Butt et al: scRNAseq analysis - QC

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Contents

1 Libraries and load data

1.1 Load library

```
library(Seurat) #v2.3.0
```

1.2 Load 10X data

```
hypoxia_1<-Read10X("Hypoxia_1")
hypoxia_2<-Read10X("Hypoxia_2")
normoxia_1<-Read10X("Normoxia_1")
normoxia_2<-Read10X("Normoxia_2")
```

2 Prepare Seurat objects

```
# Paste sample identifier in front of cell ID
colnames(hypoxia_1)<-paste0('hyp1_', colnames(hypoxia_1))
colnames(hypoxia_2)<-paste0('hyp2_', colnames(hypoxia_2))
colnames(normoxia_1)<-paste0('nor1_', colnames(normoxia_1))
colnames(normoxia_2)<-paste0('nor2_', colnames(normoxia_2))
```

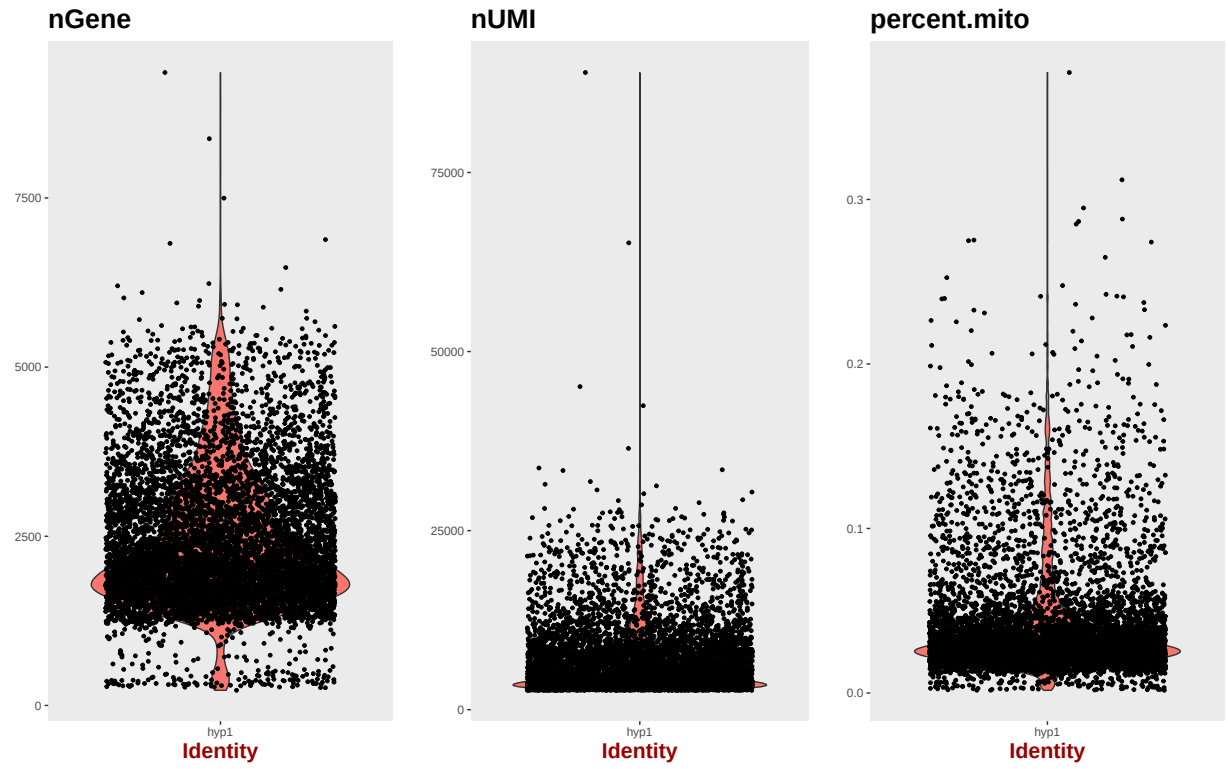
```
# create Seurat objects
hypoxia_1<-CreateSeuratObject(hypoxia_1, min.cells=3, min.genes=200, project="hypoxia_1")
hypoxia_2<-CreateSeuratObject(hypoxia_2, min.cells=3, min.genes=200, project="hypoxia_2")
normoxia_1<-CreateSeuratObject(normoxia_1, min.cells=3, min.genes=200, project="normoxia_1")
normoxia_2<-CreateSeuratObject(normoxia_2, min.cells=3, min.genes=200, project="normoxia_2")
```

```
# make list of Seurat objects for easier handling
obj<-list(hypoxia_1=hypoxia_1,hypoxia_2=hypoxia_2, normoxia_1=normoxia_1, normoxia_2=normoxia_2)
# create list of sample names
samnam<-c('hypoxia_1', 'hypoxia_2', 'normoxia_1', 'normoxia_2')
# set name for objects in list
names(obj)<-samnam
```

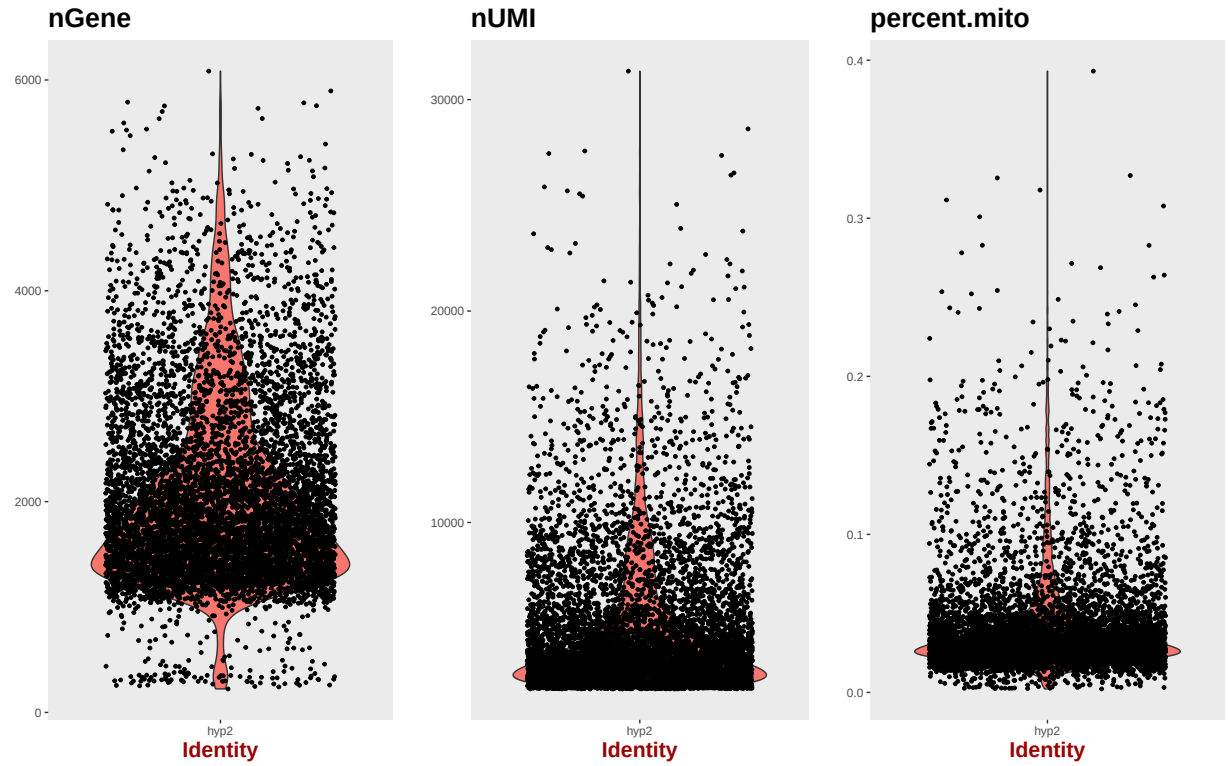
3 Calculate and plot basic features

```
# select all mitochondrial gene names (13 genes, same in all samples)
mito<-lapply(obj, function(x) grep("^mt", rownames(x@data), value=T))
# calculate percentage of mitochondrial gene per cell and sample
percent.mito<-lapply(obj, function(x) Matrix::colSums(x@raw.data[mito$hypoxia_1, ])/Matrix::colSums(x@ra
# add mito percentage to metadata
obj<-lapply(seq_along(samnam), function(x) AddMetaData(obj[[x]], metadata=percent.mito[[x]], col.name="
# plot basic featuers nGene, nUMI and percent.mito
mitoplot<-lapply(obj, function(x) VlnPlot(x, features.plot=c("nGene", "nUMI", "percent.mito"), nCol = 3
print(mitoplot)
```

```
## [[1]]
```

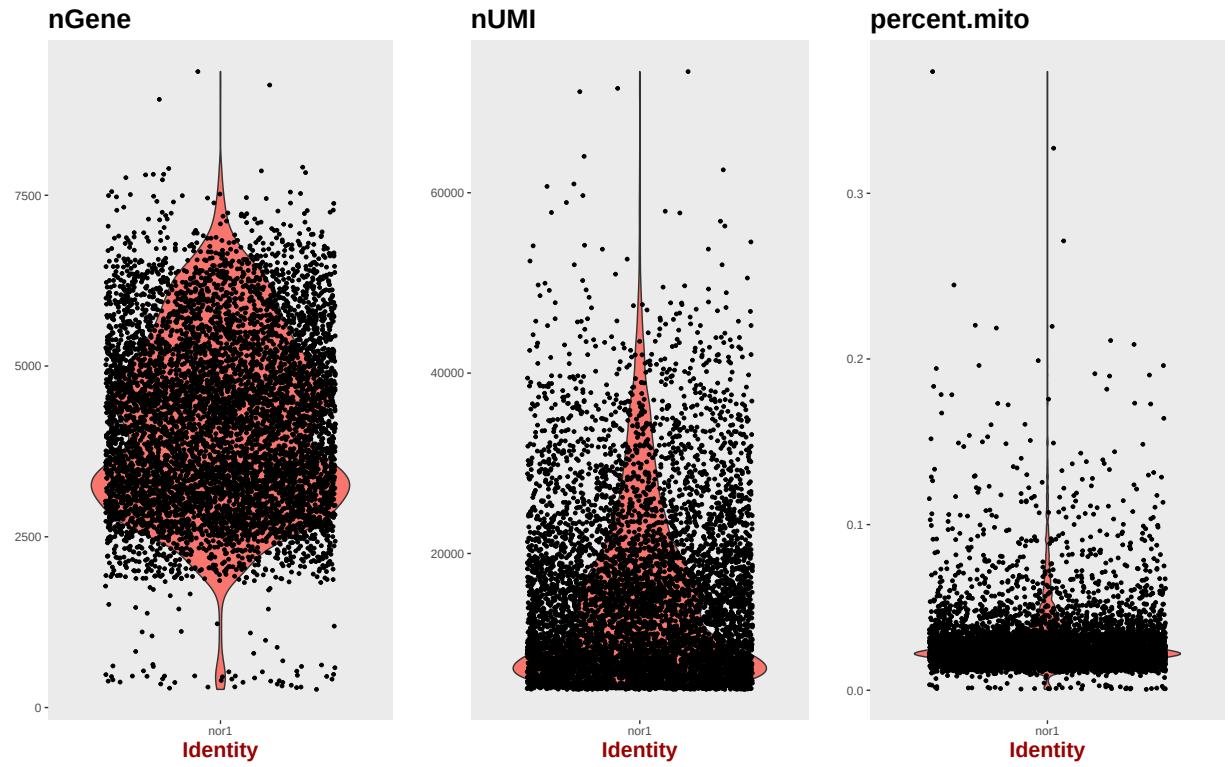

nGene
nUMI
percent.mito
7500
5000
2500
0
75000
50000
25000
0
0.3
0.2
0.1
0.0
hyp1
Identity

##
[[2]]

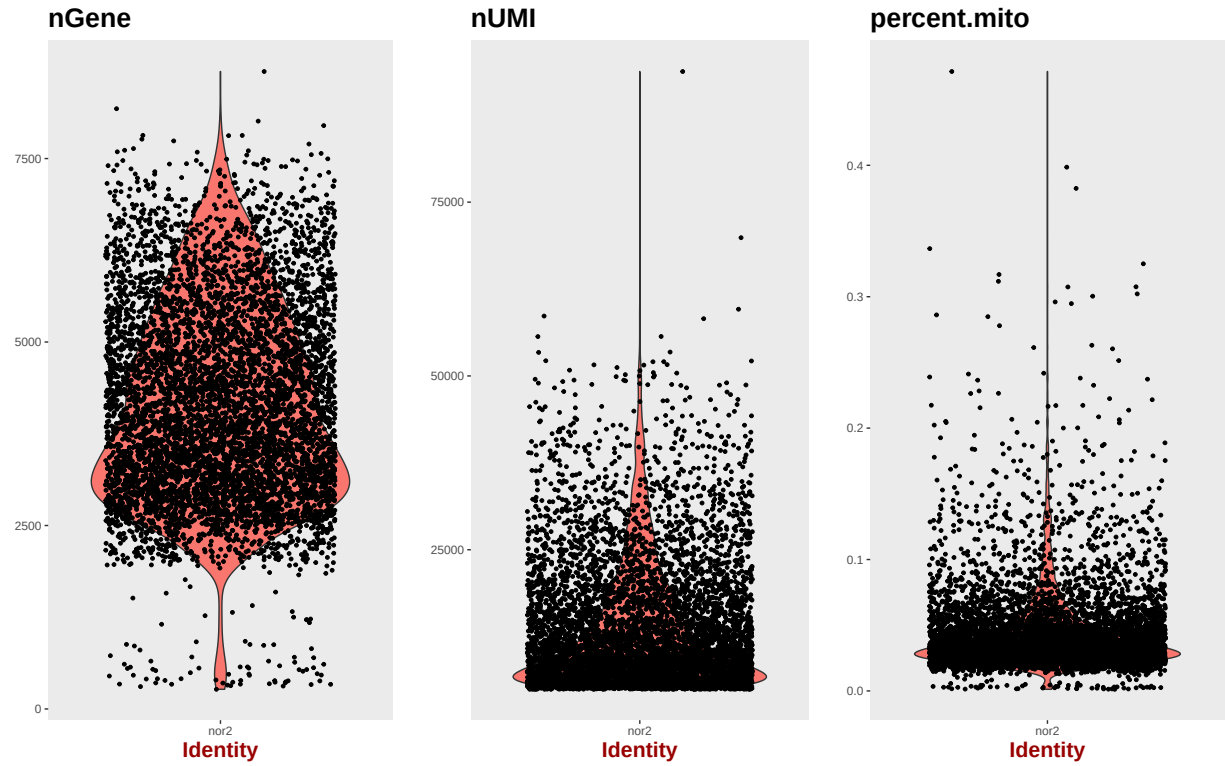

nGene
nUMI
percent.mito
6000
4000
2000
0
30000
20000
10000
0.4
0.3
0.2
0.1
0.0
hyp2
Identity

##

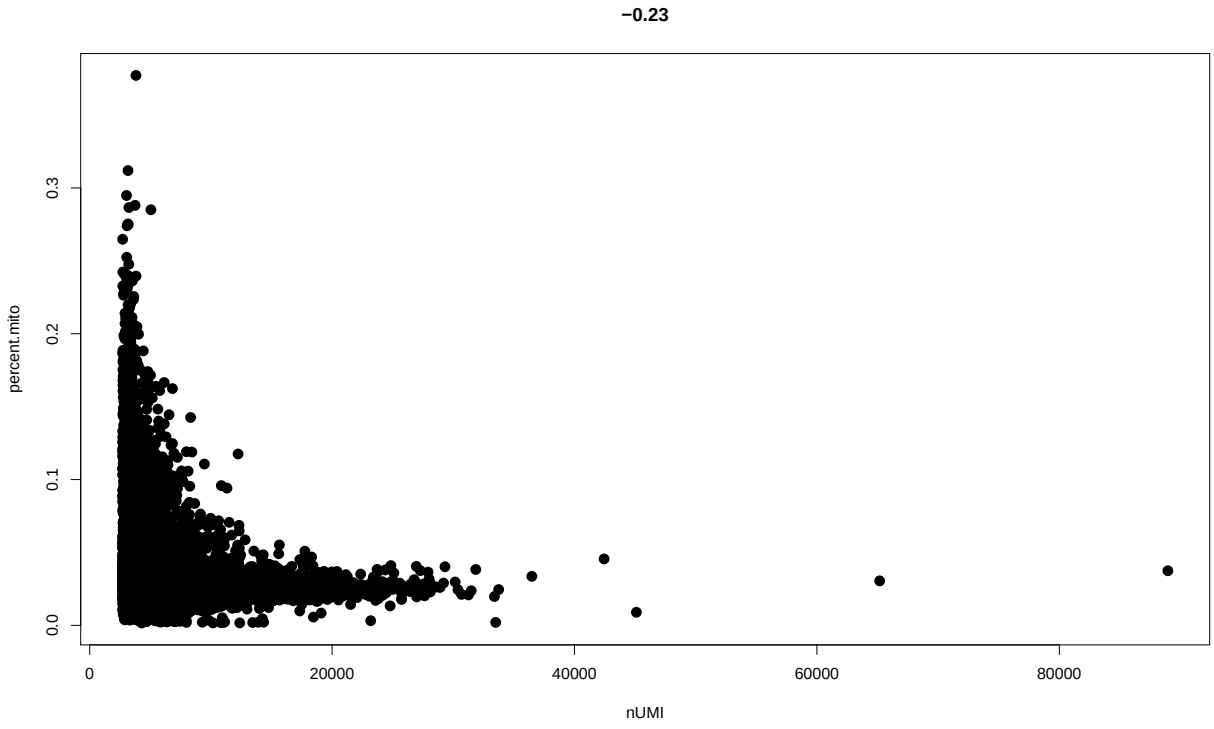
```
## [[3]]
```

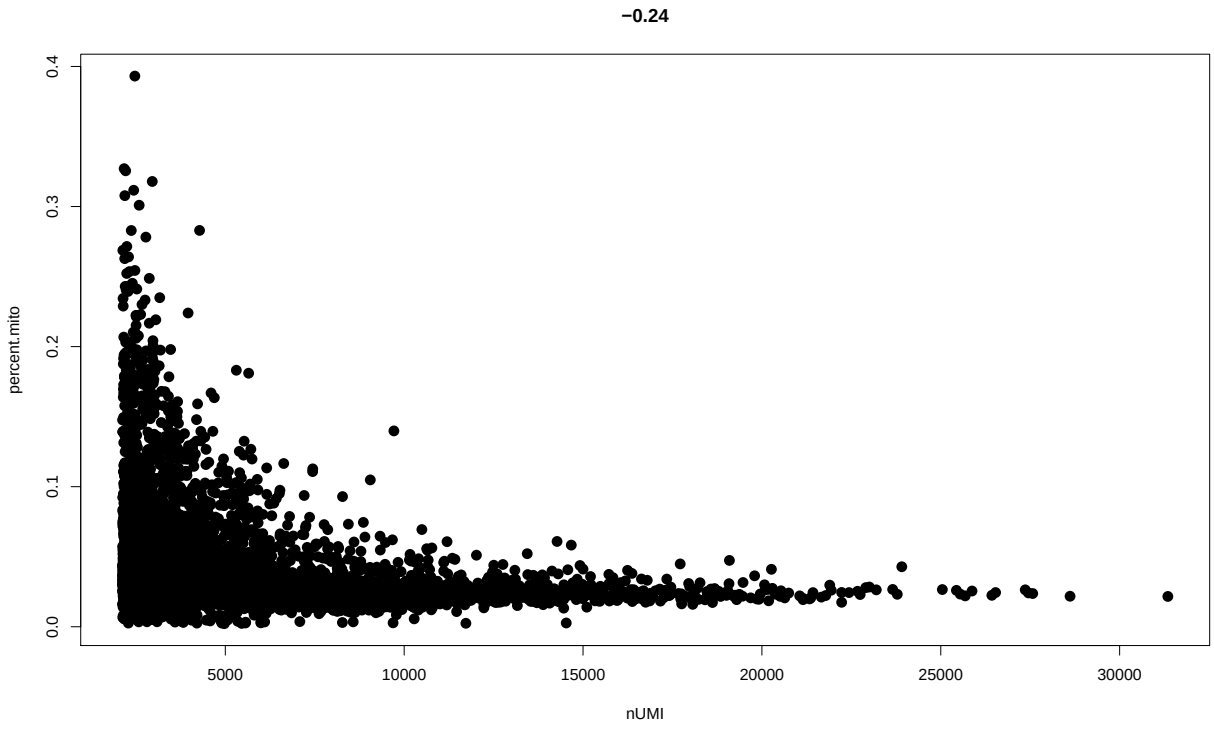

nGene
nUMI
percent.mito
7500
5000
2500
0
60000
40000
20000
0.3
0.2
0.1
0.0
nor1
Identity

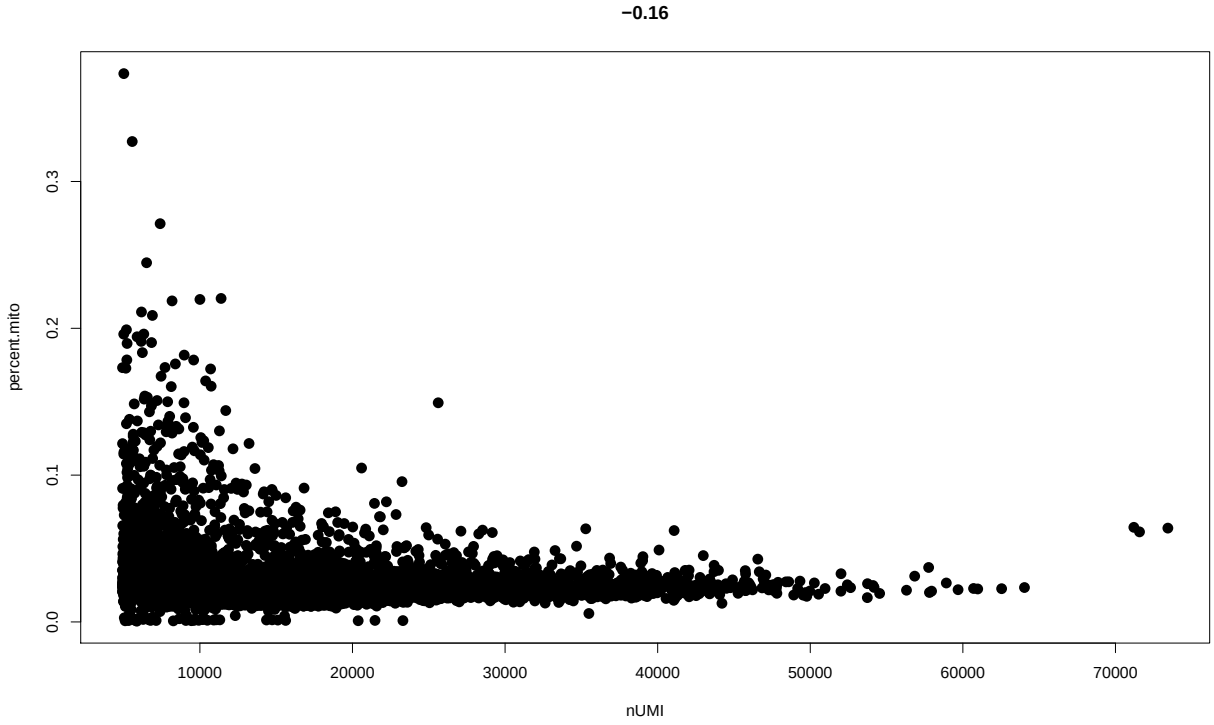
```
##
## [[4]]
```

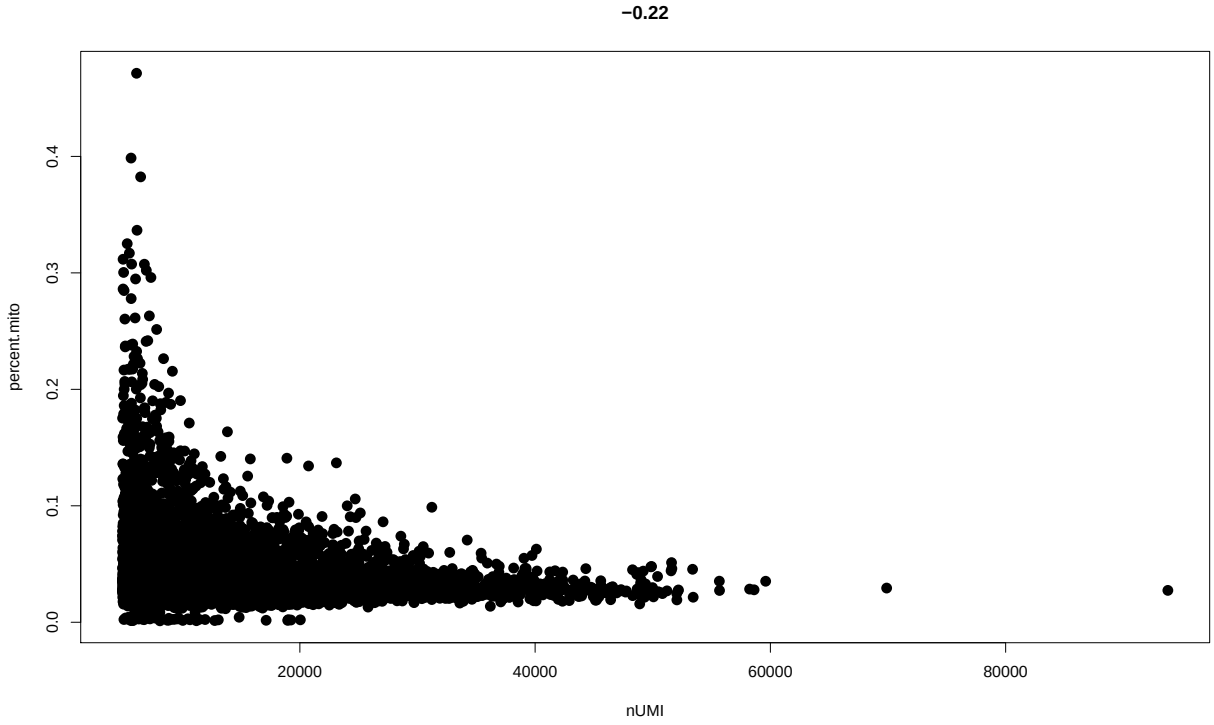

nGene
nUMI
percent.mito
7500
5000
2500
0
75000
50000
25000
0.4
0.3
0.2
0.1
0.0
nor2
Identity

```
# plot nUMI by percent.mito/nGene to see distribution and decide on filtering cutoffs
lapply(obj, function(x) GenePlot(x, gene1 = "nUMI", gene2 = "percent.mito"))
```





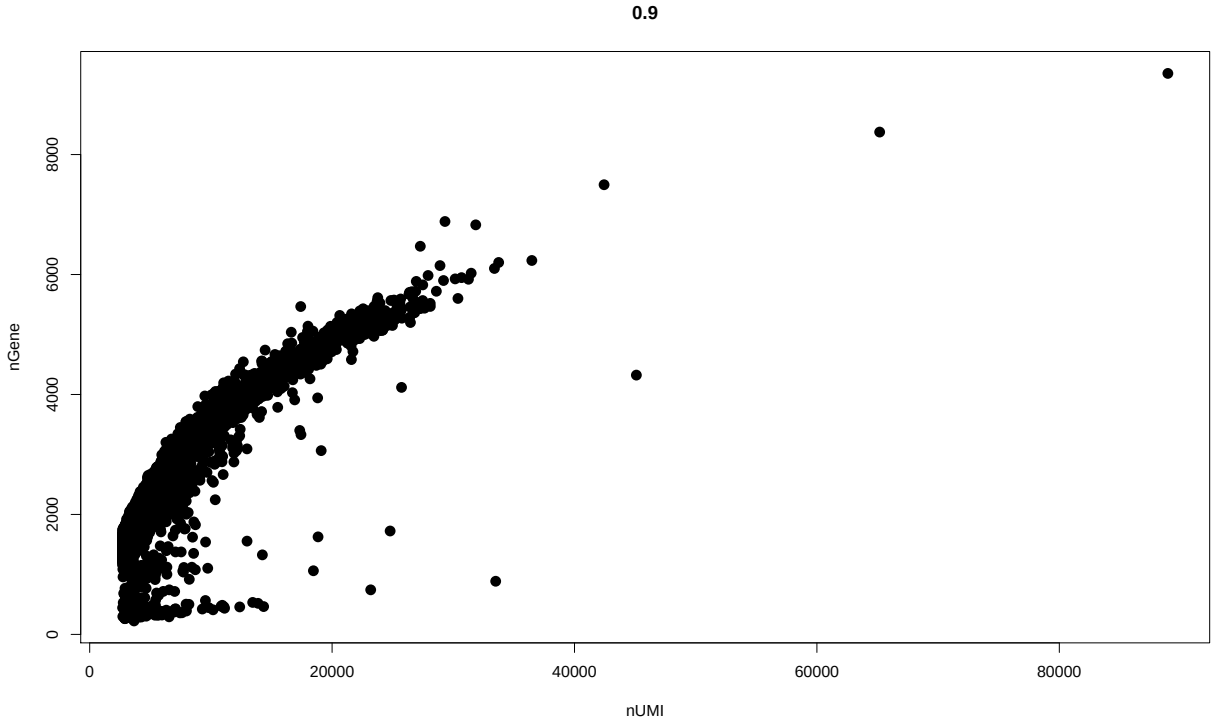

−0.16
percent.mito
0.0
0.1
0.2
0.3
10000
20000
30000
40000
50000
60000
70000
nUMI

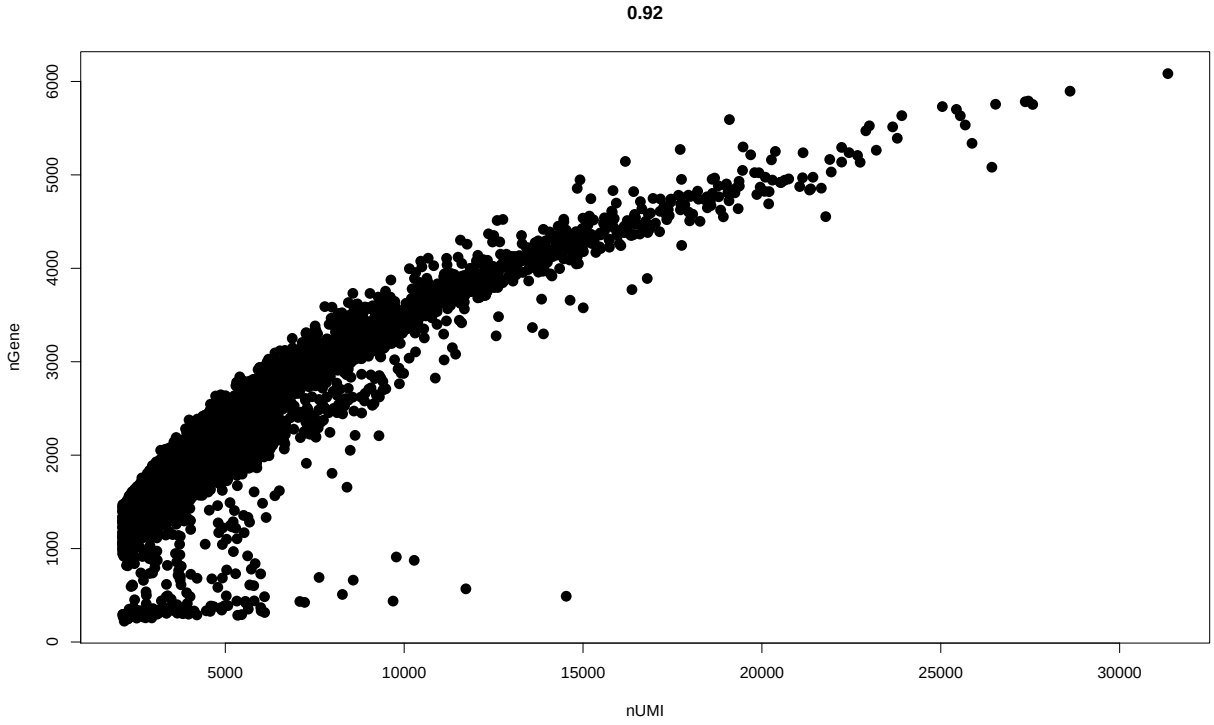

−0.22
percent.mito
0.0
0.1
0.2
0.3
0.4
20000
40000
60000
80000
nUMI

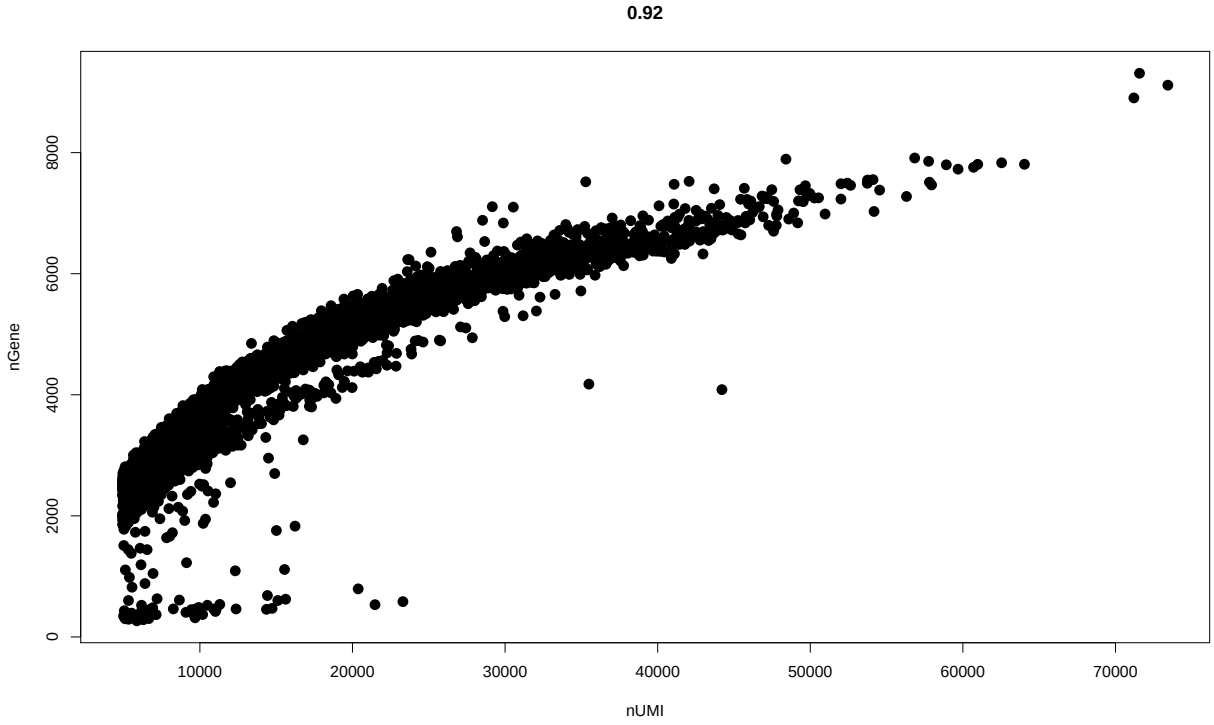
```
## [[1]]
## NULL
##
## [[2]]
## NULL
```

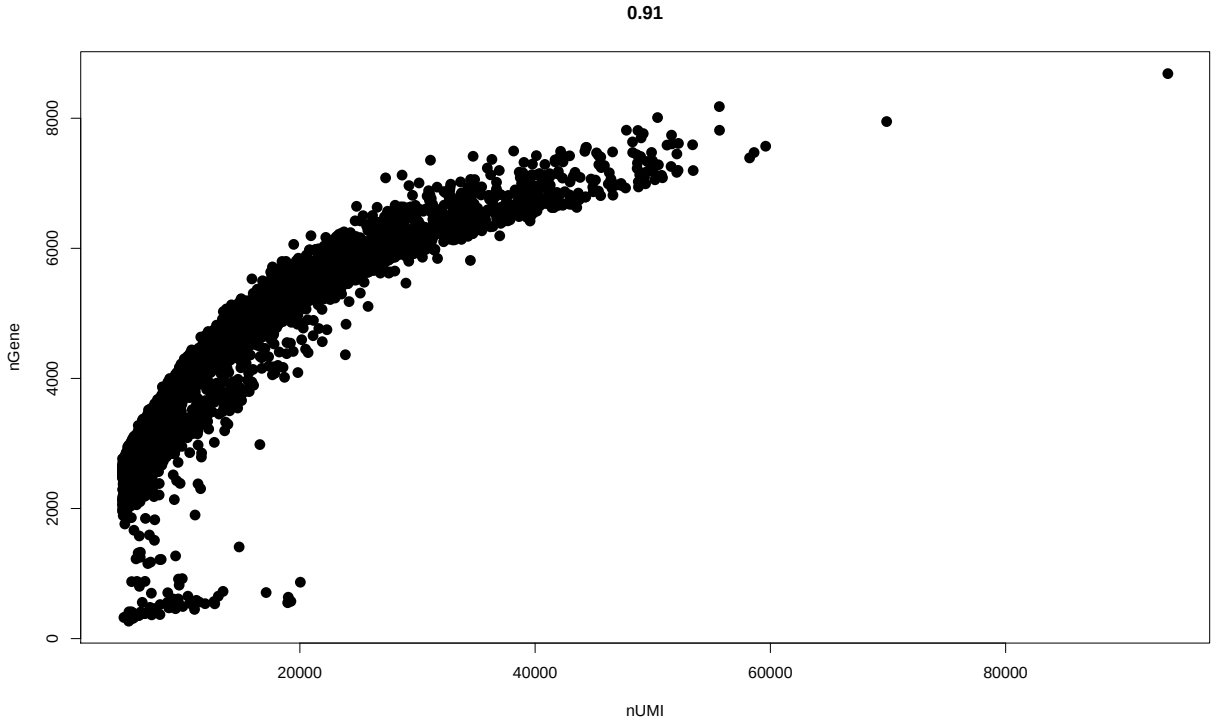
```
##
## [[3]]
## NULL
##
## [[4]]
## NULL
```

```
lapply(obj, function(x) GenePlot(x, gene1 = "nUMI", gene2 = "nGene"))
```




0.92
nGene
nUMI
0
1000
2000
3000
4000
5000
6000
5000
10000
15000
20000
25000
30000


0.92
nGene
nUMI
0
2000
4000
6000
8000
10000
20000
30000
40000
50000
60000
70000




```
## [[1]]
## NULL
##
## [[2]]
## NULL
##
## [[3]]
## NULL
##
## [[4]]
## NULL
```

4 Filter cells

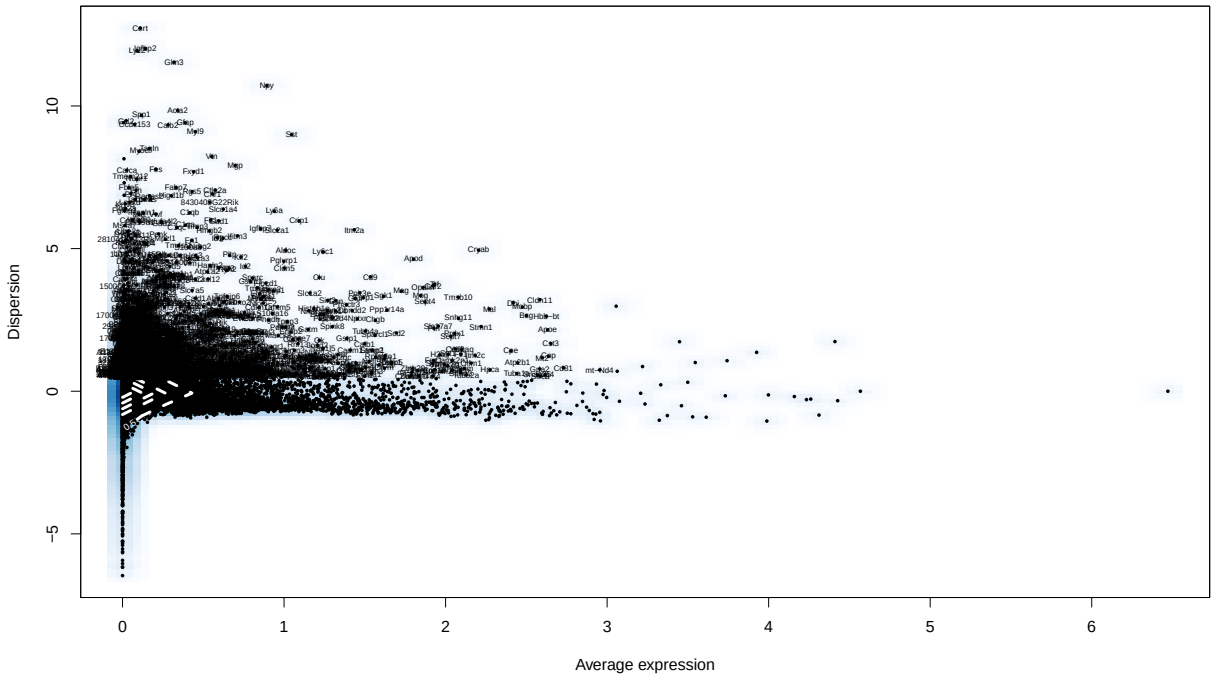
```
obj[[1]] <- FilterCells(obj[[1]], subset.names = c("nGene", "percent.mito"), low.thresholds = c(500, -I
obj[[2]] <- FilterCells(obj[[2]], subset.names = c("nGene", "percent.mito"), low.thresholds = c(500, -I
obj[[3]] <- FilterCells(obj[[3]], subset.names = c("nGene", "percent.mito"), low.thresholds = c(500, -I
obj[[4]] <- FilterCells(obj[[4]], subset.names = c("nGene", "percent.mito"), low.thresholds = c(500, -I
```

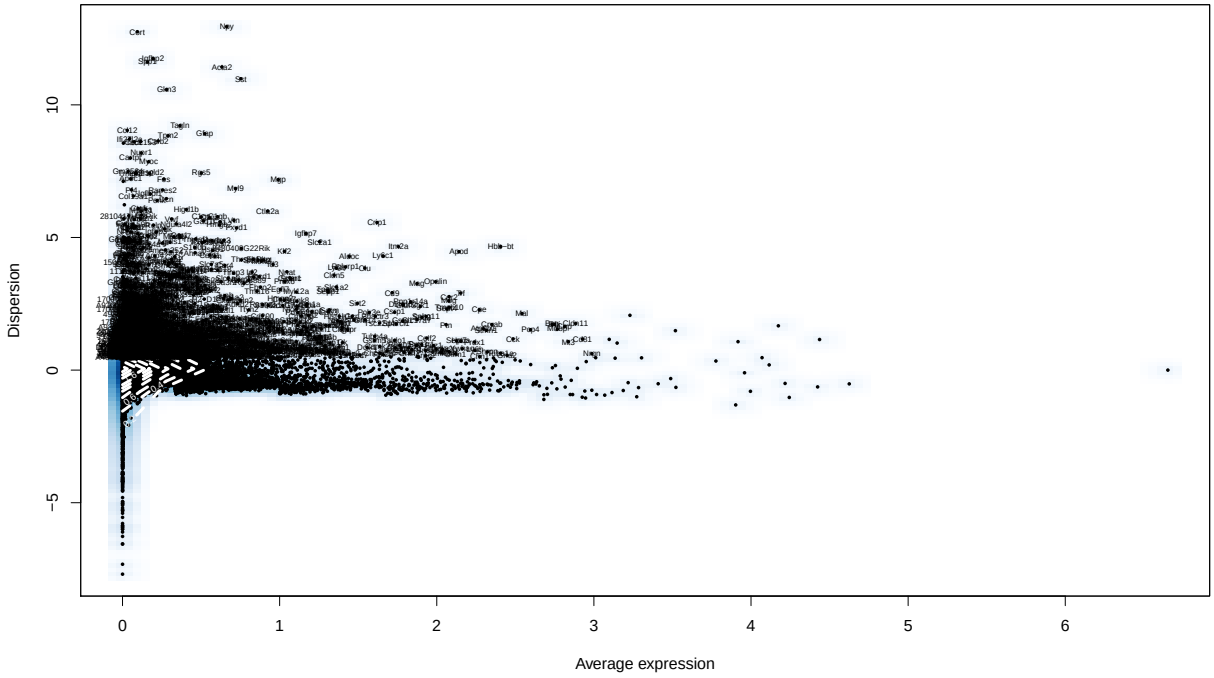
5 Normalize data

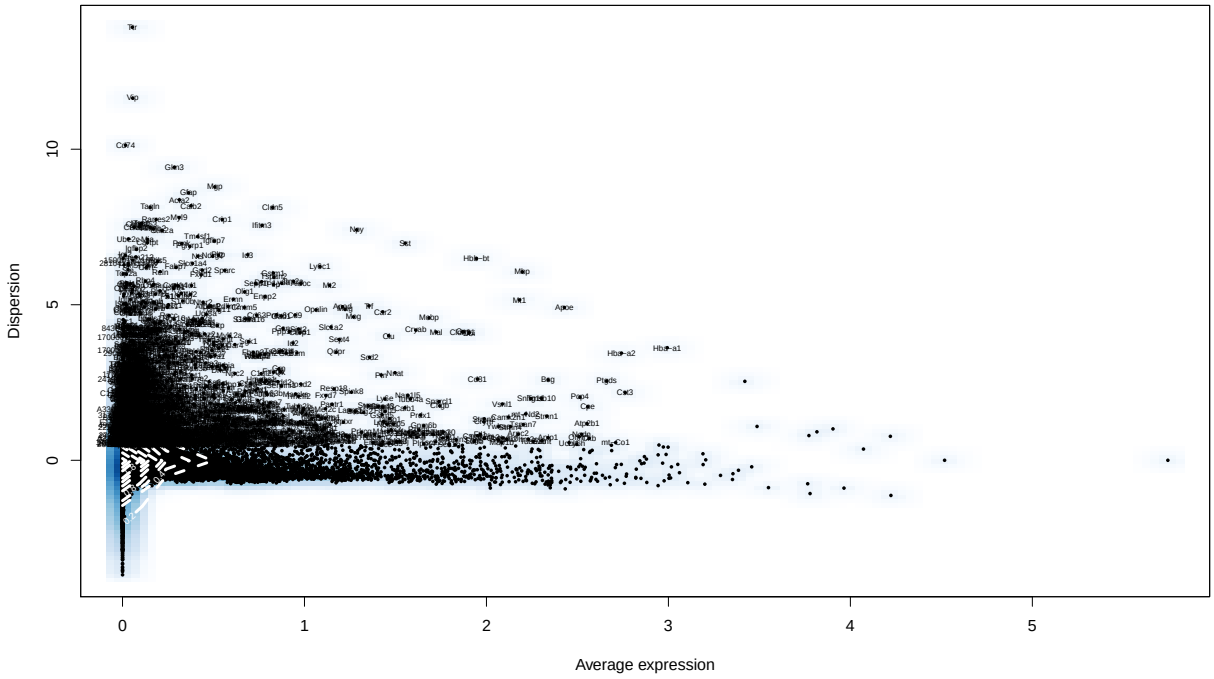
```
obj <- lapply(obj, function(x) NormalizeData(x, normalization.method = "LogNormalize", scale.factor = 1
```

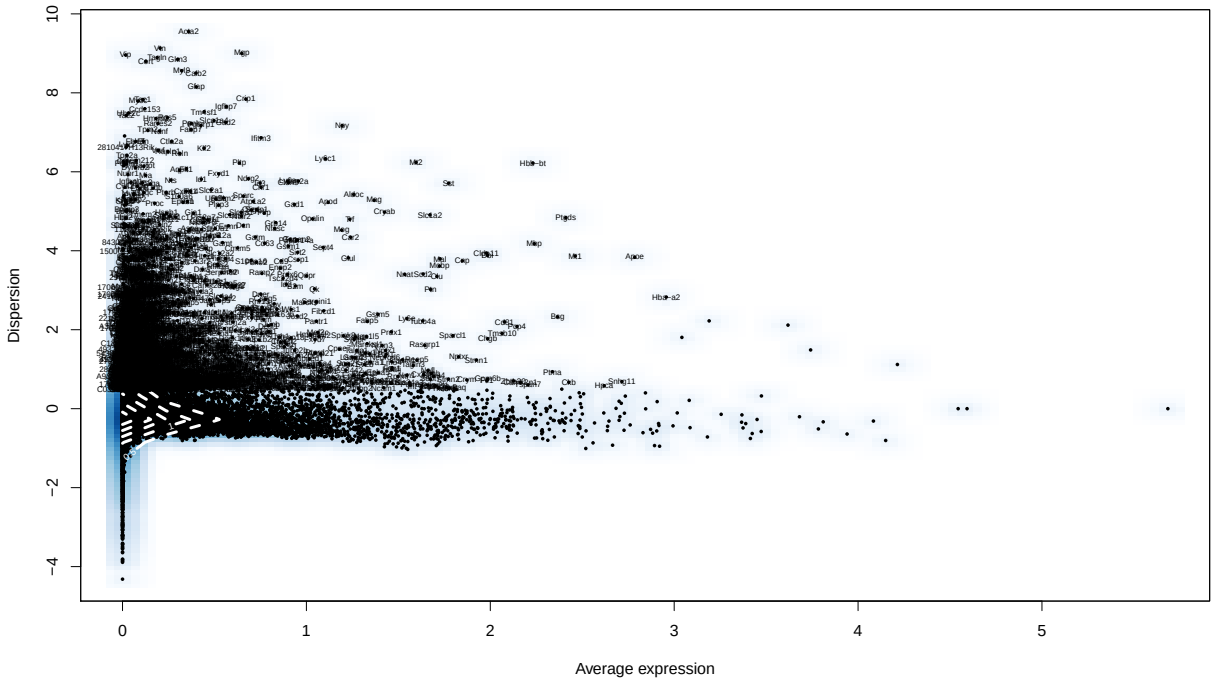
6 Define variable genes

```
obj<- lapply(obj, function(x) FindVariableGenes(object = x, mean.function = ExpMean, dispersion.functio
    x.low.cutoff = 0.0125, x.high.cutoff = 3, y.cutoff = 0.5))
```




Dispersion
Average expression
0
1
2
3
4
5
6
−5
0
5
10


Dispersion
Average expression
0
1
2
3
4
5
0
5
10



7 Scale data and regress out nUMI and percent.mito

```
obj<- lapply(obj, function(x) ScaleData(object = x, vars.to.regress = c("nUMI", "percent.mito")))
```

8 Save objects

```
lapply(seq_along(samnam), function(x) saveRDS(obj[[x]], file=paste0(samnam[x], ".rds")))
```

```
sessionInfo()
```

```
## R version 3.5.2 (2018-12-20)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 7 x64 (build 7601) Service Pack 1
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=German_Germany.1252  LC_CTYPE=German_Germany.1252
## [3] LC_MONETARY=German_Germany.1252 LC_NUMERIC=C
## [5] LC_TIME=German_Germany.1252
##
## attached base packages:
```

```
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] Seurat_2.3.0  Matrix_1.2-18 cowplot_1.0.0 ggplot2_3.2.1
##
## loaded via a namespace (and not attached):
##   [1] snow_0.4-3           backports_1.1.5      Hmisc_4.3-1
##   [4] VGAM_1.1-2           sn_1.5-5             plyr_1.8.5
##   [7] igraph_1.2.5         lazyeval_0.2.2       splines_3.5.2
##  [10] TH.data_1.0-10       digest_0.6.24        foreach_1.4.8
##  [13] htmltools_0.4.0      lars_1.2             gdata_2.18.0
##  [16] magrittr_1.5         checkmate_2.0.0      cluster_2.1.0
##  [19] mixtools_1.2.0       ROCR_1.0-7           recipes_0.1.9
##  [22] gower_0.2.1          R.utils_2.9.2        sandwich_2.5-1
##  [25] colorspace_1.4-1     xfun_0.12            dplyr_0.8.4
##  [28] crayon_1.3.4         survival_3.1-8       zoo_1.8-7
##  [31] iterators_1.0.12     ape_5.3              glue_1.3.1
##  [34] gtable_0.3.0         ipred_0.9-9          kernlab_0.9-29
##  [37] prabclus_2.3-2       BiocGenerics_0.28.0  DEoptimR_1.0-8
##  [40] scales_1.1.0         mvtnorm_1.0-12       bibtex_0.4.2.2
##  [43] Rcpp_1.0.3           metap_1.3            dtw_1.21-3
##  [46] plotrix_3.7-7        htmlTable_1.13.3     tclust_1.4-1
##  [49] foreign_0.8-75       proxy_0.4-23         mclust_5.4.5
##  [52] SDMTools_1.1-221.1   Formula_1.2-3        tsne_0.1-3
##  [55] stats4_3.5.2         lava_1.6.6           prodlim_2019.11.13
##  [58] htmlwidgets_1.5.1    FNN_1.1.3            gplots_3.0.1.2
##  [61] RColorBrewer_1.1-2   fpc_2.2-5            acepack_1.4.1
##  [64] TFisher_0.2.0        modeltools_0.2-22    ica_1.0-2
##  [67] farver_2.0.3         pkgconfig_2.0.3      R.methodsS3_1.8.0
##  [70] flexmix_2.3-15       nnet_7.3-12          caret_6.0-85
##  [73] labeling_0.3         tidyselect_1.0.0     rlang_0.4.4
##  [76] reshape2_1.4.3       munsell_0.5.0        tools_3.5.2
##  [79] generics_0.0.2       ranger_0.12.1        ggridges_0.5.2
##  [82] evaluate_0.14        stringr_1.4.0        yaml_2.2.1
##  [85] npsurv_0.4-0         ModelMetrics_1.2.2.1 knitr_1.28
##  [88] fitdistrplus_1.0-14  robustbase_0.93-5    caTools_1.17.1.2
##  [91] purrr_0.3.3          RANN_2.6.1           pbapply_1.4-3
##  [94] nlme_3.1-137         formatR_1.7          R.oo_1.23.0
##  [97] compiler_3.5.2       rstudioapi_0.11      png_0.1-7
## [100] lsei_1.2-0           tibble_2.1.3         stringi_1.4.6
## [103] lattice_0.20-40      vctrs_0.2.3          multtest_2.38.0
## [106] mutoss_0.1-12        diffusionMap_1.2.0   pillar_1.4.3
## [109] lifecycle_0.1.0      Rdpack_0.11-1        lmtest_0.9-37
## [112] data.table_1.12.8    bitops_1.0-6         irlba_2.3.3
## [115] gbRd_0.4-11          R6_2.4.1             latticeExtra_0.6-28
## [118] KernSmooth_2.23-16   gridExtra_2.3        codetools_0.2-16
## [121] MASS_7.3-51.5        gtools_3.8.1         assertthat_0.2.1
## [124] withr_2.1.2          mnormt_1.5-6         multcomp_1.4-12
## [127] diptest_0.75-7       parallel_3.5.2       doSNOW_1.0.18
## [130] grid_3.5.2           rpart_4.1-15         timeDate_3043.102
## [133] tidyr_1.0.2          class_7.3-15         rmarkdown_2.1
## [136] segmented_1.1-0      Rtsne_0.15           pROC_1.16.1
## [139] numDeriv_2016.8-1.1  scatterplot3d_0.3-41 Biobase_2.42.0
## [142] lubridate_1.7.4      base64enc_0.1-3
```