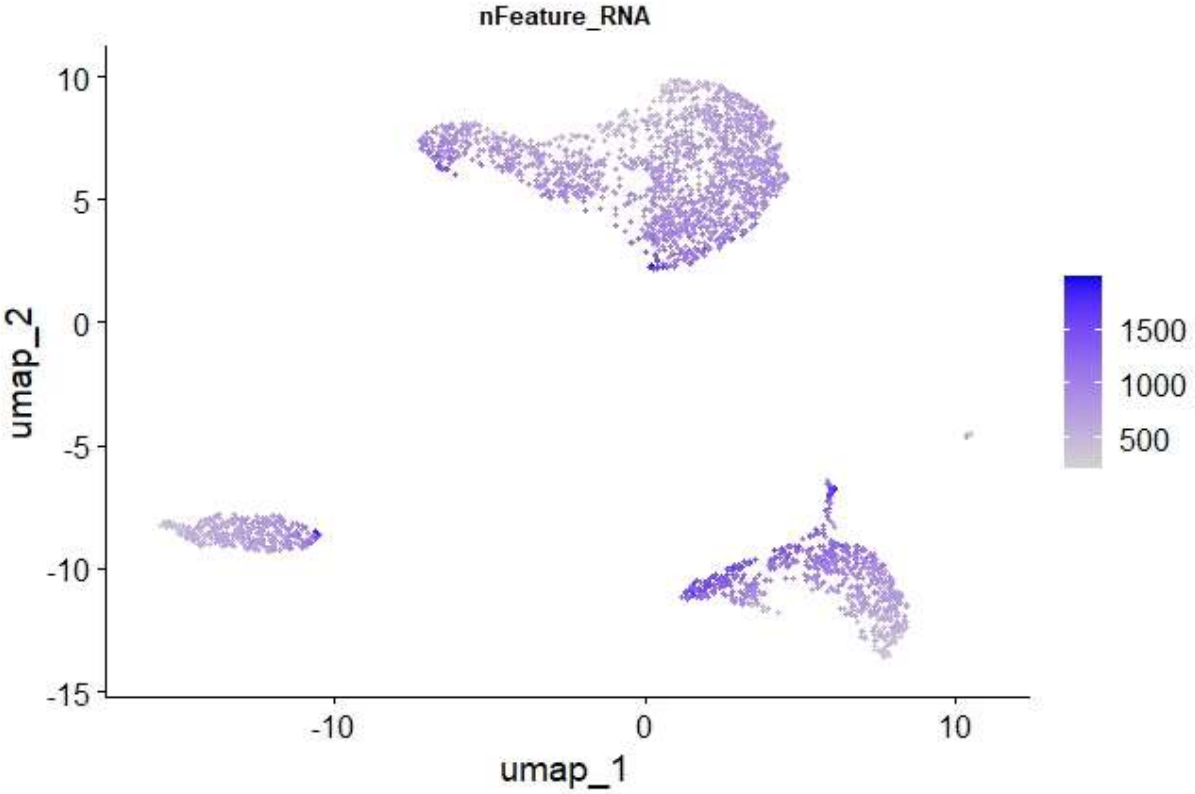

n
1.00
0.75
0.50
0.25
0.00
pbmc3k
orig.ident
ident
0
1
2
3
4
5
6
7
8
9

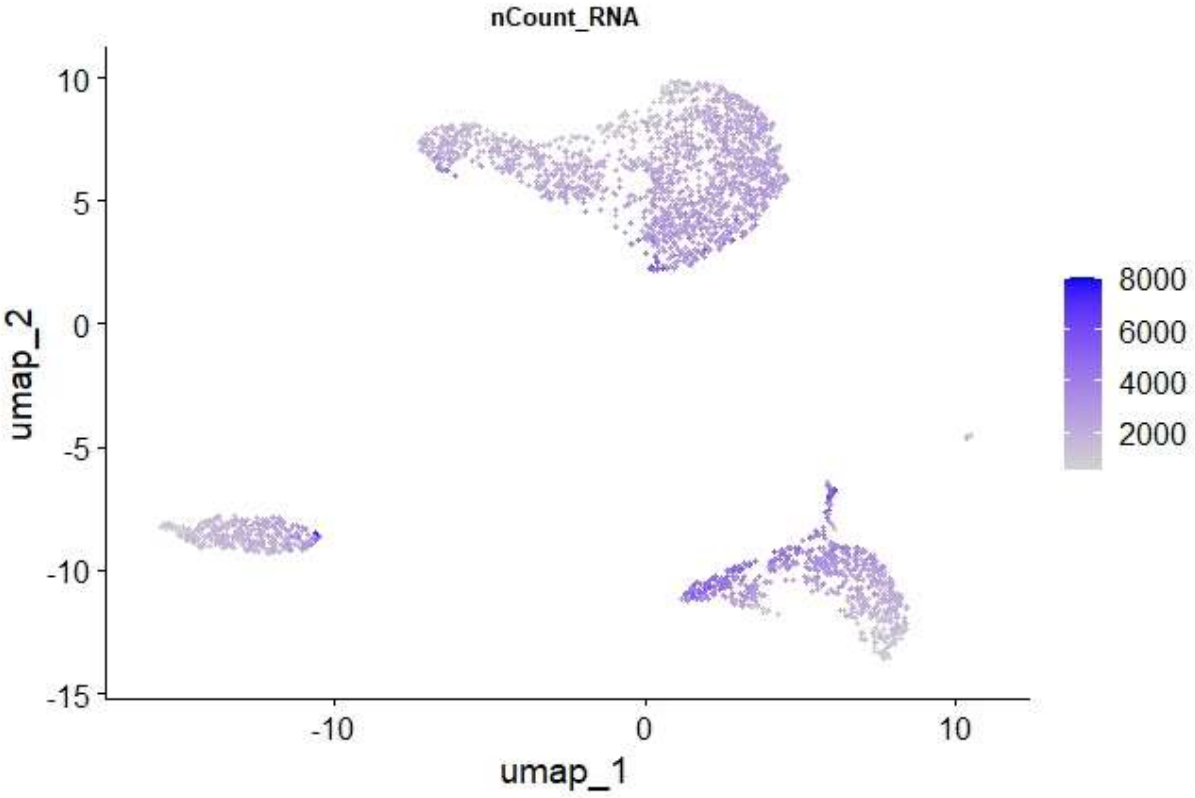
Проверка QC:

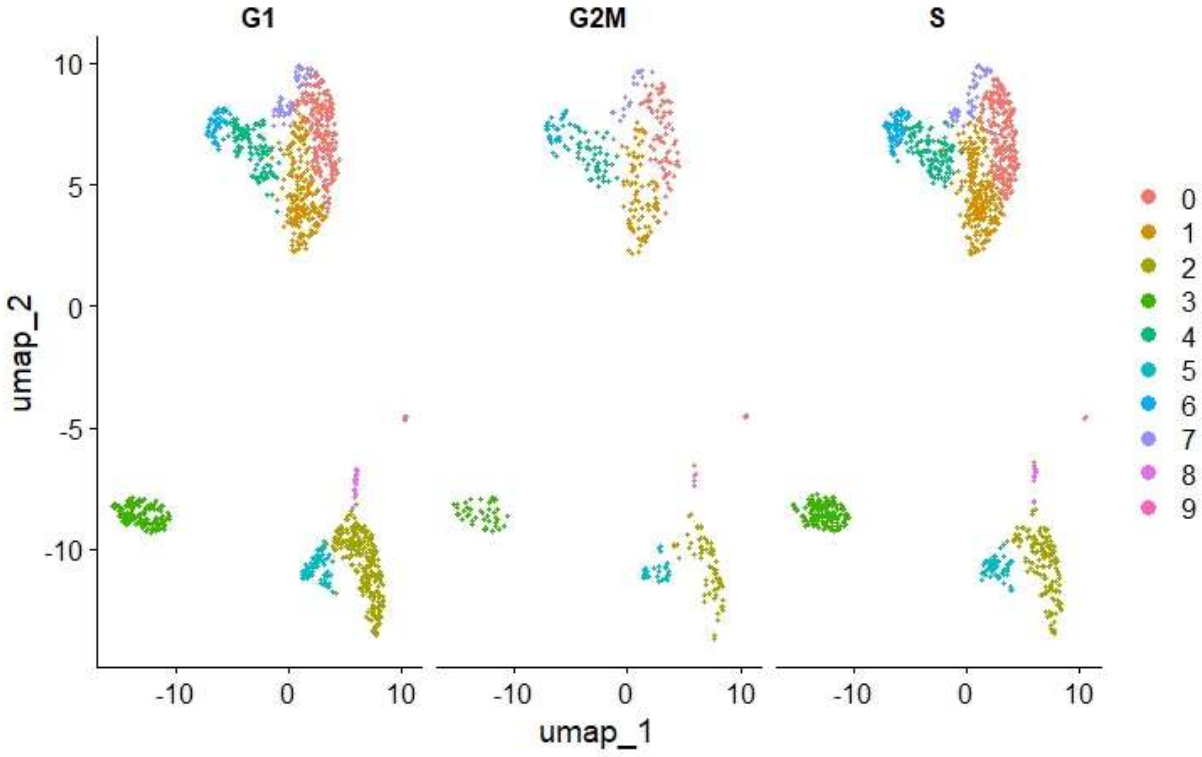

LILRA4
PPBP
MS4A1
NKG7
umap_1
umap_2
10
5
0
-5
-10
-15
-10
0
10
3
2
1
0
6
4
2
4
5

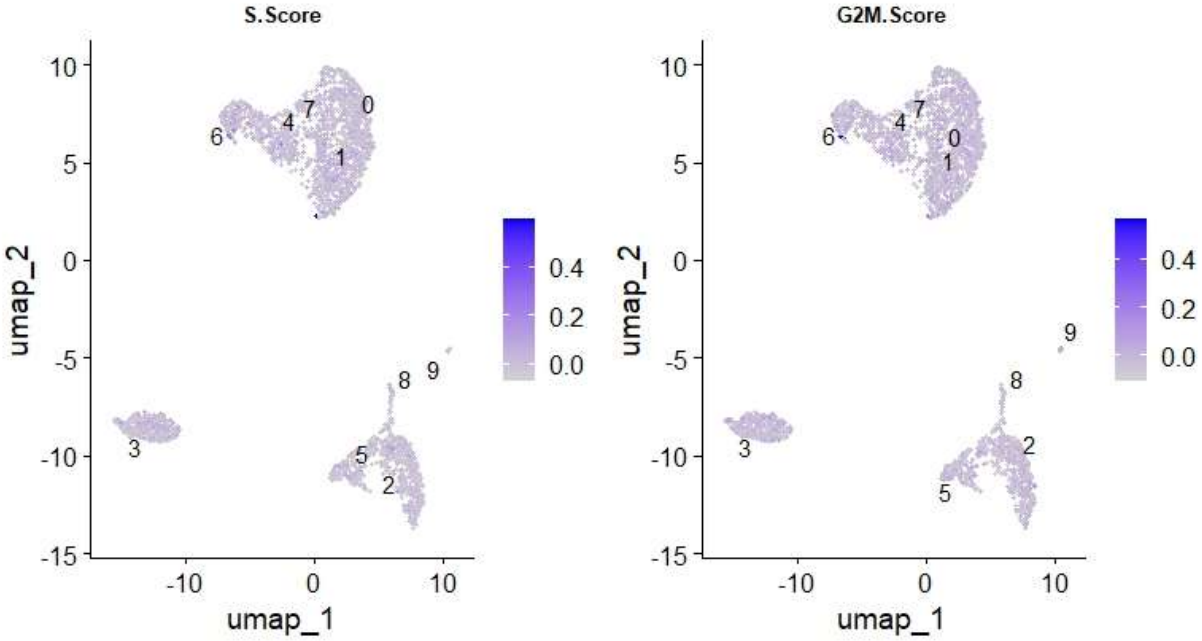

percent.mt
10
5
0
-5
-10
-15
umap_2
-10
0
10
umap_1
4
3
2
1
0

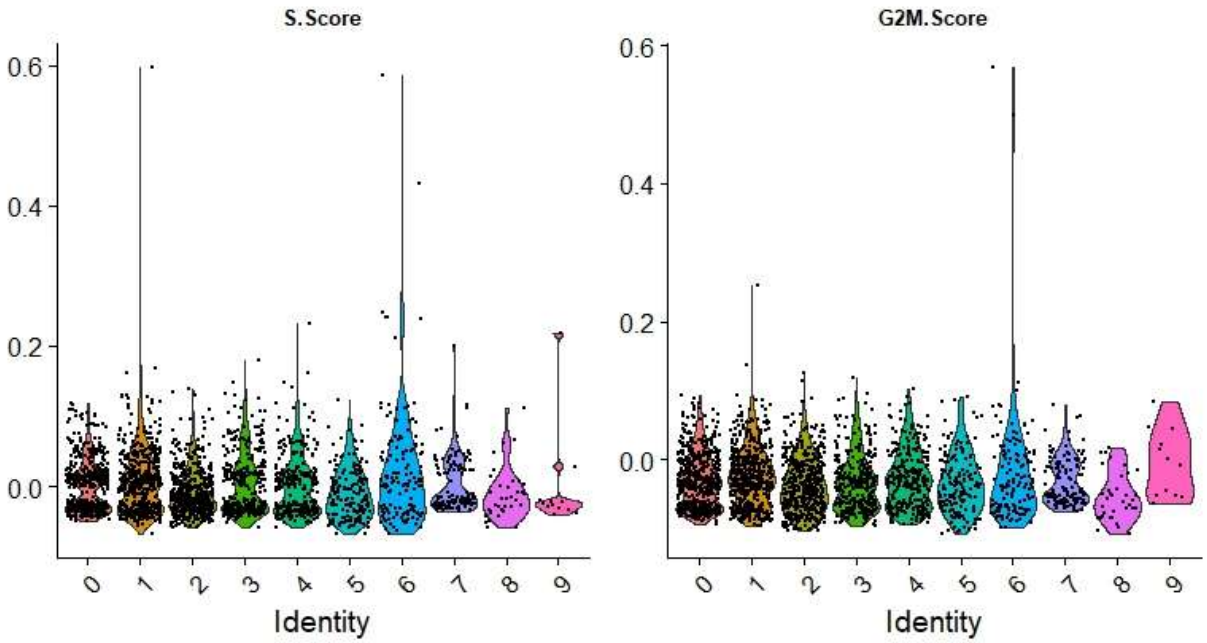

percent.rb
10
5
0
-5
-10
-15
umap_2
-10
0
10
umap_1
50
40
30
20
10


nFeature_RNA
10
5
0
-5
-10
-15
umap_2
-10
0
10
umap_1
1500
1000
500


nCount_RNA
10
5
0
-5
-10
-15
umap_2
-10
0
10
umap_1
8000
6000
4000
2000


G1
G2M
S
umap_2
umap_1
10
5
0
-5
-10
-10
0
10
0
1
2
3
4
5
6
7
8
9


S.Score
G2M.Score
umap_2
umap_1
0.4
0.2
0.0
0
1
2
3
4
5
6
7
8
9



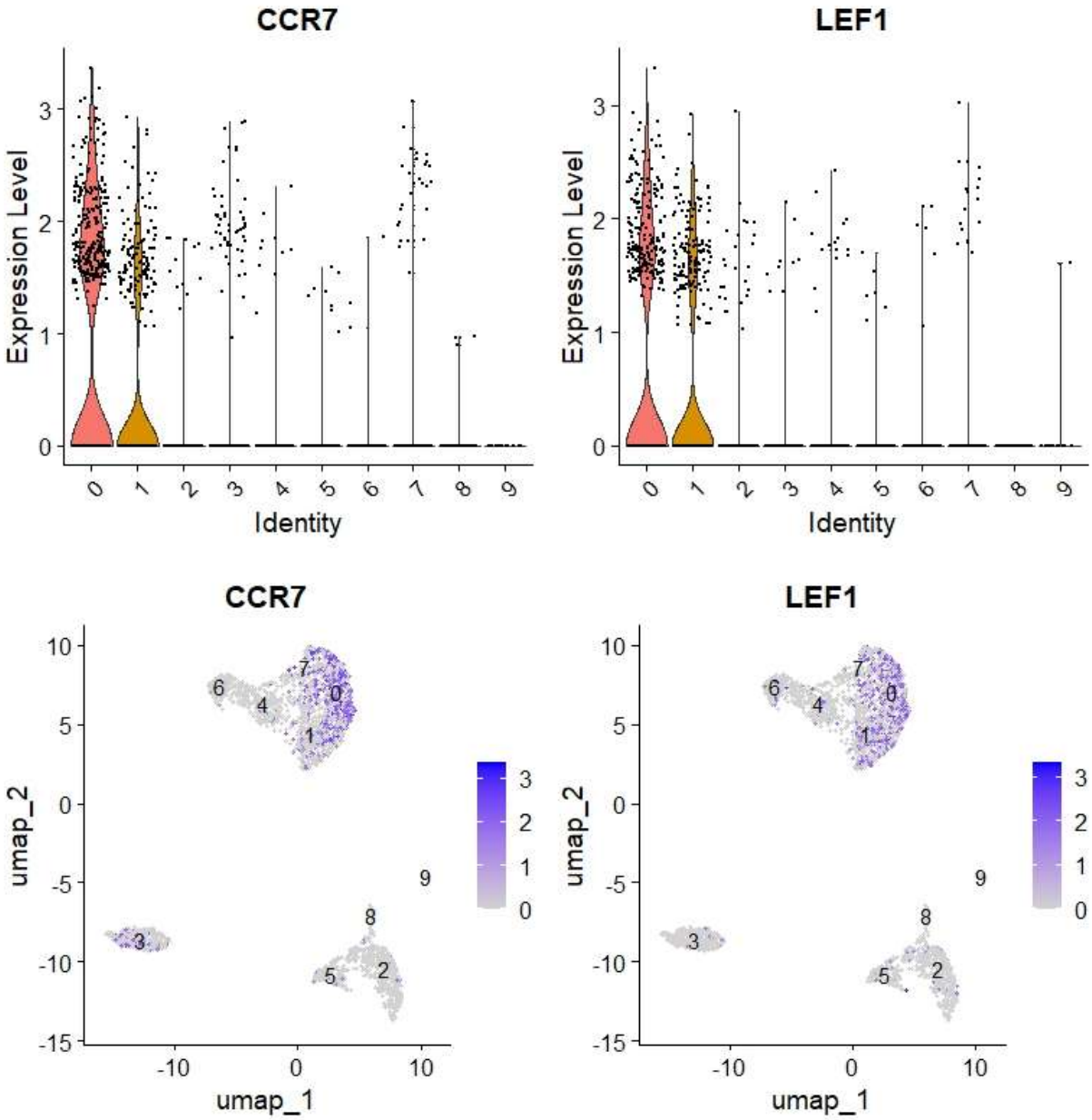

5. Аннотируем некоторые кластеры.
Используйте функцию FindAllMarkers() для идентификации дифференциально ап-регулированных генов для каждого кластера. По представленной таблице маркеров попробуйте определить тип клеток кластеров. Визуализируйте некоторые маркеры с помощью функций FeaturePlot(), VlnPlot().

| Markers | Cell Type |
|---|---|
| IL7R, CCR7 | Naive CD4+ T |
| CD14, LYZ | CD14+ Mono |
| IL7R, S100A4 | Memory CD4+ |
| MS4A1 | B |
| CD8A | CD8+ T |
| FCGR3A, MS4A7 | FCGR3A+ Mono |
| GNLY, NKG7 | NK |
| FCER1A, CST3 | DC |
| PPBP | Platelet |

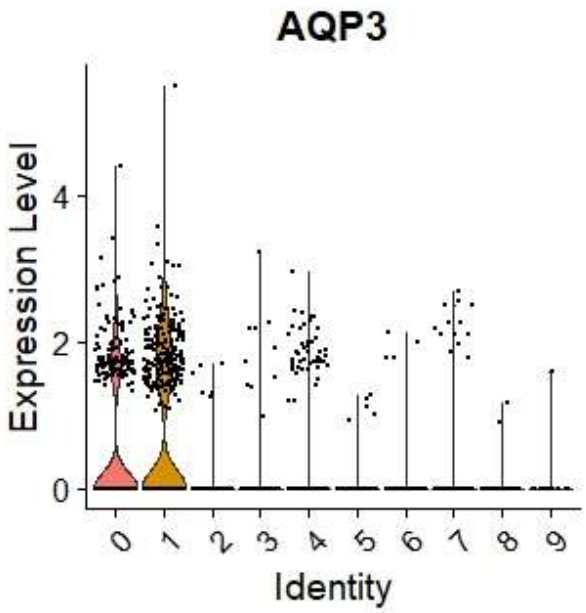
```
> # find markers for every cluster compared to all remaining cells, report on
ly the positive
> # ones
> pbmc.markers <- FindAllMarkers(pbmc, only.pos = TRUE, min.pct = 0.25, logfc
.threshold = 0.25)
Calculating cluster 0
For a (much!) faster implementation of the Wilcoxon Rank Sum Test,
(default method for FindMarkers) please install the presto package
--------------------------------------------
install.packages('devtools')
devtools::install_github('immunogenomics/presto')
--------------------------------------------
```

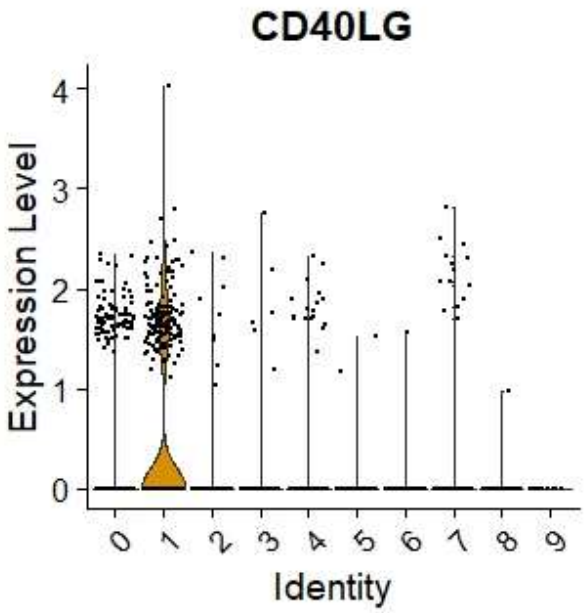
```
After installation of presto, Seurat will automatically use the more
efficient implementation (no further action necessary).
This message will be shown once per session
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=02s
Calculating cluster 1
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=03s
Calculating cluster 2
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=05s
Calculating cluster 3
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=02s
Calculating cluster 4
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=03s
Calculating cluster 5
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=06s
Calculating cluster 6
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=04s
Calculating cluster 7
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=01s
Calculating cluster 8
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=06s
Calculating cluster 9
  |++++++++++++++++++++++++++++++++++++++++++++++++++| 100% elapsed=03s
> pbmc.markers %>%
+   group_by(cluster) %>%
+   slice_max(n = 2, order_by = avg_log2FC)
# A tibble: 20 × 7
# Groups:   cluster [10]
       p_val avg_log2FC pct.1 pct.2 p_val_adj cluster gene
       <dbl>      <dbl> <dbl> <dbl>     <dbl> <fct>   <chr>
 1 2.27e- 92       2.32 0.501 0.118 3.11e- 88 0       CCR7
 2 1.64e- 57       2.10 0.387 0.108 2.25e- 53 0       LEF1
 3 5.95e- 60       2.16 0.41  0.108 8.16e- 56 1       AQP3
 4 2.47e- 42       2.07 0.273 0.064 3.38e- 38 1       CD40LG
 5 7.33e-141       7.30 0.303 0.004 1.01e-136 2       FOLR3
 6 5.87e-123       6.76 0.28  0.006 8.05e-119 2       S100A12
 7 5.18e-272       7.38 0.564 0.009 7.10e-268 3       LINC00926
 8 5.41e-237       7.13 0.488 0.007 7.42e-233 3       VPREB3
 9 5.63e-166       4.32 0.592 0.056 7.72e-162 4       GZMK
10 2.89e- 92       3.60 0.437 0.061 3.97e- 88 4       GZMH
11 2.12e-165       5.86 0.366 0.005 2.91e-161 5       CKB
12 1.22e-211       5.45 0.5   0.009 1.67e-207 5       CDKN1C
13 3.97e-185       6.21 0.493 0.013 5.45e-181 6       AKR1C3
14 2.05e-269       5.98 0.986 0.07  2.80e-265 6       GZMB
15 1.99e-  4       1.95 0.094 0.266 1    e+  0 7       NDUFA2
16 5.38e-  3       1.47 0.157 0.314 1    e+  0 7       TBCB
17 1.65e-198       8.06 0.457 0.002 2.27e-194 8       SERPINF1
18 7.97e-269       8.05 0.857 0.01  1.09e-264 8       FCER1A
19 0              14.4  0.583 0     0         9       LY6G6F
20 2.46e-192      14.0  0.333 0     3.38e-188 9       RP11-879F14.2
```

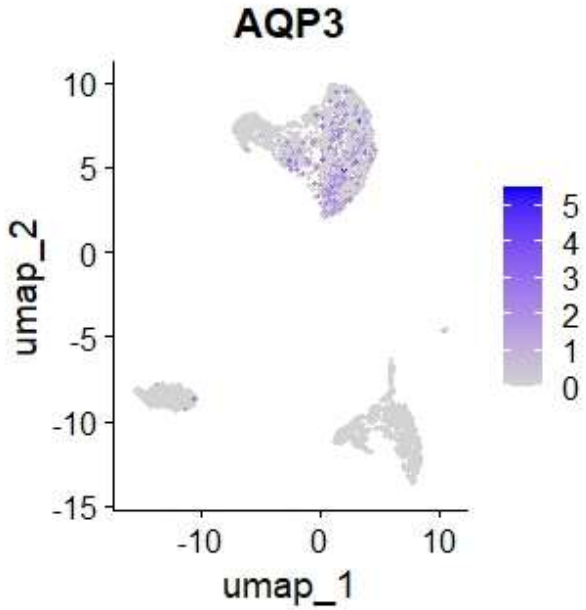
*кластер 0:*

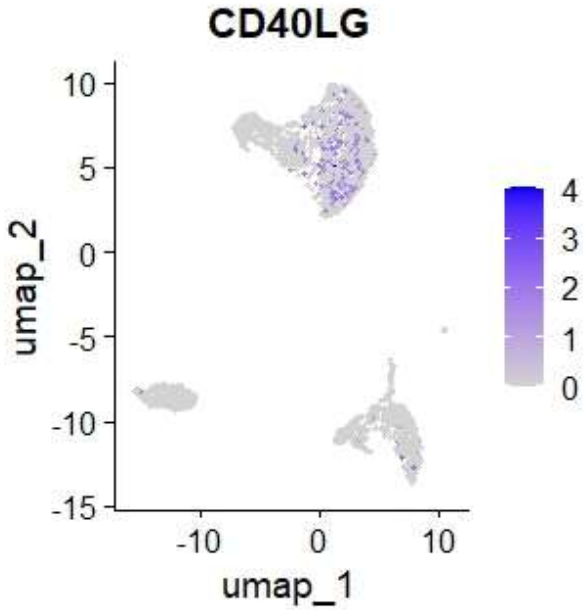

CCR7
LEF1
Expression Level
Identity
0 1 2 3 4 5 6 7 8 9
CCR7
LEF1
umap_2
umap_1
0 1 2 3 4 5 6 7 8 9

*кластер 1:*

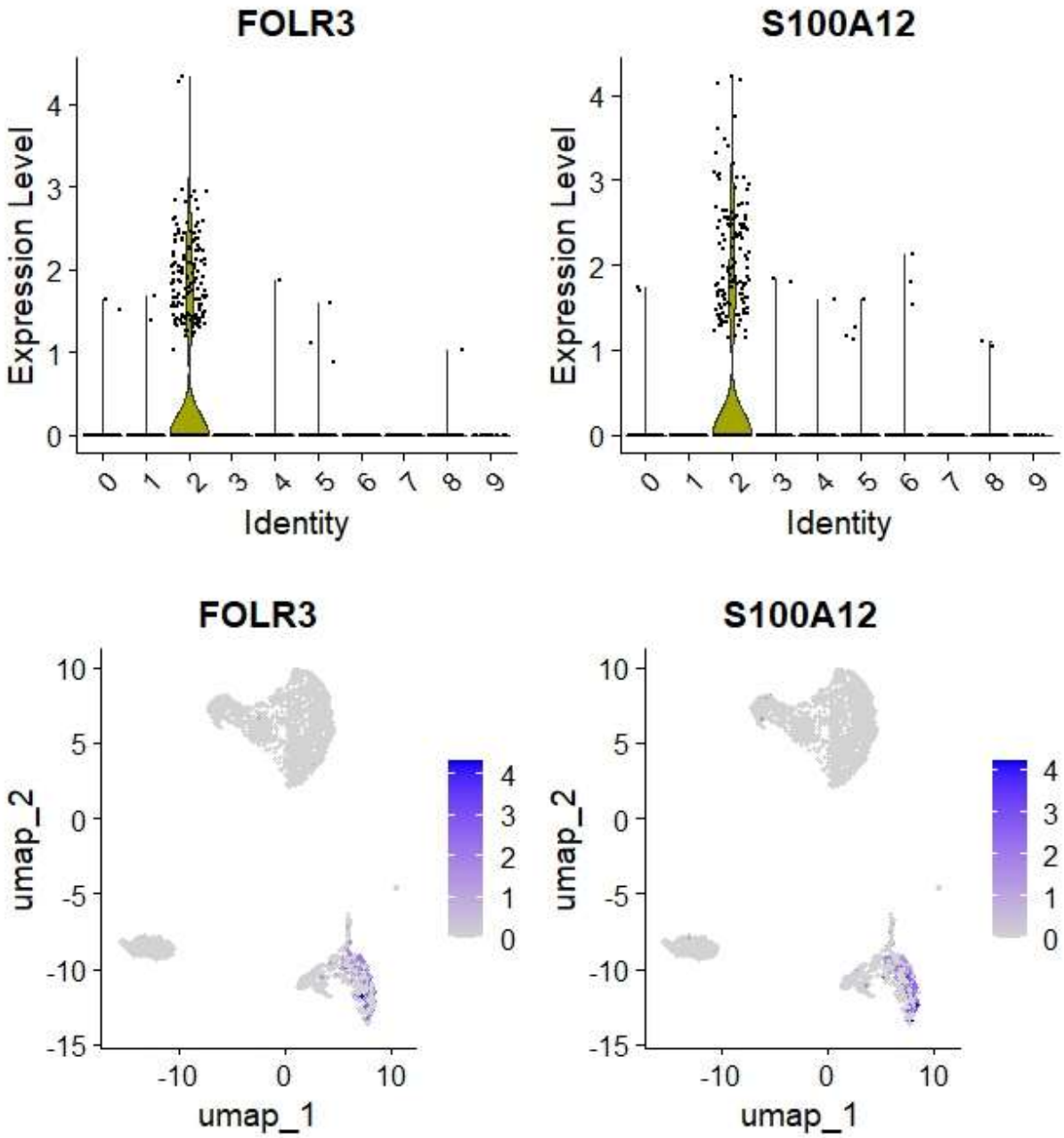

AQP3
Expression Level
Identity


CD40LG
Expression Level
Identity

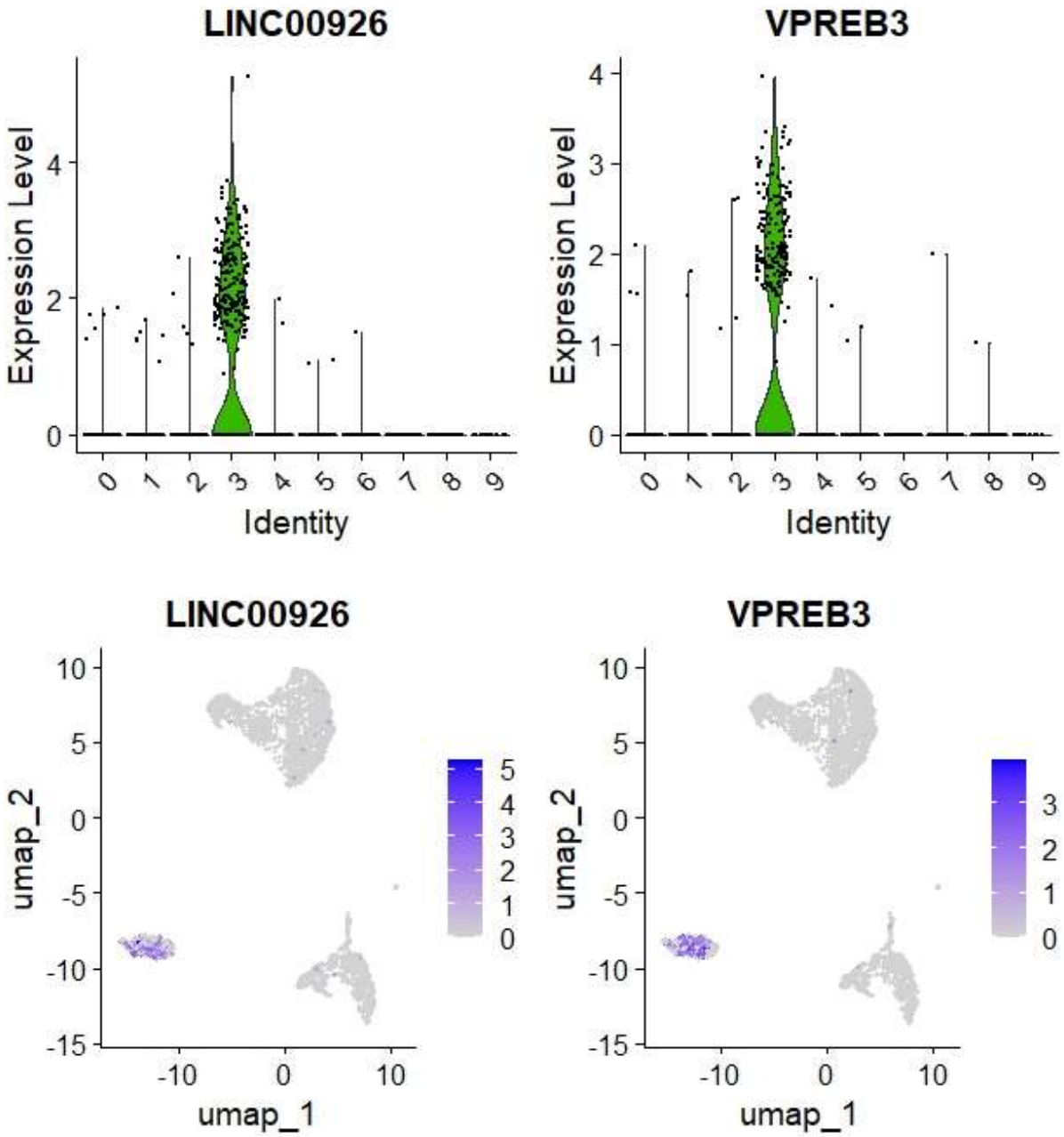

AQP3
umap_2
umap_1


CD40LG
umap_2
umap_1

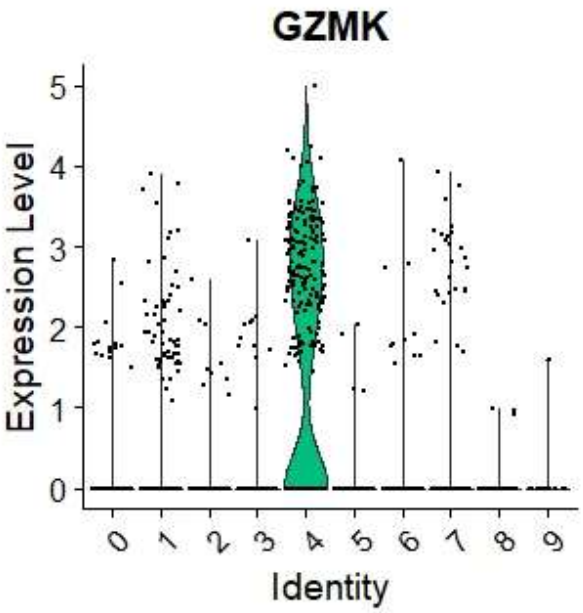
*кластер 2:*

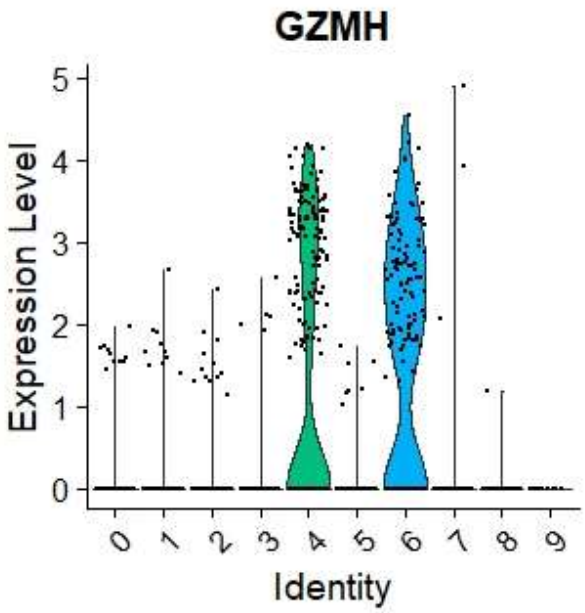


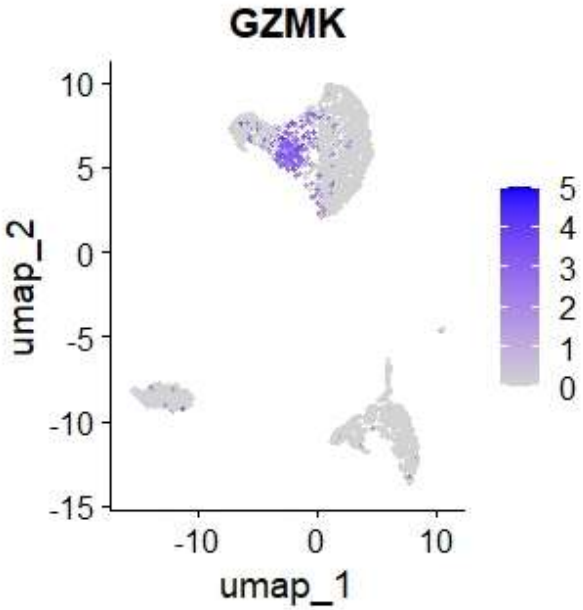

*кластер 3:*

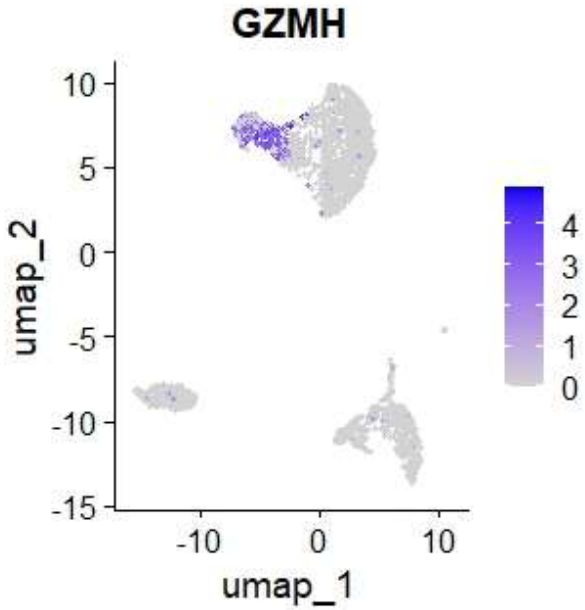



*кластер 4:*

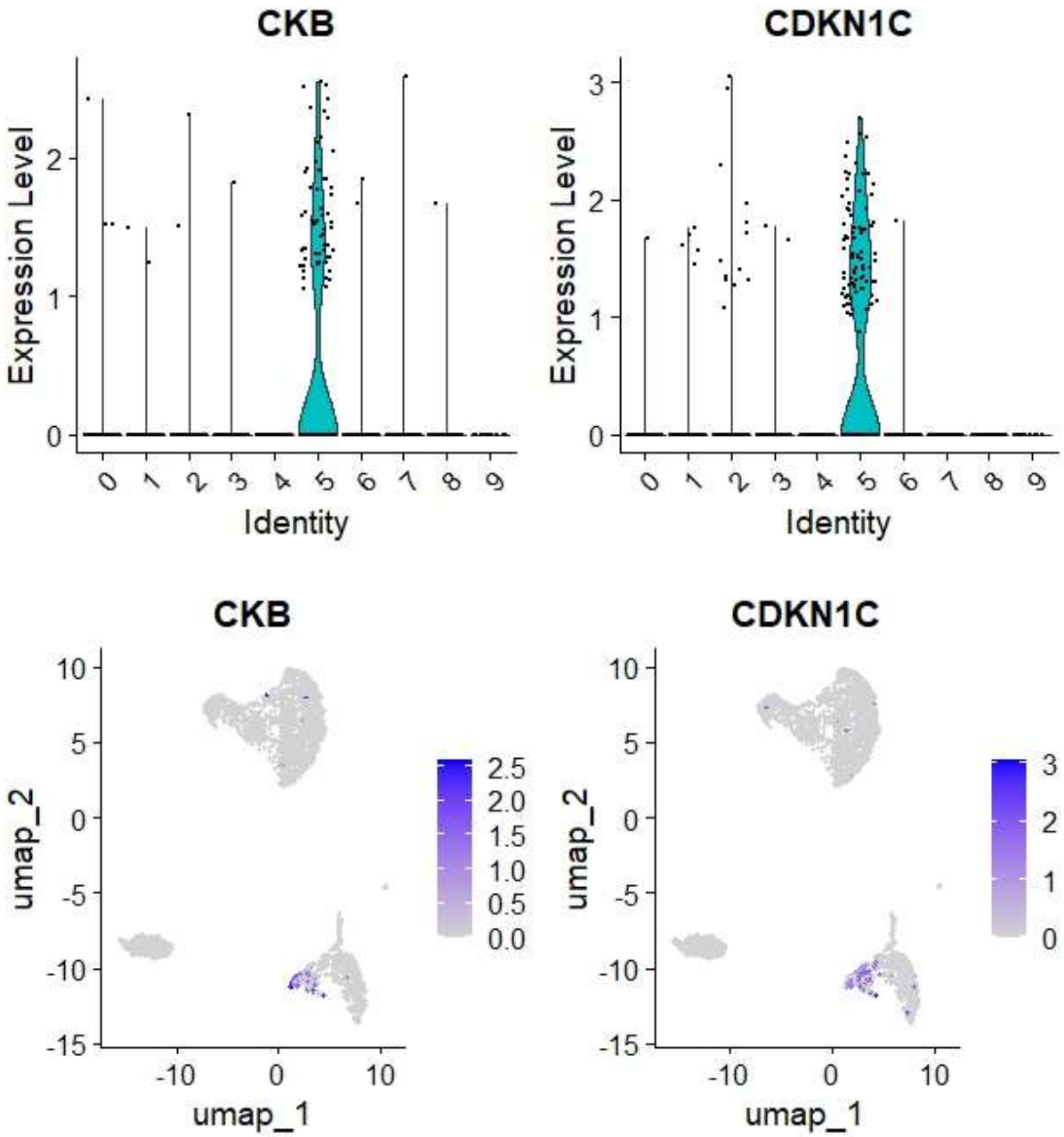

GZMK
Expression Level
Identity


GZMH
Expression Level
Identity

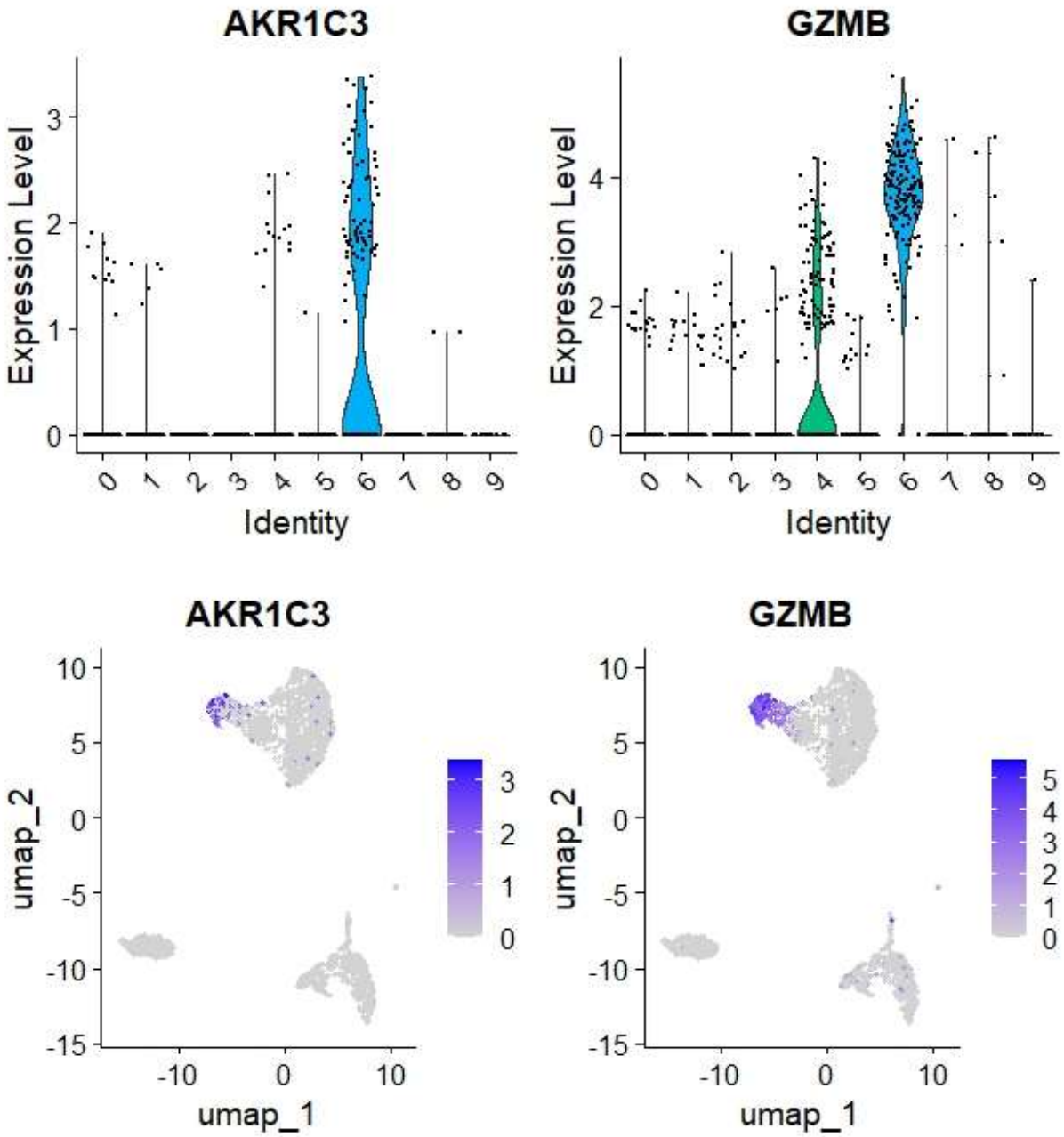

GZMK
umap_2
umap_1


GZMH
umap_2
umap_1

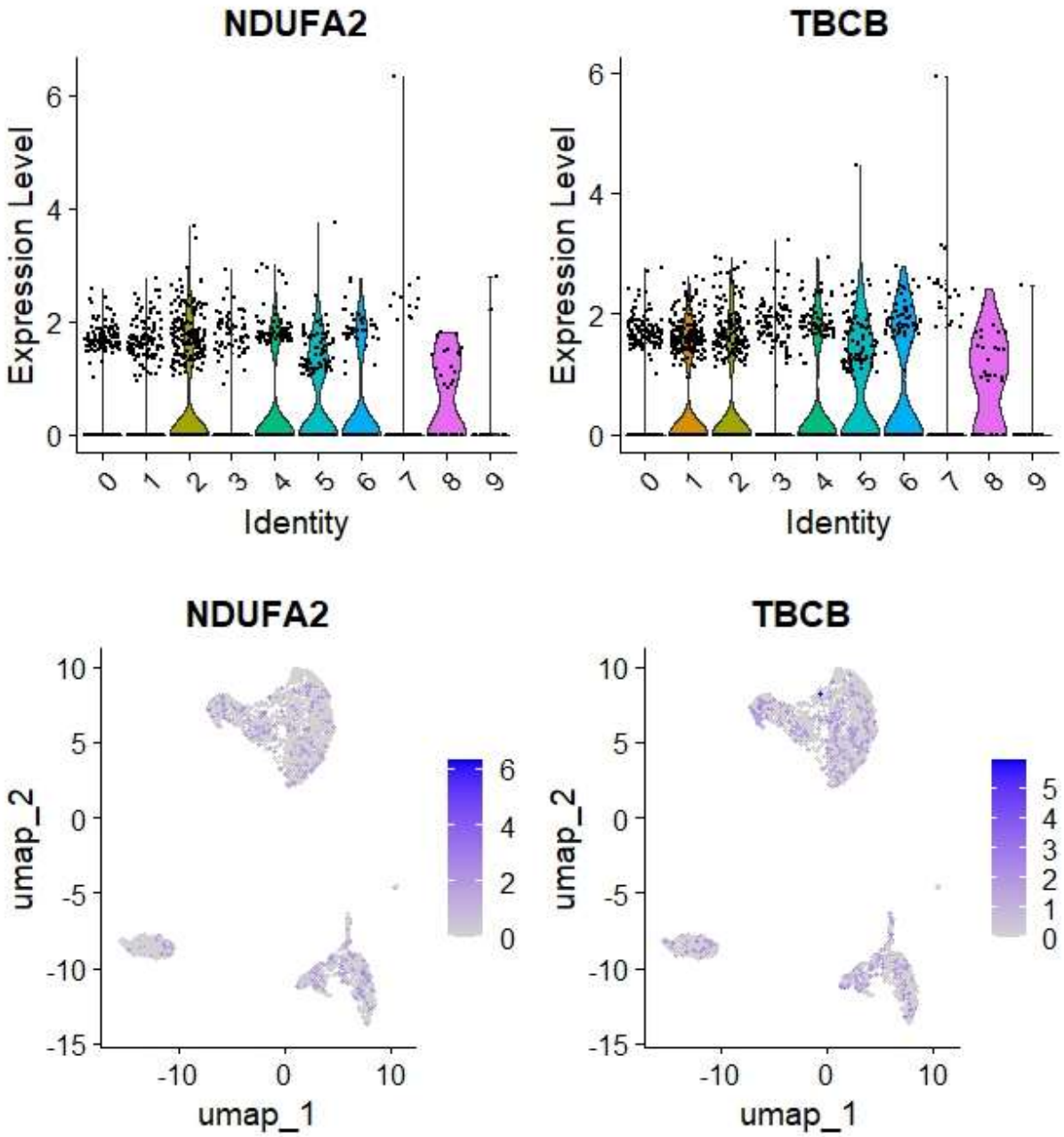
*кластер 5:*



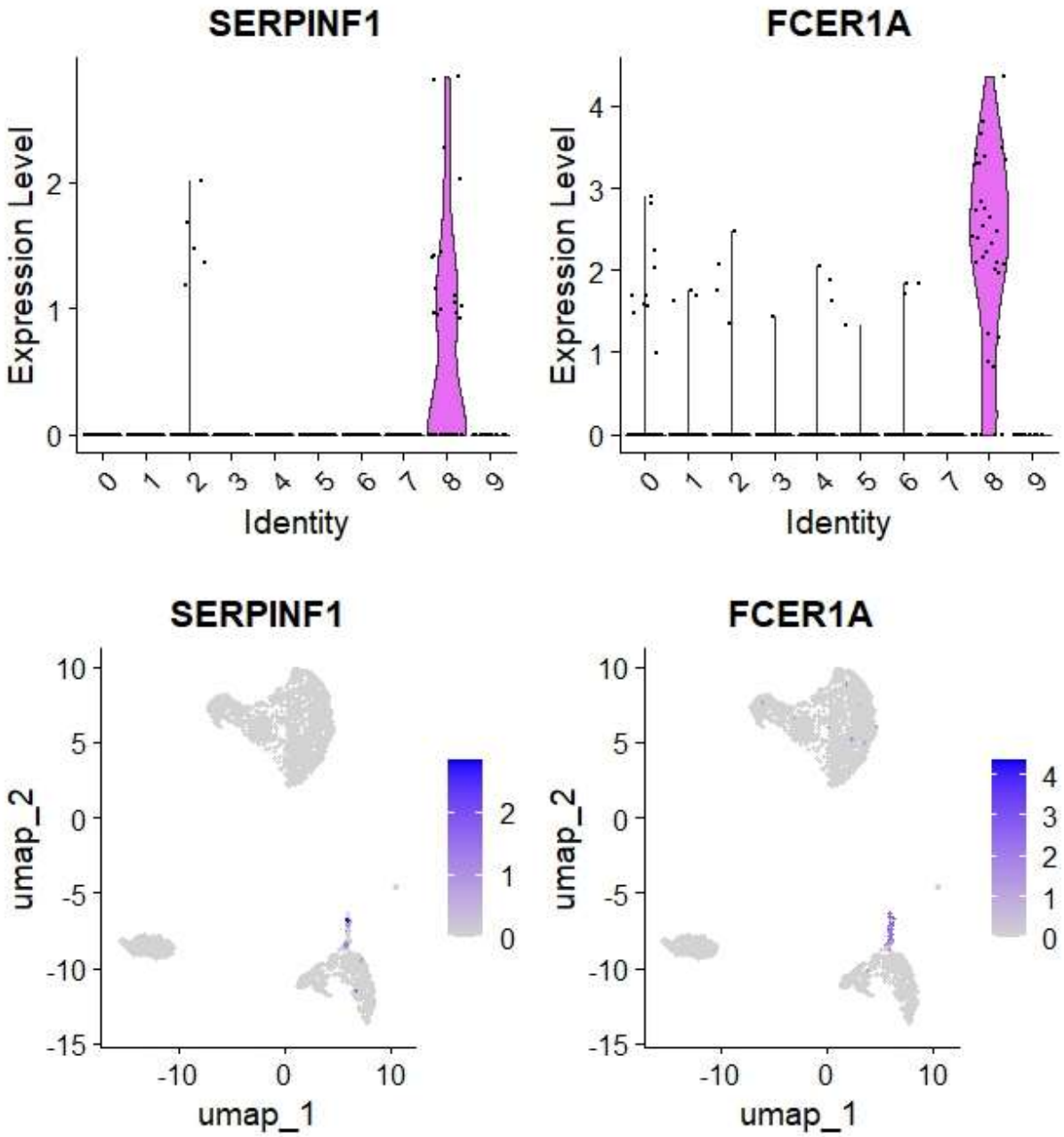

*кластер 6:*


AKR1C3
GZMB
Expression Level
Identity
0 1 2 3 4 5 6 7 8 9
AKR1C3
GZMB
umap_2
umap_1

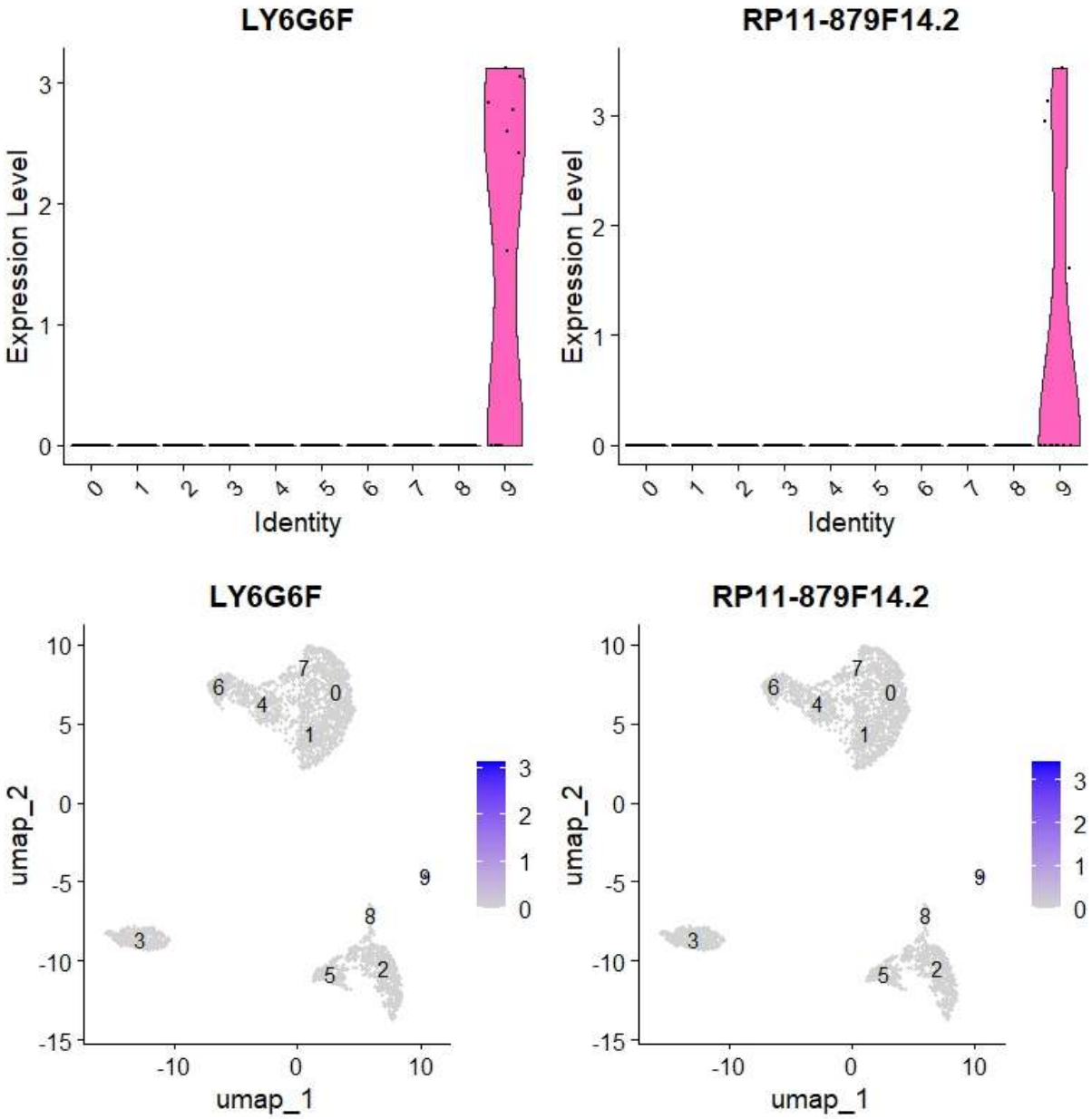
*кластер 7:*


NDUFA2
TBCB
Expression Level
Identity
0 1 2 3 4 5 6 7 8 9
NDUFA2
TBCB
umap_2
umap_1

*кластер 8:*




*Кластер 9:*




*Можно использовать базу https://panglaodb.se/search.html - по гену подскажет в каком типе клеток обычно экспрессируется этот ген.

В результате проаннотируйте хотя бы какое-то количество кластеров; можно присвоить одинаковую аннотацию нескольким кластерам, если они похожи.

Результат: UMAP с (частично) проаннотированными кластерами.

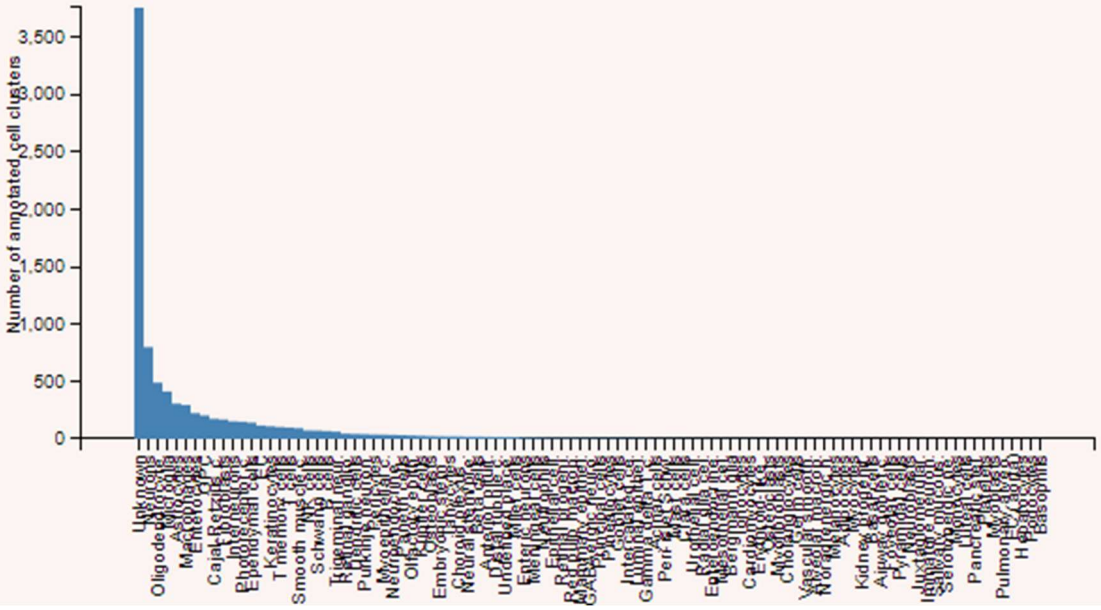
*CCR7, LEF1, AQP3, CD40LG, FOLR3, S100A12, LINC00926, VPREB3, GZMK, GZMH, CKB, CDKN1C, AKR1C3, GZMB, NDUFA2, TBCB, SERPINF1, FCER1A, LY6G6F, RP11-879F14.2*

*CCR7, AQP3, GZMK, CKB, GZMB*

Summary of search results

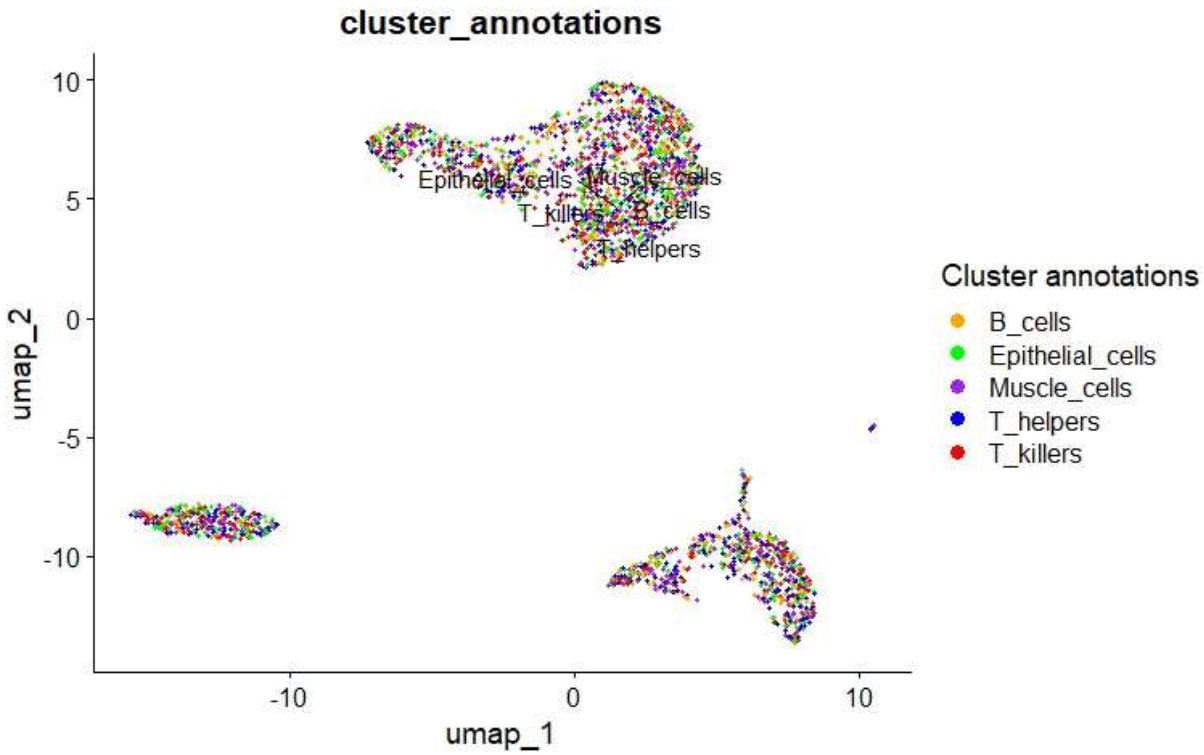
| Gene | Description | Type | No. samples | No. cell clusters |
|---|---|---|---|---|
| CCR7 | C-C motif chemokine receptor 7 | protein-coding gene | 91 | 181 |
| AQP3 | aquaporin 3 (Gill blood group) | protein-coding gene | 71 | 248 |
| GZMK | granzyme K | protein-coding gene | 12 | 26 |
| CKB | creatine kinase B | protein-coding gene | 793 | 8176 |
| GZMB | granzyme B | protein-coding gene | 82 | 144 |

Barplot of cell clusters (Y-axis) and cell types (X-axis) where the gene is expressed.




```
library(Seurat)
library(ggplot2)

seurat_data <- readRDS("C:/Users/nasty/Desktop/Магистратура/ДЗ RNA/hw3/pbmc_norm.rds")

# Определяем типы клеток для каждого кластера на основе экспрессии генов
# кластер 0 представляет Т-хелперы,
# кластер 1 представляет эпителиальные клетки, кластер 4 представляет Т-киллеры,
# кластер 5 представляет мышечные клетки, а кластер 6 представляет В-клетки.
cluster_annotations <- list(
  T_helpers = "0",
  Epithelial_cells = "1",
  T_killers = "4",
```

```
  Muscle_cells = "5",
  B_cells = "6"
)

# Преобразуем список аннотаций кластеров в столбец данных
cluster_annotations_df <- data.frame(
  cluster = as.numeric(unlist(cluster_annotations)),
  annotation = rep(names(cluster_annotations), lengths(cluster_annotations)),
stringsAsFactors = FALSE
)

seurat_data[["cluster_annotations"]] <-
as.character(cluster_annotations_df$annotation)

seurat_data$cluster_annotations <-
as.character(cluster_annotations_df$annotation)

# Визуализируем аннотированные кластеры с помощью UMAP
DimPlot(seurat_data, reduction = "umap", label = TRUE, group.by =
"cluster_annotations", repel = TRUE) +
  scale_color_manual(values = c(
    T_helpers = "blue",
    Epithelial_cells = "green",
    T_killers = "red",
    Muscle_cells = "purple",
    B_cells = "orange"
  )) +
  labs(color = "Cluster annotations")
```


cluster_annotations
Epithelial_cells
Muscle_cells
T_killers
B_cells
T_helpers
umap_2
umap_1
10
5
0
-5
-10
-10
0
10
Cluster annotations
B_cells
Epithelial_cells
Muscle_cells
T_helpers
T_killers

Single-cell RNA-seq typical Analysis