A sampling of bioinformatics using R

Abhijit Dasgupta, PhD

Spring 2019

Expectations

1. We will explore
    - the basics of using Bioconductor packages for bioinformatics
    - visualizations useful for bioinformatics
    - ways to annotate our graphics
2. We will not explore
    - how to do bioinformatics using R
    - bioinformatic workflows
    - data cleaning, modeling, analytics

This area is too broad and interests too varied to cover this with any sort of breadth or depth.

A very simple example

```
fix_names <- function(d) d %>% set_names(make.names(names(.)))
clinical <- rio::import('data/BreastCancer_Clinical.xlsx') %>% fix_names()
proteome <- rio::import('data/BreastCancer_Expression.xlsx') %>% fix_names()
final_data <- clinical %>%
    inner_join(proteome, by = c('Complete.TCGA.ID' = 'TCGA_ID')) %>%
    filter(Gender == 'FEMALE') %>%
    select(Complete.TCGA.ID, Age.at.Initial.Pathologic.Diagnosis, ER.Status, starts_with("NP"))
head(final_data)
```

```
#>    Complete.TCGA.ID Age.at.Initial.Pathologic.Diagnosis ER.Status
#>  1     TCGA-A2-A0CM                                  40  Negative
#>  2     TCGA-BH-A18Q                                  56  Negative
#>  3     TCGA-A7-A0CE                                  57  Negative
#>  4     TCGA-D8-A142                                  74  Negative
#>  5     TCGA-AO-A0J6                                  61  Negative
#>  6     TCGA-A2-A0YM                                  67  Negative
#>     NP_958782  NP_958785  NP_958786  NP_000436  NP_958781  NP_958780
#>  1  0.6834035  0.6944241  0.6980976  0.6870771  0.6870771  0.6980976
#>  2  0.1953407  0.2154129  0.2154129  0.2053768  0.2154129  0.2154129
#>  3 -1.1231731 -1.1231731 -1.1168605 -1.1294857 -1.1294857 -1.1200168
#>  4  0.5385958  0.5422105  0.5422105  0.5349810  0.5422105  0.5422105
#>  5  0.8311317  0.8565398  0.8565398  0.8367780  0.8650092  0.8565398
#>  6  0.6558497  0.6581426  0.6558497  0.6558497  0.6512639  0.6581426
#>     NP_958783  NP_958784  NP_112598  NP_001611
#>  1  0.6980976  0.6980976 -2.6521501 -0.9843733
#>  2  0.2154129  0.2154129 -1.0357599 -0.5172257
#>  3 -1.1231731 -1.1231731  2.2445844 -2.5750648
#>  4  0.5422105  0.5422105 -0.1482049  0.2674902
```

A very simple example

```
results <- final_data %>%
    summarise_at(vars(starts_with('NP')),
                 ~wilcox.test(. ~ ER.Status)$p.value)
results
```

```
#>    NP_958782 NP_958785 NP_958786 NP_000436 NP_958781 NP_958780 NP_958783
#>  1 0.6988415 0.6910103 0.6832121 0.6910103 0.6832121 0.6910103 0.6910103
#>    NP_958784 NP_112598    NP_001611
#>  1 0.6832121 0.9957714 0.0001218627
```

`.` is the placeholder for what's specified inside the `vars()`.

This isn't in the right format for me to plot

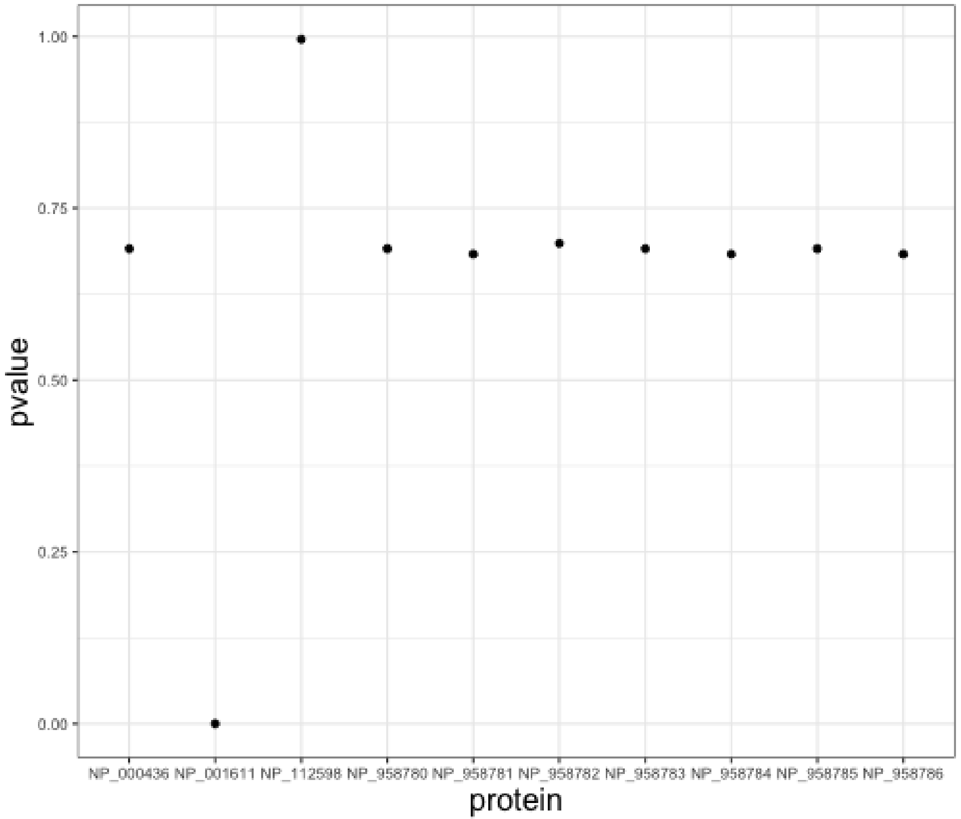
A very simple example

```
results %>% tidyr::gather(protein, pvalue)
```

```
#>       protein       pvalue
#>  1  NP_958782 0.6988415415
#>  2  NP_958785 0.6910103484
#>  3  NP_958786 0.6832120796
#>  4  NP_000436 0.6910103484
#>  5  NP_958781 0.6832120796
#>  6  NP_958780 0.6910103484
#>  7  NP_958783 0.6910103484
#>  8  NP_958784 0.6832120796
#>  9  NP_112598 0.9957713985
#>  10 NP_001611 0.0001218627
```

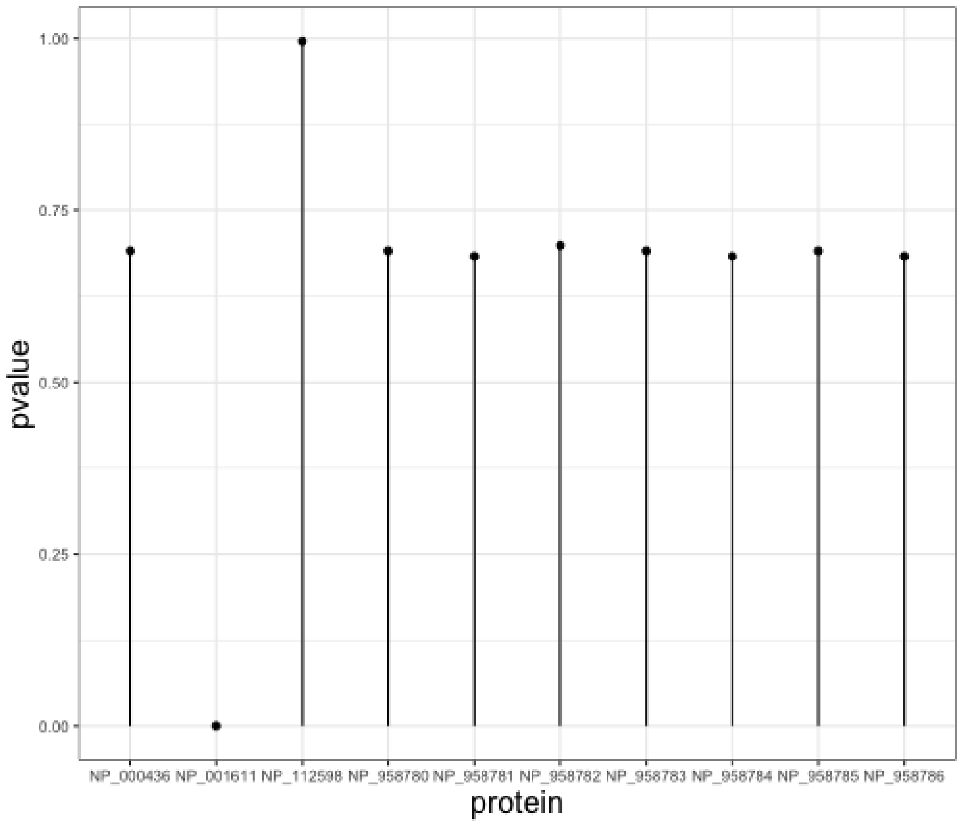
A very simple example

```
theme_439 <- theme_bw() +
    theme(axis.title = element_text(size=16),
          axis.text = element_text(size=8))


results %>% tidyr::gather(protein, pvalue) %>%
    ggplot(aes(x = protein, y = pvalue)) +
      geom_point() +
    theme_439
```



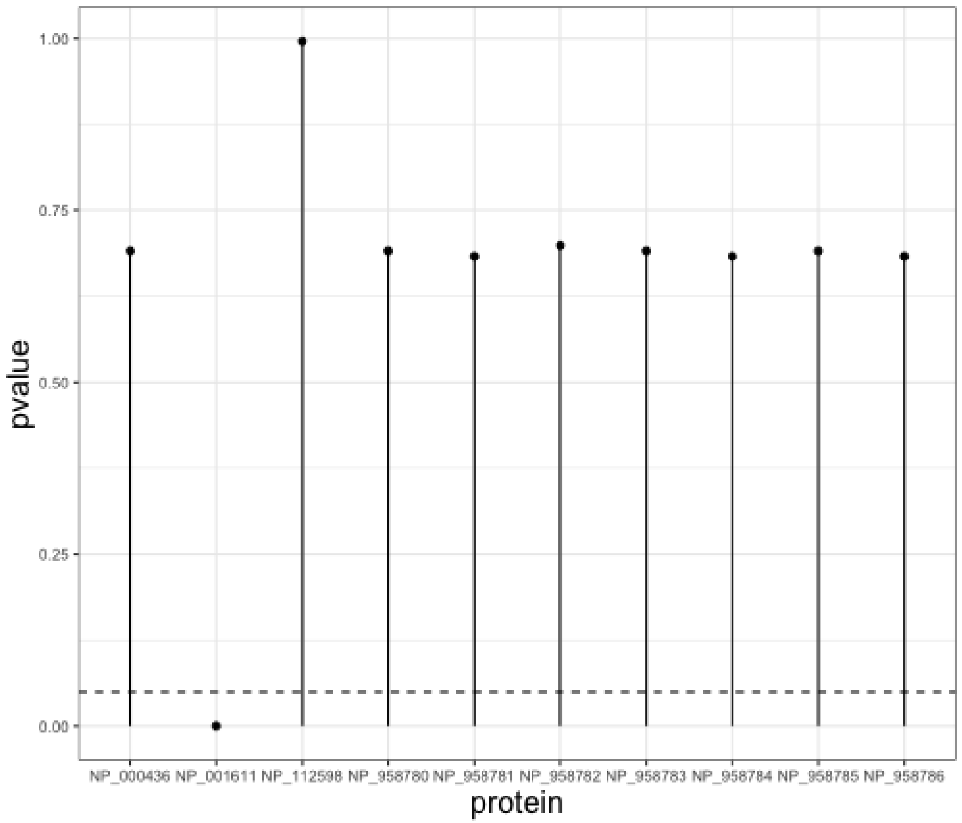
A very simple example

```
results %>% tidyr::gather(protein, pvalue) %>%
    ggplot(aes(x = protein, y = pvalue)) +
      geom_point() +
    geom_segment(aes(x = protein, xend = protein,
                     y = 0, yend = pvalue))+
    theme_439
```



A very simple example

```
results %>% tidyr::gather(protein, pvalue) %>%
    ggplot(aes(x = protein, y = pvalue)) +
      geom_point() +
    geom_segment(aes(x = protein, xend = protein,
                     y = 0, yend = pvalue))+
    geom_hline(yintercept = 0.05, linetype=2)+
    theme_439
```

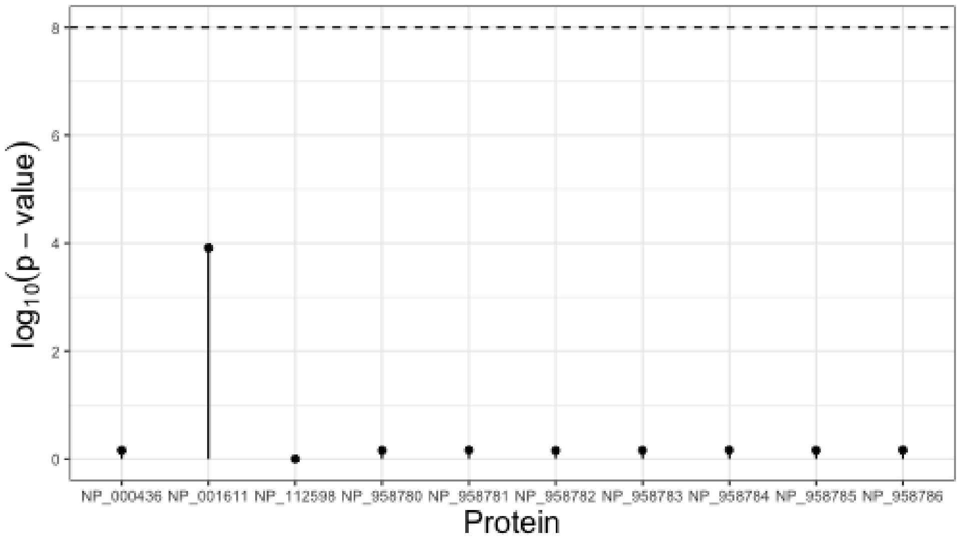


Manhattan plot

A Manhattan plot is used to visualize a set of p-values from unit-based tests

It plots the negative log p-value at each unit

```
results %>% tidyr::gather(protein, pval) %>%
    ggplot(aes(x = protein, y = -log10(pval))) +
    geom_point() +
    geom_segment(aes(xend = protein, yend = 0))+
    geom_hline(yintercept = 8, linetype=2)+
    labs(x = 'Protein',
         y = expression(log[10](p-value))) +
    theme_439
```



Manhattan plot

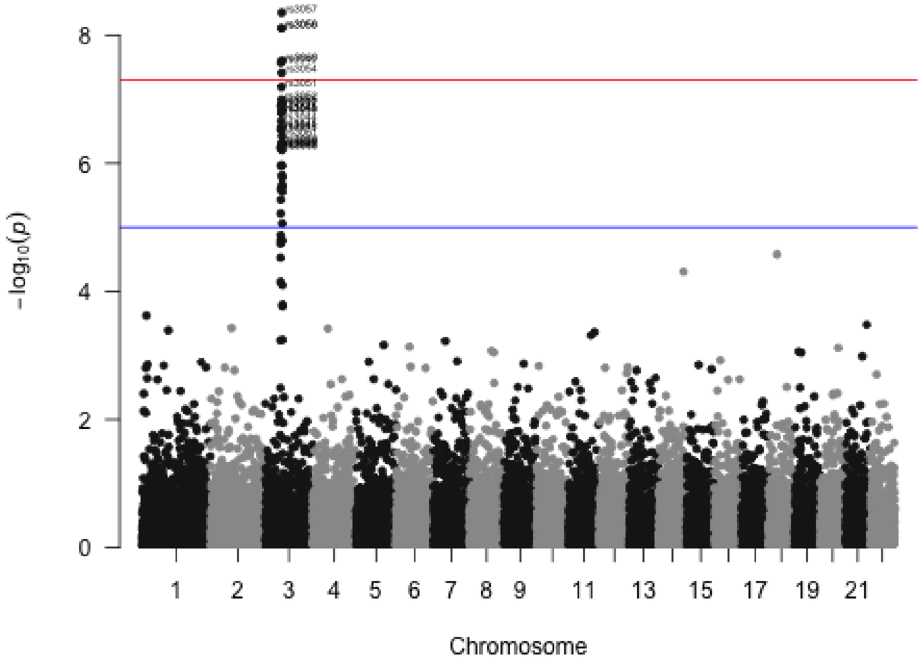
There is a specialized package for doing Manhattan plots and quantile plots for GWAS data

This package is meant to work with PLINK output, but the function is generic

```r
library(qqman)
manhattan(gwasResults)
```

Manhattan plot

```
library(qqman)
manhattan(gwasResults,
          annotatePval = 1e-6,
          annotateTop=F)
```



ggplot tours Manhattan

Data prep

```
head(gwasResults)
```

```
#>    SNP CHR BP         P
#>  1 rs1   1  1 0.9148060
#>  2 rs2   1  2 0.9370754
#>  3 rs3   1  3 0.2861395
#>  4 rs4   1  4 0.8304476
#>  5 rs5   1  5 0.6417455
#>  6 rs6   1  6 0.5190959
```

ggplot tours Manhattan

Data prep

```
plt_x_position <- gwasResults %>%
    group_by(CHR) %>%
    summarize(chr_len = max(BP)) %>%
    mutate(tot = cumsum(chr_len) - chr_len)
head(plt_x_position)
```

```
#>  # A tibble: 6 x 3
#>      CHR chr_len   tot
#>    <int>   <int> <int>
#>  1     1    1500     0
#>  2     2    1191  1500
#>  3     3    1040  2691
#>  4     4     945  3731
#>  5     5     877  4676
#>  6     6     825  5553
```

ggplot tours Manhattan

Data prep

```
plt_dat <- gwasResults %>%
    left_join(plt_x_position %>% select(-chr_len),
              by = c('CHR'='CHR')) %>%
    arrange(CHR, BP) %>%
    mutate(BPcumul = BP + tot)
tail(plt_dat)
```

```
#>           SNP CHR  BP         P   tot BPcumul
#> 16465 rs16465  22 530 0.5643702 15935   16465
#> 16466 rs16466  22 531 0.1382863 15935   16466
#> 16467 rs16467  22 532 0.3936999 15935   16467
#> 16468 rs16468  22 533 0.1778749 15935   16468
#> 16469 rs16469  22 534 0.2393020 15935   16469
#> 16470 rs16470  22 535 0.2630441 15935   16470
```

ggplot tours Manhattan

Data for plotting x-axis labels

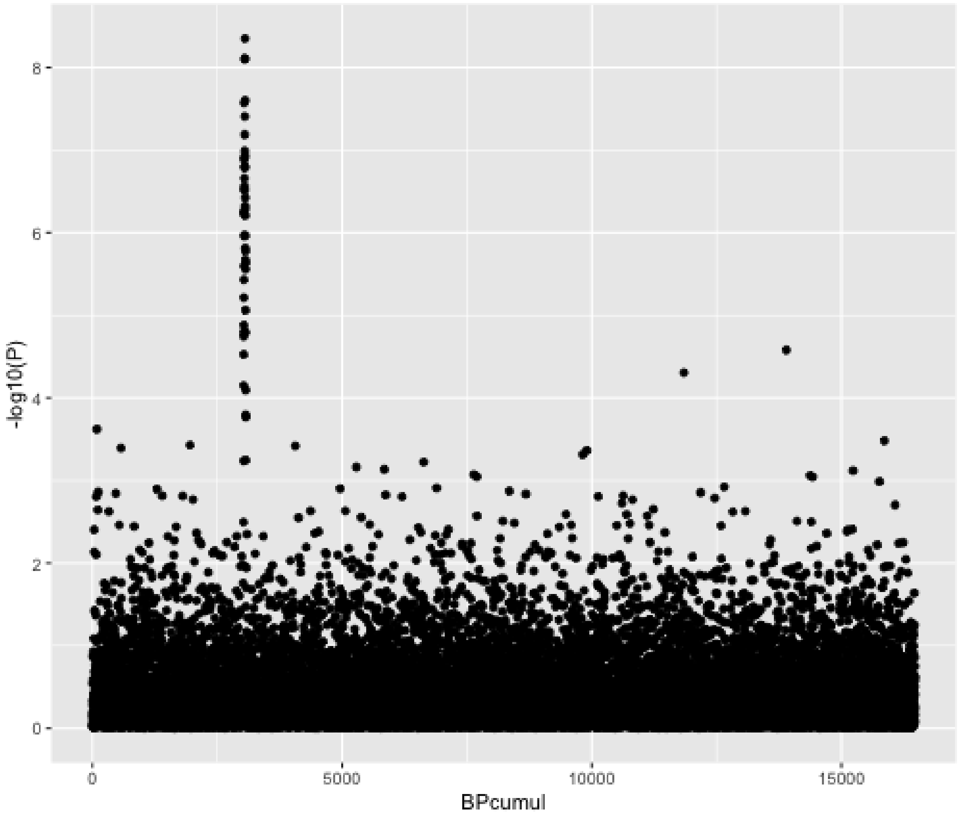
```
axisdf <- plt_dat %>%
    group_by(CHR) %>%
    summarize(center = (max(BPcumul)+min(BPcumul))/2)
axisdf
```

```
#>  # A tibble: 22 x 2
#>        CHR center
#>      <int>  <dbl>
#>    1     1   750.
#>    2     2  2096
#>    3     3  3212.
#>    4     4  4204
#>    5     5  5115
#>    6     6  5966
#>    7     7  6770.
#>    8     8  7538.
#>    9     9  8273
#>   10    10  8982.
#>  # … with 12 more rows
```

ggplot tours Manhattan

Plotting

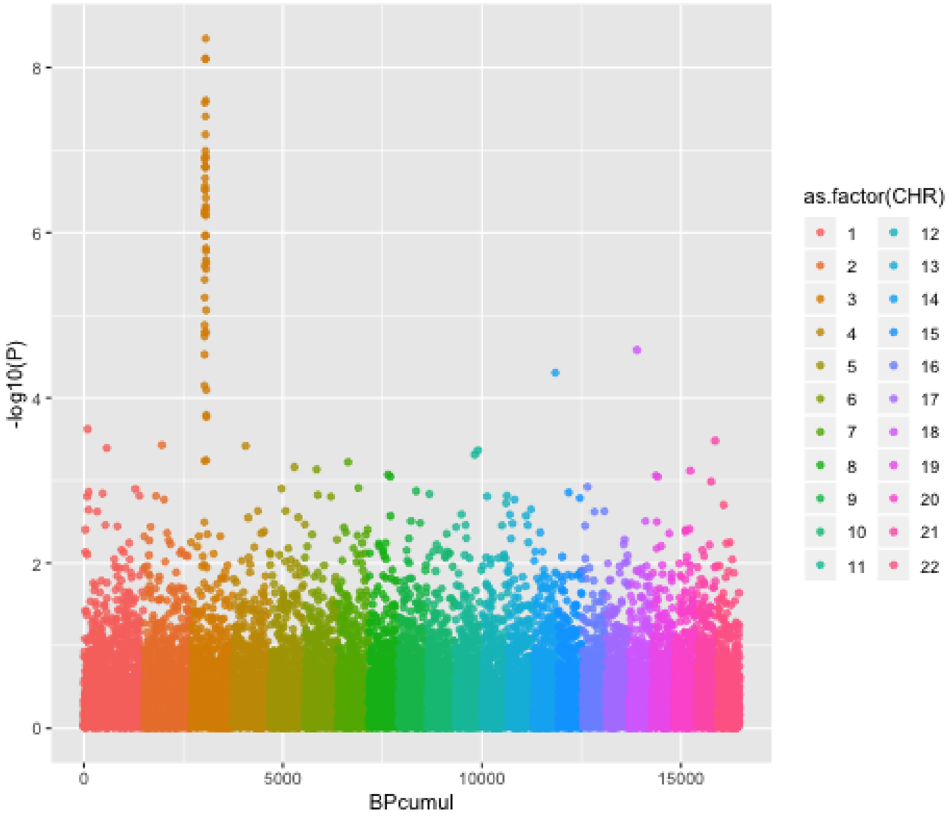
```
ggplot(plt_dat,
       aes(x = BPcumul, y = -log10(P)))+
    geom_point()
```



ggplot tours Manhattan

Plotting

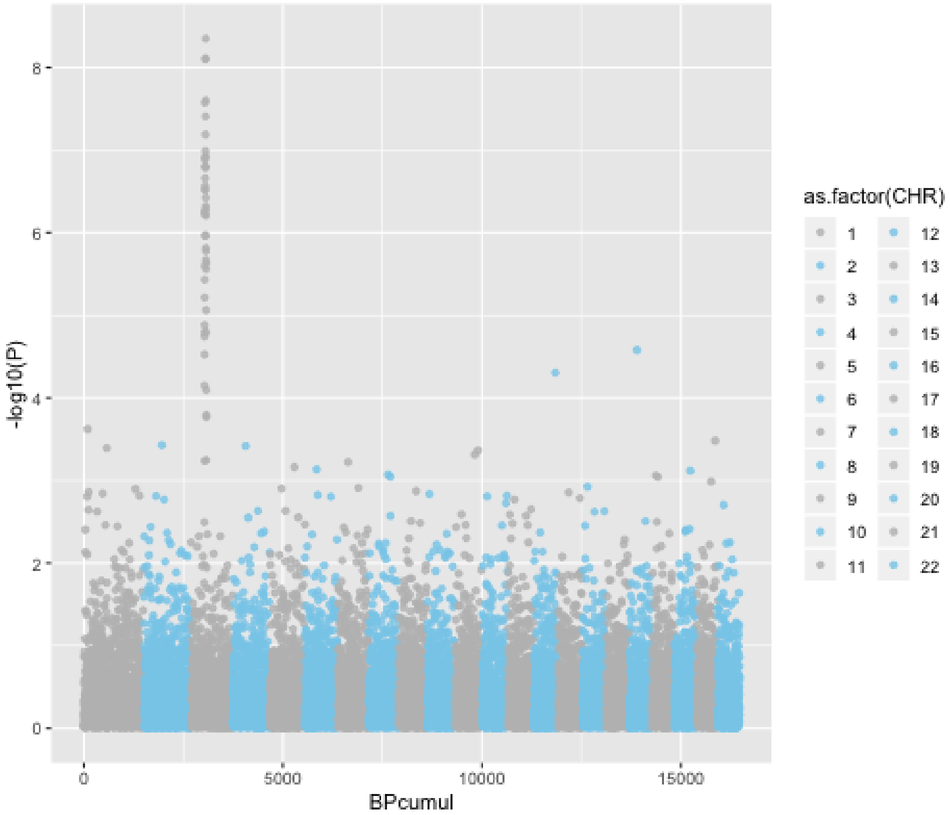
```
ggplot(plt_dat,
       aes(x = BPcumul, y = -log10(P)))+
    geom_point(aes(color = as.factor(CHR)),
               alpha = 0.8, size=1.3)
```



ggplot tours Manhattan

Plotting

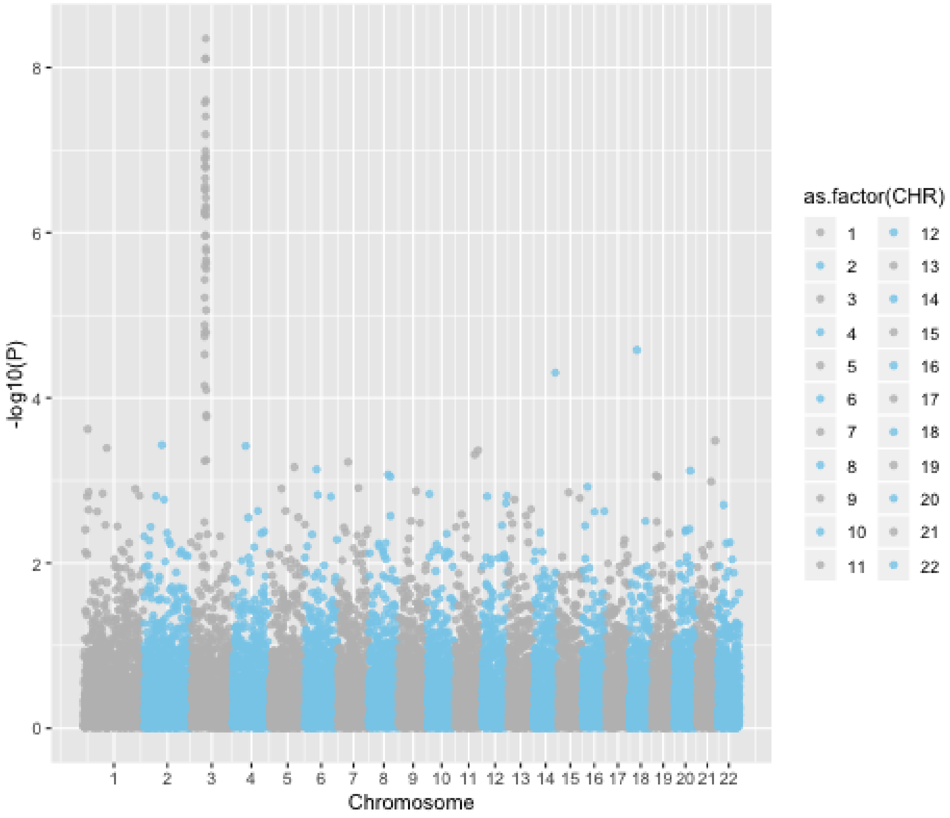
```
ggplot(plt_dat,
       aes(x = BPcumul, y = -log10(P)))+
    geom_point(aes(color = as.factor(CHR)),
               alpha = 0.8, size=1.3) +
    scale_color_manual(
        values = rep(c('grey','skyblue'), 22))
```



ggplot tours Manhattan

Plotting

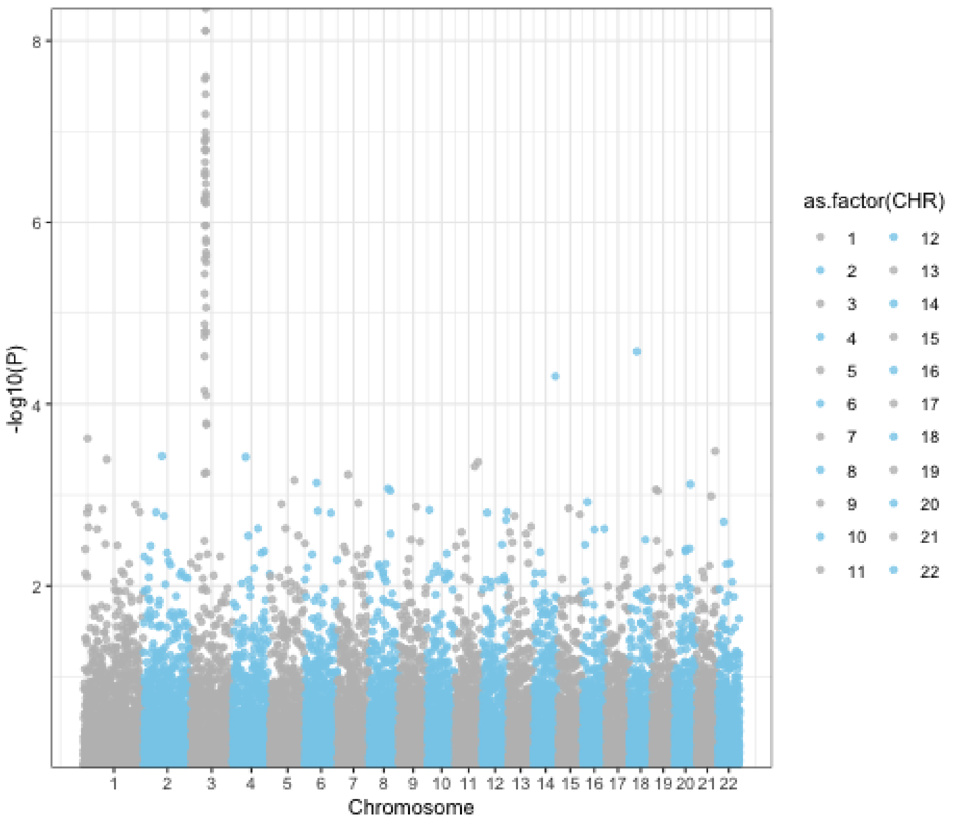
```
ggplot(plt_dat,
       aes(x = BPcumul, y = -log10(P)))+
    geom_point(aes(color = as.factor(CHR)),
               alpha = 0.8, size=1.3) +
    scale_color_manual(
        values = rep(c('grey','skyblue'), 22))+
    scale_x_continuous(
        name = 'Chromosome',
        breaks = axisdf$center,
        labels = axisdf$CHR
    )
```



ggplot tours Manhattan

Plotting

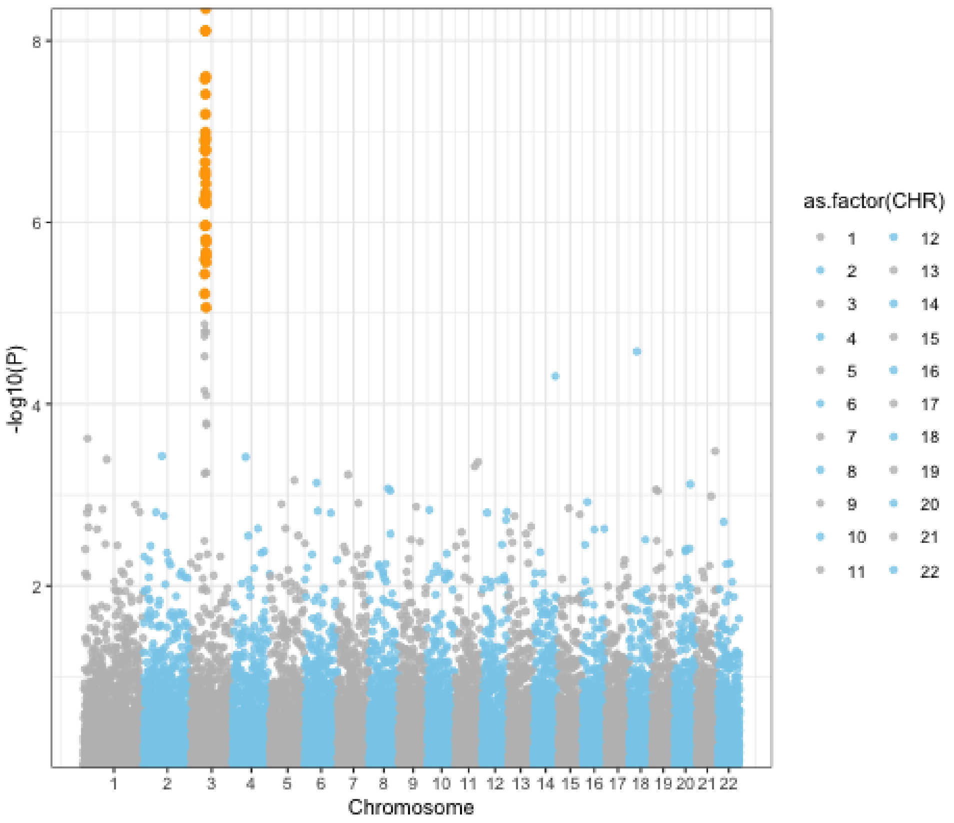
```
ggplot(plt_dat,
       aes(x = BPcumul, y = -log10(P)))+
    geom_point(aes(color = as.factor(CHR)),
               alpha = 0.8, size=1.3) +
    scale_color_manual(
      values = rep(c('grey','skyblue'), 22))+
    scale_x_continuous(
      name = 'Chromosome',
      breaks = axisdf$center,
      labels = axisdf$CHR
    ) +
    scale_y_continuous(expand = c(0,0)) +
    theme_bw()
```



ggplot tours Manhattan

Plotting

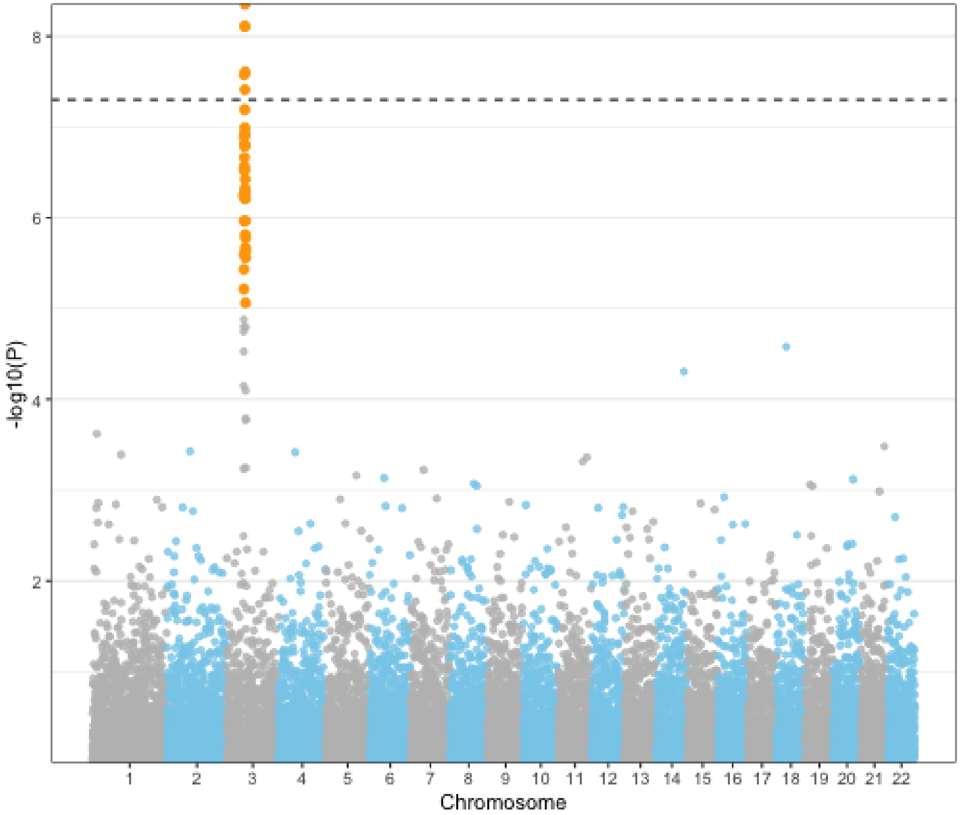
```
ggplot(plt_dat,
       aes(x = BPcumul, y = -log10(P)))+
    geom_point(aes(color = as.factor(CHR)),
               alpha = 0.8, size=1.3) +
    scale_color_manual(
        values = rep(c('grey','skyblue'), 22))+
    scale_x_continuous(
        name = 'Chromosome',
        breaks = axisdf$center,
        labels = axisdf$CHR
    ) +
    scale_y_continuous(expand = c(0,0)) +
    theme_bw() +
    geom_point(data = plt_dat %>% filter(P < 1e-5),
               color = 'orange',
               size=2)
```



ggplot tours Manhattan

Plotting

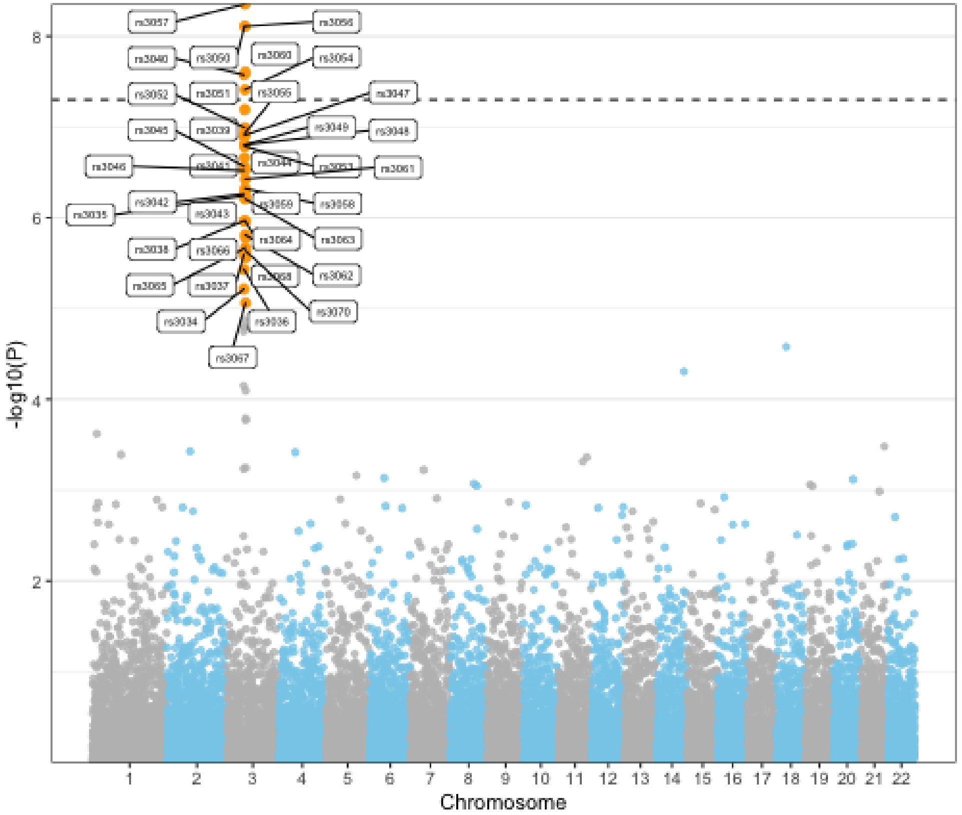
```
(plt_manhattan <- ggplot(plt_dat,
       aes(x = BPcumul, y = -log10(P)))+
    geom_point(aes(color = as.factor(CHR)),
               alpha = 0.8, size=1.3) +
    scale_color_manual(
        values = rep(c('grey','skyblue'), 22))+
    scale_x_continuous(
        name = 'Chromosome',
        breaks = axisdf$center,
        labels = axisdf$CHR
    ) +
    scale_y_continuous(expand = c(0,0)) +
    theme_bw() +
    geom_point(data = plt_dat %>% filter(P < 1e-5),
               color = 'orange',
               size=2)+
    geom_hline(yintercept = -log10(5e-8), linetype=2)
    theme(legend.position='none',
          panel.grid.major.x=element_blank(),
          panel.grid.minor.x = element_blank())
)
```



ggplot tours Manhattan

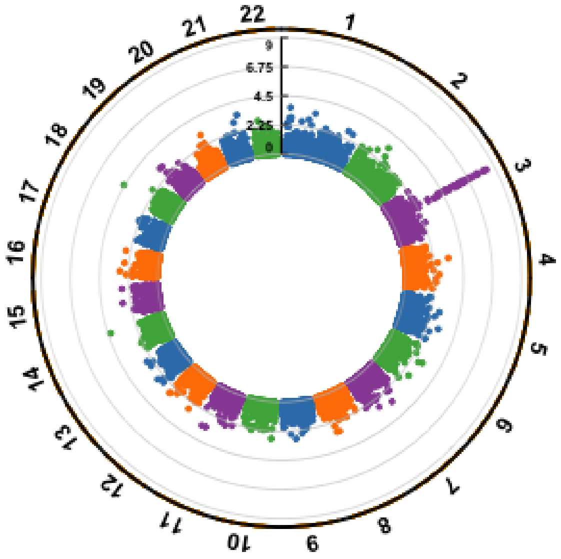
Annotation

```r
library(ggrepel)
plt_manhattan +
    geom_label_repel(
        data = plt_dat %>% filter(P < 1e-5),
        aes(label = SNP),
        size = 2)
```



Circular Manhattan plots

```r
library(CMplot)
CMplot(gwasResults, plot.type = 'c',
       r = 1.6,
       cir.legend = T,
       outward=T,
       cir.legend.col='black',
       cir.chr.h=.1,
       chr.den.col='orange',
       file.output=F)
```



```
#>  [1] "Circular_Manhattan Plotting P..."
```

Heatmaps

Let us count the ways

There are several ways of doing heatmaps in R:

- http://sebastianraschka.com/Articles/heatmaps_in_r.html
- https://plot.ly/r/heatmaps/
- http://moderndata.plot.ly/interactive-heat-maps-for-r/
- http://www.siliconcreek.net/r/simple-heatmap-in-r-with-ggplot2
- https://rud.is/b/2016/02/14/making-faceted-heatmaps-with-ggplot2/

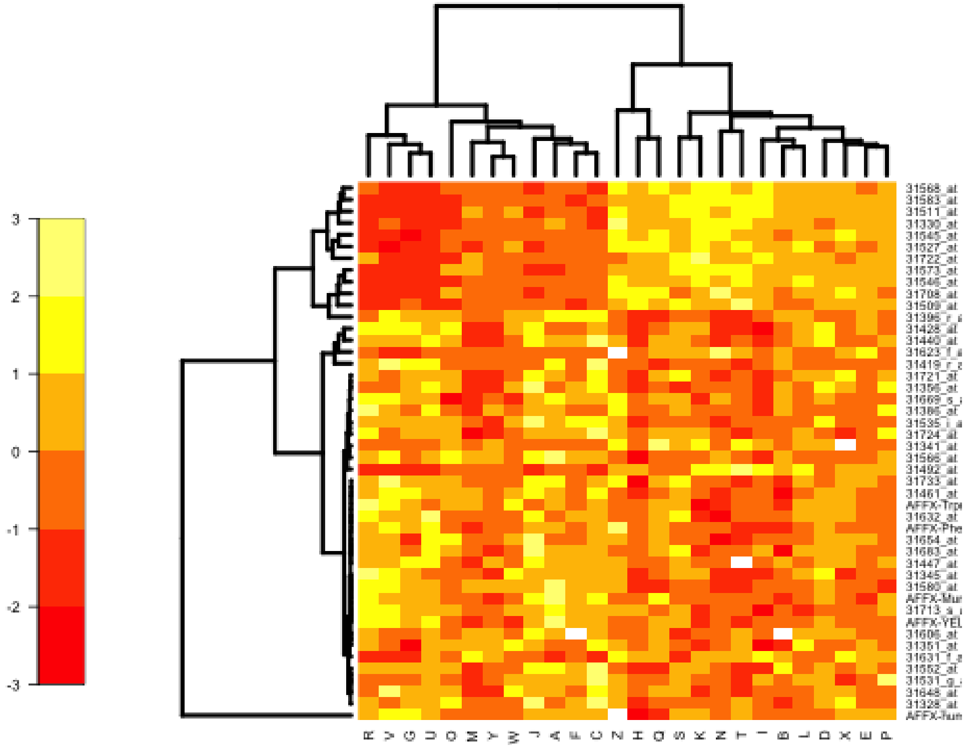
Some example data

```r
library(Biobase)
#data(sample.ExpressionSet)
exdat <-  readRDS('data/exprset.rds')
library(limma)
design1 <- model.matrix(~type, data=pData(exdat))
lm1 <- lmFit(exprs(exdat), design1)
lm1 <- eBayes(lm1) # compute linear model for each probeset
geneID <- rownames(topTable(lm1, coef = 2, number = 100,
                            adjust.method = 'none',
                            p.value = 0.05))
exdat2 <- exdat[geneID,] # Keep features with p-values < 0.05

head(exdat2)
```

```
#>  ExpressionSet (storageMode: lockedEnvironment)
#>  assayData: 1 features, 26 samples
#>    element names: exprs, se.exprs
#>  protocolData: none
#>  phenoData
#>    sampleNames: A B ... Z (26 total)
#>    varLabels: sex type score
#>    varMetadata: labelDescription
#>  featureData: none
#>  experimentData: use 'experimentData(object)'
#>  Annotation: hgu95av2
```

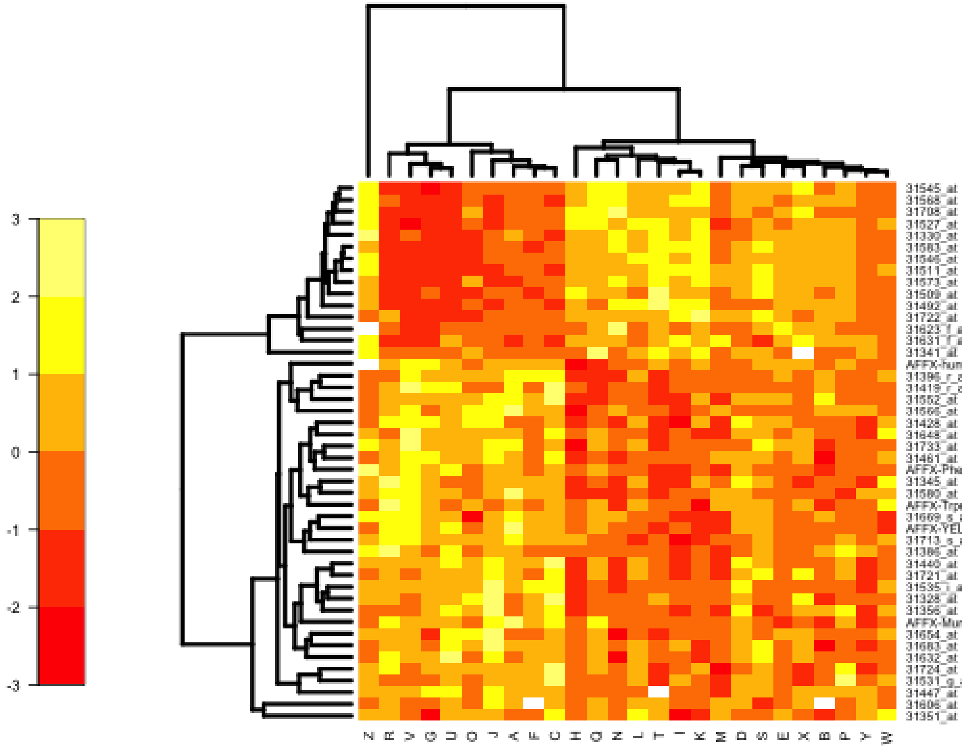
Using Heatplus

```
# BiocManager::install('Heatplus')
library(Heatplus)
reg1 <- regHeatmap(exprs(exdat2), legend=2, col=heat.
                   breaks=-3:3)
plot(reg1)
```



Using Heatplus

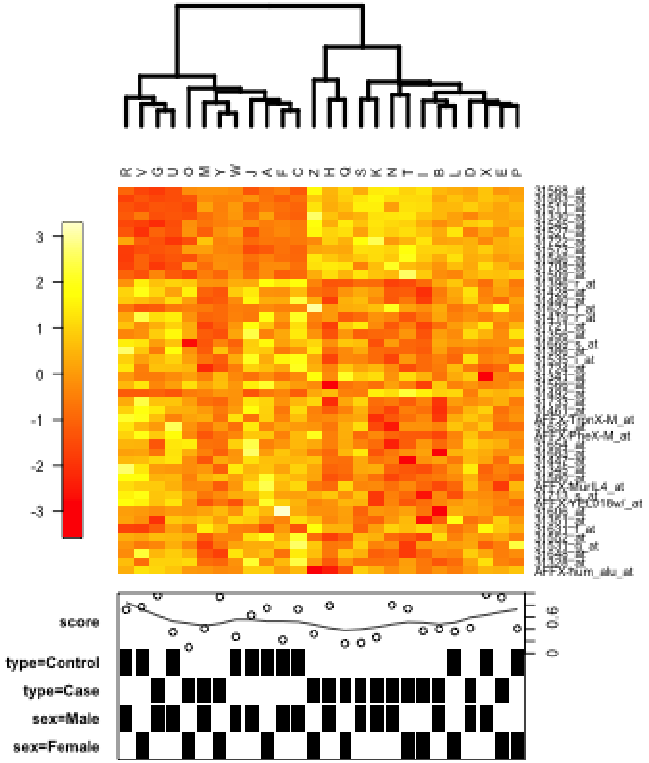
```
corrdist <- function(x) as.dist(1-cor(t(x)))
hclust.avl <- function(x) hclust(x, method='average')
reg2 <- regHeatmap(exprs(exdat2), legend=2,
                   col=heat.colors,
                   breaks=-3:3,
                   dendrogram =
                       list(clustfun=hclust.avl,
                            distfun=corrdist))
plot(reg2)
```



Using Heatplus

Adding annotations

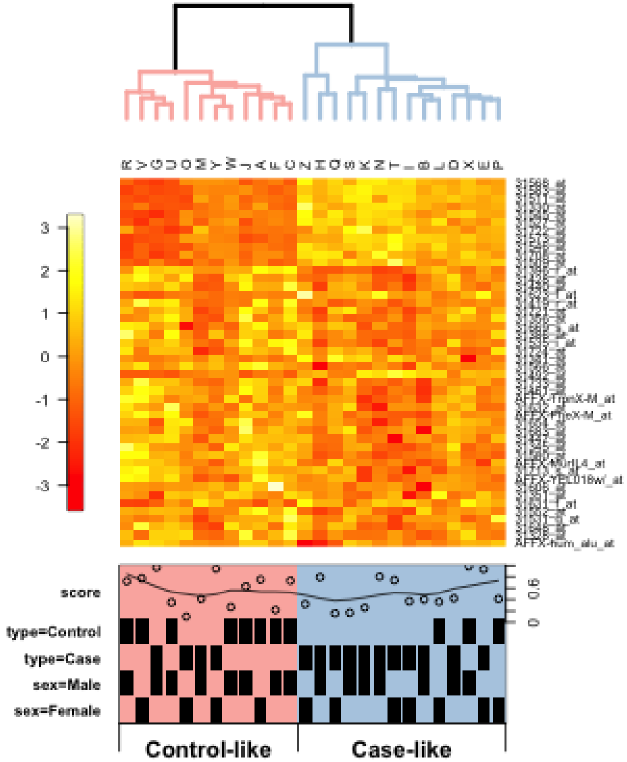
```
ann1 <- annHeatmap(exprs(exdat2),
                   ann=pData(exdat2),
                   col = heat.colors)
plot(ann1)
```



Using Heatplus

Adding annotations

```
ann2 <- annHeatmap(exprs(exdat2),
                   ann=pData(exdat2),
                   col = heat.colors,
        cluster =
            list(cuth=7500,
                 label=c('Control-like','Case-like'))
plot(ann2)
```



Playing with Seurat

Example data

```
library(Seurat)
# pbmc.data <- Read10X(data.dir='data/hg19/')
# pbmc <- CreateSeuratObject(counts = pbmc.data, project='pbmc3k', min.cells=3, min.features=200)
pbmc <- readRDS('data/pbmc.rds')
pbmc
```

```
#>  An object of class Seurat
#>  13714 features across 2700 samples within 1 assay
#>  Active assay: RNA (13714 features)
```

```
names(pbmc)
```

```
#>  [1] "RNA"
```

```
slotNames(pbmc)
```

```
#>   [1] "assays"       "meta.data"    "active.assay" "active.ident"
#>   [5] "graphs"       "neighbors"    "reductions"   "project.name"
#>   [9] "misc"         "version"      "commands"     "tools"
```

Adding QC metrics and plotting

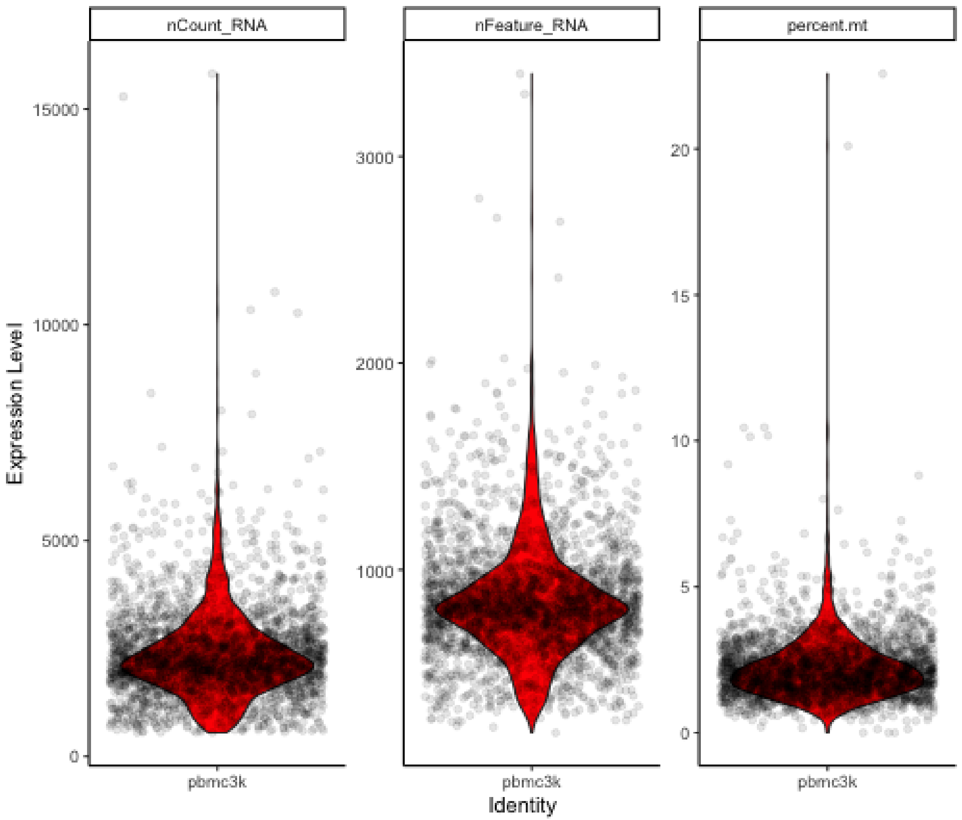
We'll calculate mitochondrial QC metrics (percentage counts originating from mitochondrial genes)

```
pbmc[['percent.mt']] <- PercentageFeatureSet(pbmc, pattern = '^MT-')
head(pbmc@meta.data)
```

```
#>                orig.ident nCount_RNA nFeature_RNA percent.mt
#> AAACATACAACCAC     pbmc3k       2419          779  3.0177759
#> AAACATTGAGCTAC     pbmc3k       4903         1352  3.7935958
#> AAACATTGATCAGC     pbmc3k       3147         1129  0.8897363
#> AAACCGTGCTTCCG     pbmc3k       2639          960  1.7430845
#> AAACCGTGTATGCG     pbmc3k        980          521  1.2244898
#> AAACGCACTGGTAC     pbmc3k       2163          781  1.6643551
```

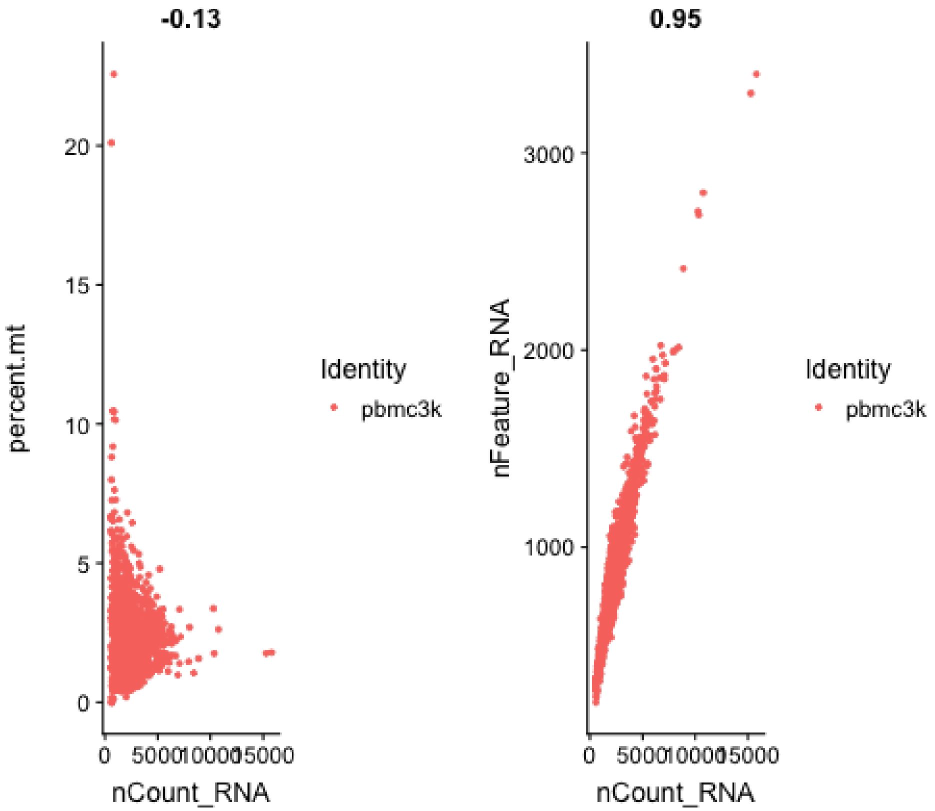
Visualizing metrics

```
# plt <- VlnPlot(object = pbmc,
#   features = c('nFeature_RNA',
#                'nCount_RNA',
#                'percent.mt'))

plot_data <- pbmc@meta.data %>%
  tidyr::gather(variable, value, -orig.ident)

ggplot(plot_data, aes(orig.ident, value)) +
  geom_violin(fill = 'red') +
  geom_jitter(width=0.5, alpha = 0.1) +
  facet_wrap(~variable, nrow = 1,
             scales = 'free_y') +
  labs(x = 'Identity',y = 'Expression Level') +
  theme_classic()
```



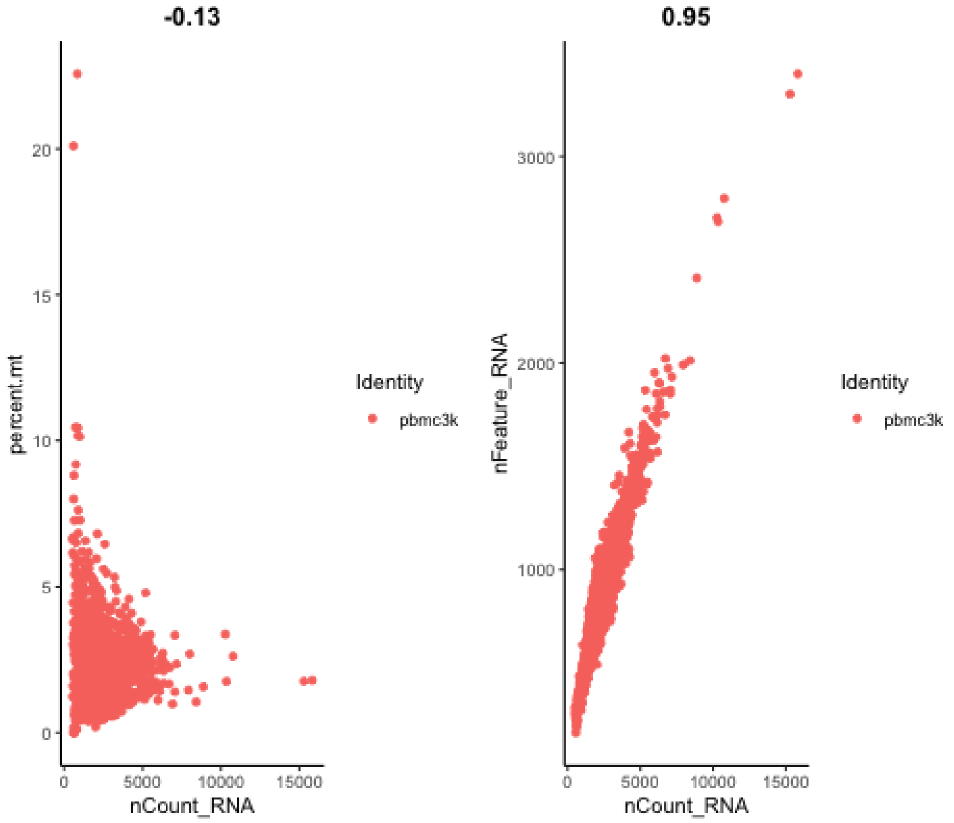
Visualizing feature-feature relationships

```r
plot1 <- FeatureScatter(object = pbmc,
                        feature1 = "nCount_RNA",
                        feature2 = "percent.mt")
plot2 <- FeatureScatter(object = pbmc,
                        feature1 = "nCount_RNA",
                        feature2 = "nFeature_RNA")
CombinePlots(plots = list(plot1, plot2))
```



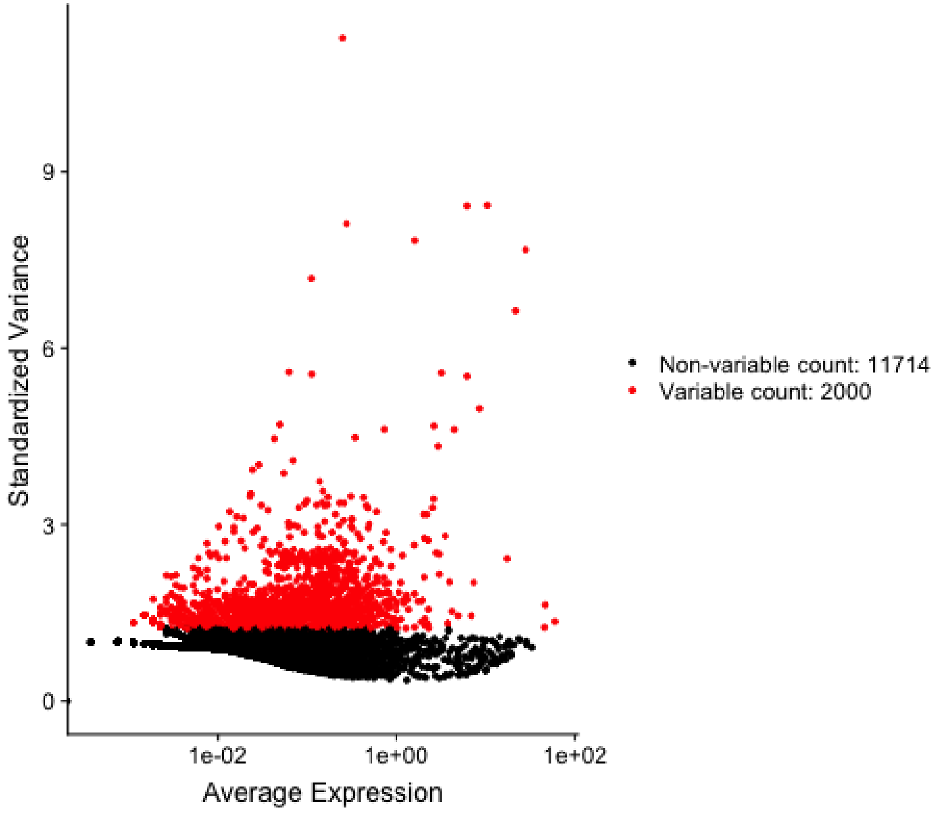
Visualizing feature-feature relationships

```
cormatrix <- cor(pbmc@meta.data %>% select(-orig.iden
plt1 <-
  ggplot(pbmc@meta.data,
         aes(x = nCount_RNA,
             y = percent.mt,
             group = orig.ident,
             color = orig.ident)) +
  geom_point() +
    theme_classic() +
    labs(color = 'Identity',
         title=as.character(round(cormatrix['nCount_R
  theme(plot.title = element_text(face = 'bold', hjus

plt2 <-
  ggplot(pbmc@meta.data,
         aes(x = nCount_RNA,
             y = nFeature_RNA,
             group = orig.ident,
             color = orig.ident)) +
  geom_point() +
  theme_classic() +
  labs(color = 'Identity',
       title=as.character(round(cormatrix['nCount_RNA
  theme(plot.title = element_text(face = 'bold', hjus

ggpubr::ggarrange(plt1, plt2, nrow = 1, ncol=2)
```



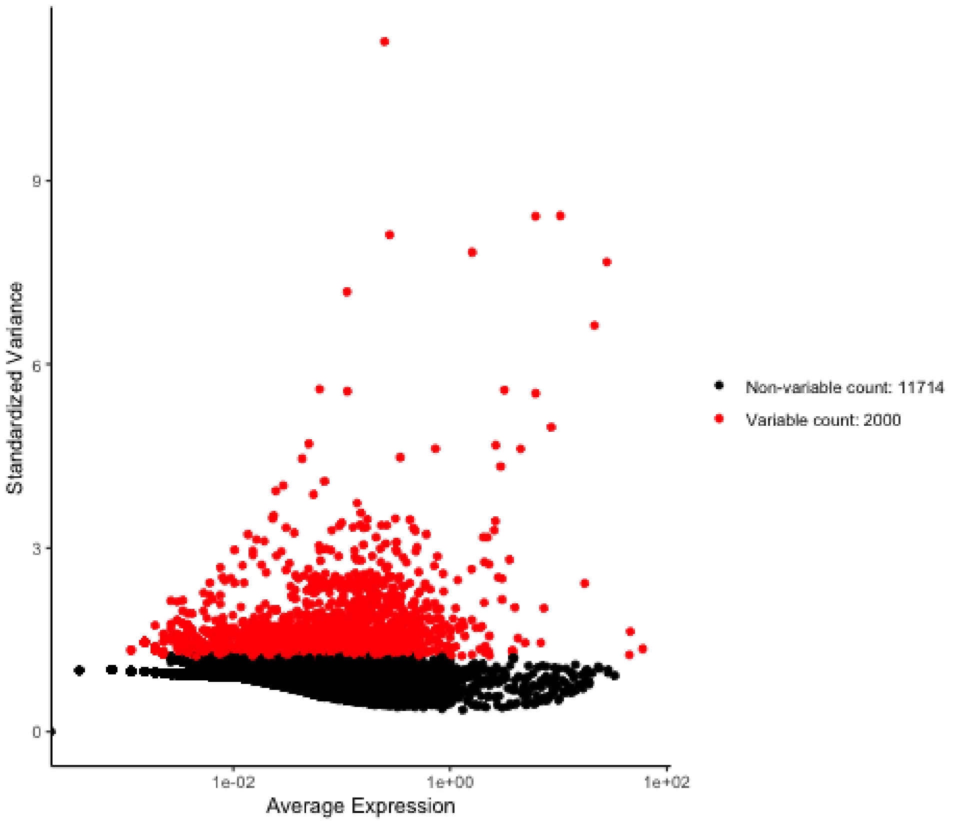
Feature selection

```
pbmc <- subset(x = pbmc,
    subset = nFeature_RNA > 200 & nFeature_RNA < 2500
pbmc <- NormalizeData(object = pbmc,
                      normalization.method = "LogNorm
                      scale.factor = 10000)
# This is stored in pbmc[['RNA']]@meta.features

pbmc <- FindVariableFeatures(object = pbmc,
                             selection.method = "vst"
                             nfeatures = 2000)


# Identify the 10 most highly variable genes
top10 <- head(x = VariableFeatures(object = pbmc), 10

# plot variable features with and without labels
plot1 <- VariableFeaturePlot(object = pbmc)
plot1
```



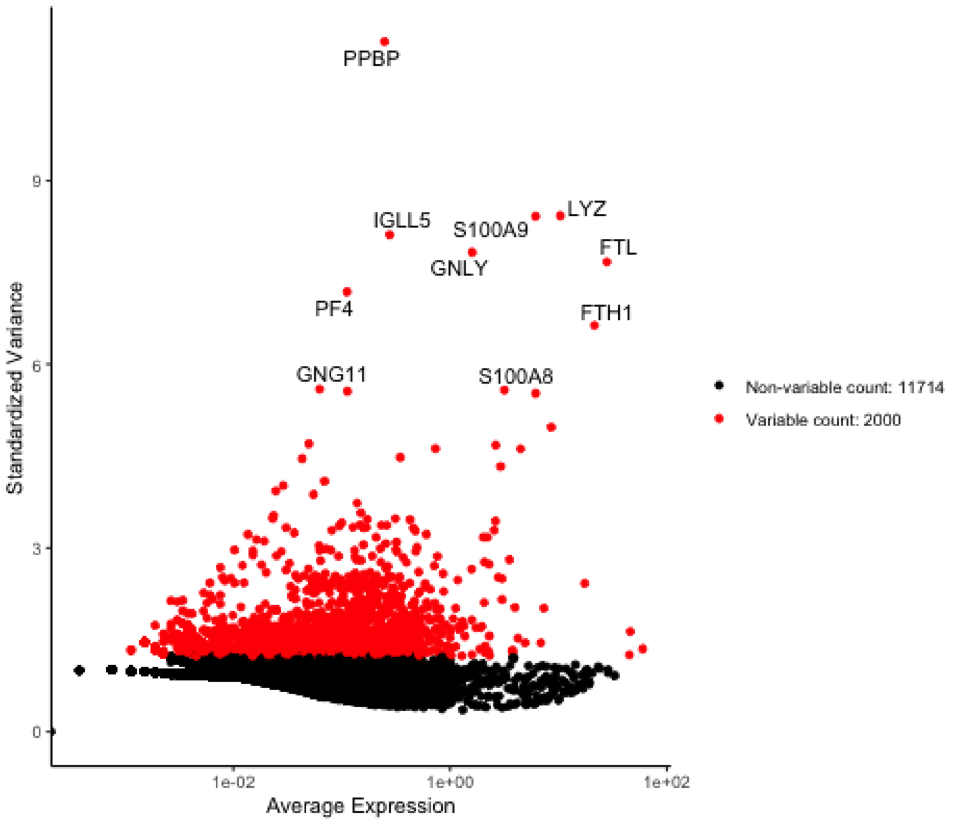
Feature selection

```
plt_data <- pbmc[['RNA']]@meta.features %>%
    rownames_to_column(var='id')
topvars <- pbmc[['RNA']]@var.features
plt_data <- plt_data %>%
    mutate(indic = ifelse(id %in% topvars,
                          'Variable count',
                          'Non-variable count'))
bl <- plt_data %>%
    count(indic) %>%
    glue::glue_data("{indic}: {n}")
names(bl) <- c('Non-variable count','Variable count')
plt_data <- plt_data %>%
  mutate(indic = bl[indic])
plt11 <- ggplot(plt_data,
                aes(x = mean,
                    y = variance.standardized,
                    color = indic)) +
  geom_point() +
  scale_x_log10() +
  scale_color_manual(values = c('black','red')) +
  labs(x = 'Average Expression', y = 'Standardized Va
  theme_classic()
plt11
```



Feature selection

```
# plot2 <- LabelPoints(plot = plot1, points = top10,
plt12 <- plt11 + ggrepel::geom_text_repel(data = plt_
                                          aes(label =
                                          color = 'bl
plt12
```



There's a lot more

We'll stop our sampling here.

- Many Bioconductor packages do use ggplot, however some use base graphics
    - Faster
- Key is to find where the data is stored, and use that to create visualizations
- Bioconductor tends to create
    - One monolithic object
    - Containing different information in slots
    - combined by lists
- `slotNames` and `names` are your friends