# Visualization in different applications

Abhijit Dasgupta, PhD

# Goals

1. Visualization in biomedical applications and models
2. Visualizing spatial data using maps
3. Bioinformatics

# Biomedical applications

# Survival analysis

# Survival analysis

Survival analysis is the analysis of time-to-event data. In biomedical research, *event* can mean

1. death
2. recurrence or relapse (cancer, infection)
3. equipment failure (pacemakers, orthopedic implants)

You need two pieces of information:

1. the time of the event or the time you last saw the subject, and
2. the status (dead/alive) the last time you saw the subject. If subject is alive, you call that observation *censored*, since you didn't observe the subject long enough to see the event happen.

# Survival analysis

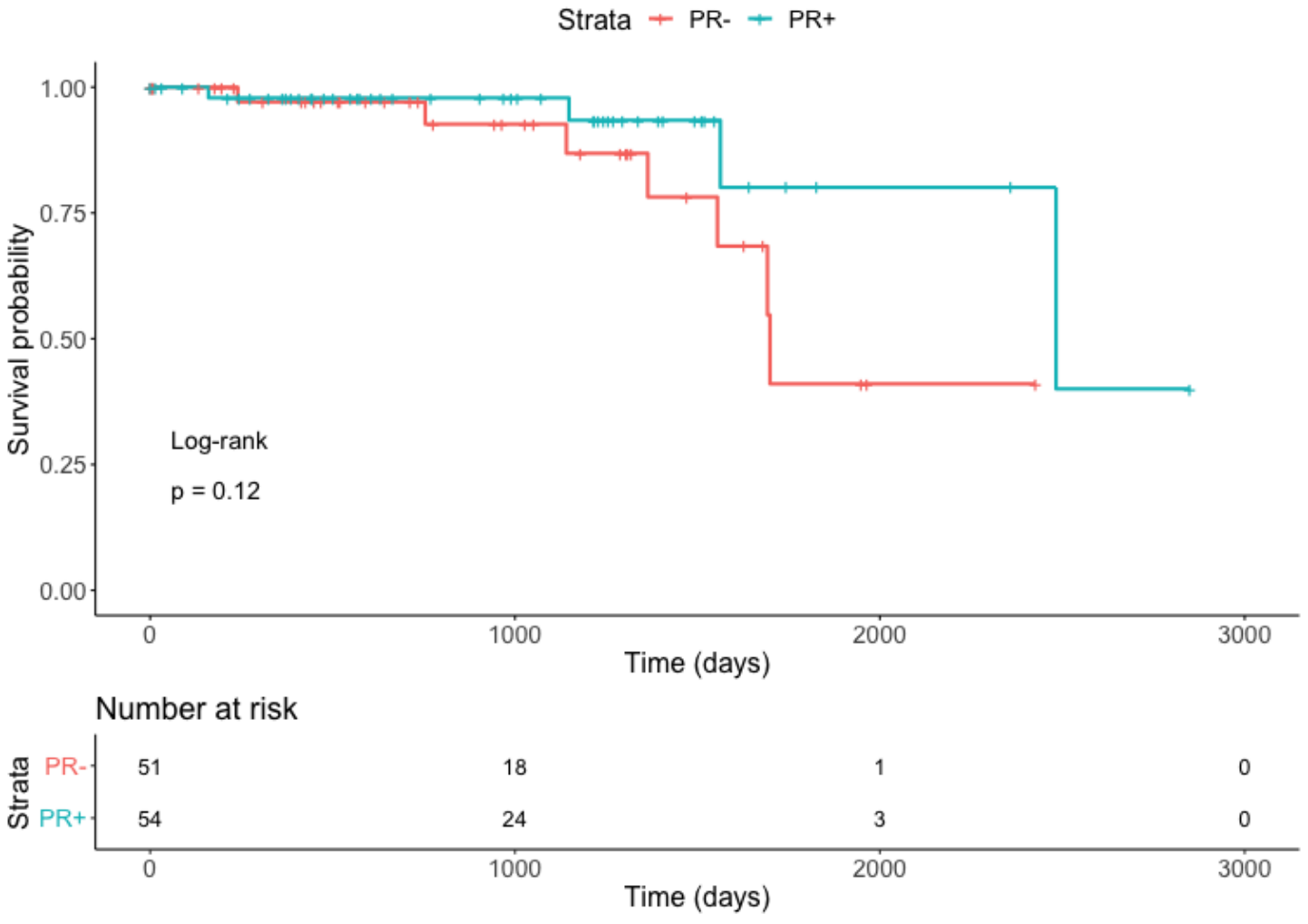
The basic graphical tool in survival analysis is the *Kaplan-Meier curve*, which shows the proportion of subjects **still surviving** at any given time, with 100% alive at time 0.

The Kaplan-Meier curve is computed using the function `survival::survfit` and uses the same formula interface as usual models, with one little quirk.

```
library(survival)
brca <- readxl::read_excel('data/BreastCancer_Clinical.xlsx', .name_repair = 'universal')
fit <- survfit(Surv(OS.Time, OS.event) ~ PR.Status, data = brca)
```

We have a composite dependent variable, comprising both the time and status of each event. It is encapsulated in the function `Surv` (note capitalization)

# Kaplan-Meier curve




- Small ticks are times when subjects are censored
- The log-rank test tests if the stratified curves are statistically different
- The risk table gives the number still alive at different times.

# Kaplan-Meier curve

The plot is generated by the following code:

```
library(survminer)
(plt <- ggsurvplot(fit, pval=TRUE, pval.method = TRUE, risk.table=TRUE,
                   legend.labs = c('PR-','PR+'),
                   xlab = 'Time (days)'))
```

- The actual plot can be accessed using `plt$plot` and is a regular **ggplot2** object
- The table can be accessed using `plt$table` and is *also* a **ggplot2** object

There is a lot of customization of these plots that are possible. See https://rpkgs.datanovia.com/survminer/index.html for tutorials and details.

# Regression results

# Displaying the results of a regression model

We're used to seeing the results of a regression analysis in tables, but graphically displaying them may make them easier to understand.

We'll use a toy example using the `mpg` data.

We fit a linear regression model using

```
mpg <- mpg %>%
  mutate(year = as.factor(year)) %>%
  mutate(trans = str_extract(trans, '[:alnum:]+')) %>%
  mutate(cyl = as.factor(cyl))
fit <- lm(cty ~ year + trans + cyl + drv + class, data=mpg)
```

This models city mpg against year, type of transmission, number of cylinders, type of drive, and class of car.

# Displaying the results of a regression model

As a table, these results look like

```
knitr::kable(broom::tidy(fit))
```

| term | estimate | std.error | statistic | p.value |
|---|---|---|---|---|
| (Intercept) | 20.5622426 | 1.1227750 | 18.3137694 | 0.0000000 |
| year2008 | 0.4624090 | 0.2712424 | 1.7047815 | 0.0896468 |
| transmanual | 0.4024746 | 0.3106162 | 1.2957299 | 0.1964264 |
| cyl5 | -2.1058003 | 1.0607113 | -1.9852718 | 0.0483563 |
| cyl6 | -3.4162456 | 0.3620780 | -9.4351106 | 0.0000000 |
| cyl8 | -5.5938201 | 0.4951472 | -11.2972877 | 0.0000000 |
| drvf | 2.5030933 | 0.4872506 | 5.1371787 | 0.0000006 |
| drvr | -0.0873527 | 0.5793938 | -0.1507656 | 0.8802988 |
| classcompact | -1.6774636 | 1.1124558 | -1.5078924 | 0.1330167 |
| classmidsize | -2.2914265 | 1.1438440 | -2.0032683 | 0.0463754 |
| classminivan | -4.3516621 | 1.2936355 | -3.3639014 | 0.0009065 |

Obviously this needs cleaning up

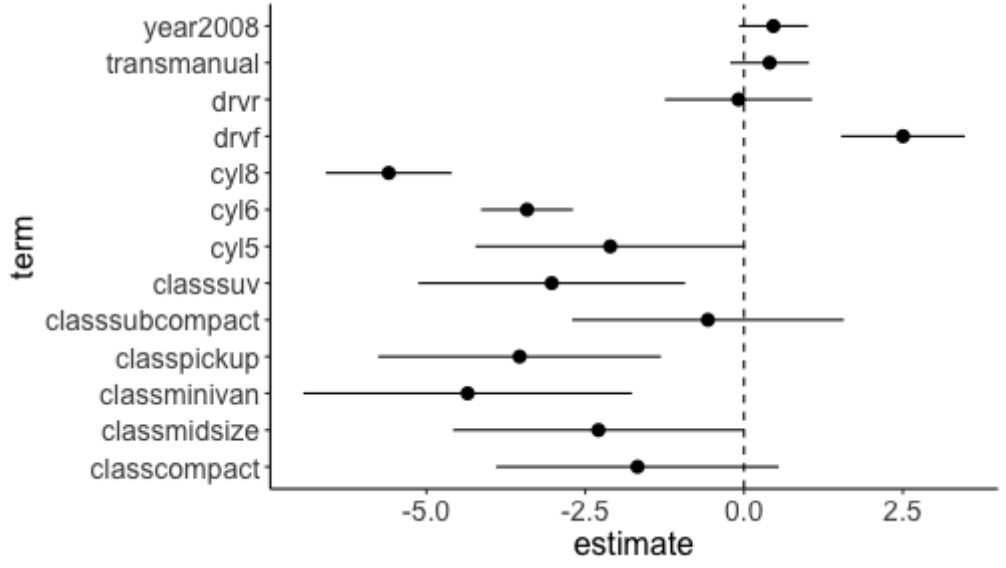
We'd fix the terms, and reduce the number of decimal places to 2

The point is, it's relatively hard to understand the relative effects of different levels.

# Displaying the results of a regression model

As a figure, it's probably a bit better

```
results = broom::tidy(fit) %>% filter(term !="(Intercept)")
ggplot(results)+
  geom_pointrange(aes(x=term, y=estimate,
                      ymin = estimate-2*std.error, ymax=estimate+2*std.error))+
  geom_hline(yintercept=0, linetype=2) +
  coord_flip() +
  theme_439
```




We plot the estimate and 95% confidence interval for each term

Provide reference line of 0

Can see increasing and decreasing patterns within variable levels

We'll work on cleaning this picture up in a tutorial

# A learning note

You've been gaining some experience in creating graphics.

understanding what the different parts are doing

I expect you to work on parsi that code and

e. So developing the skill of understanding other people's code is really useful and somewhat important.

Reach out to me if it really feels mysterious and arcane. I'm not teaching mystical arts here.

# Logistic regression

We'll use the `PimaIndianDiabetes2` data from the **mlbench** package

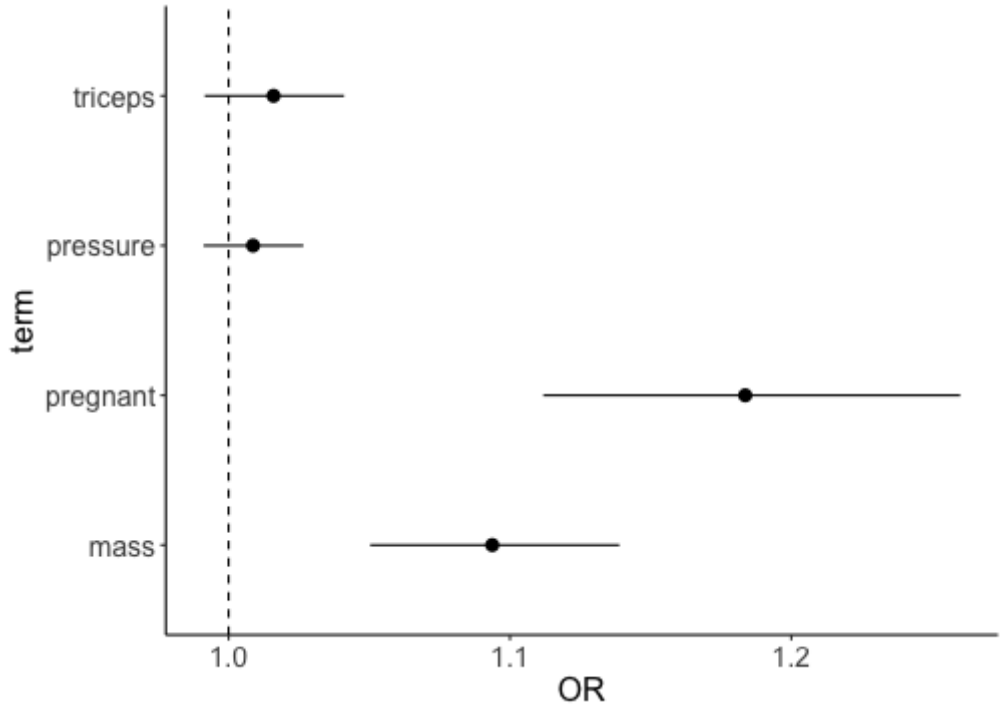
```
library(mlbench); data("PimaIndiansDiabetes2") # 2 commands separated by ;
fit_logistic <- glm(diabetes ~ pregnant + pressure + triceps + mass,
                    data = PimaIndiansDiabetes2,
                    family='binomial') # This option makes this logistic regression
```

The unifying step to displaying the results of most regression modeling is `broom::tidy`. We'll transform the results a bit, since logistic regression provides log-odds ratios and we will display the odds ratios.

```
results <- broom::tidy(fit_logistic)
results <- results %>%
  mutate(OR = exp(estimate),
         LCB = exp(estimate - 2*std.error),
         UCB = exp(estimate + 2*std.error)) %>%
  filter(term != '(Intercept)')
```

# Logistic regression

```
ggplot(results)+
  geom_pointrange(aes(x = term, y = OR, ymin = LCB, ymax = UCB))+
  geom_hline(yintercept = 1, linetype=2)+
  coord_flip()
```




The graph makes it clear that number of pregnancies and body mass significantly influence the odds of being diabetic, but blood pressure and tricep size do not

# Cox proportional hazards regression

For survival data, Cox regression is typically used to model the *hazard rate*, i.e. the instantaneous risk of having an event.

We'll use the breast cancer dataset to do this, after some data munging

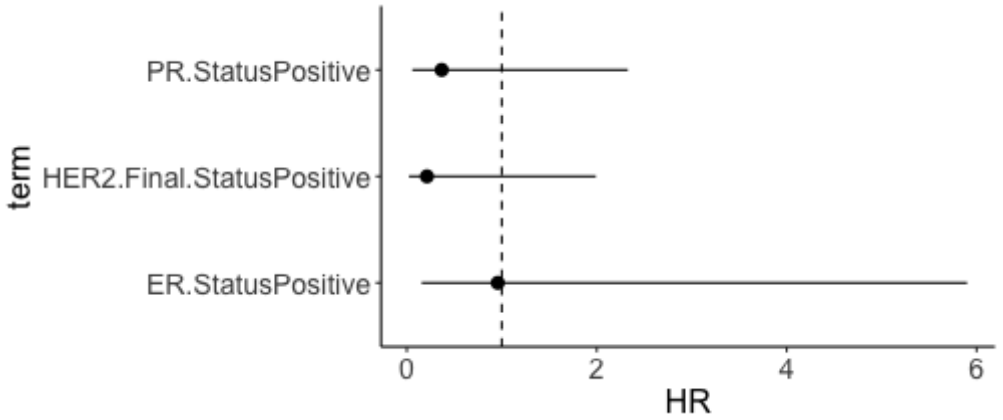
```
library(survival)
brca1 <- brca %>%
  mutate(ER.Status = ifelse(ER.Status == 'Indeterminate', NA, ER.Status),
         HER2.Final.Status = ifelse(HER2.Final.Status=='Equivocal', NA, HER2.Final.Status))
fit_cox <- coxph(Surv(OS.Time,OS.event) ~ ER.Status + PR.Status + HER2.Final.Status, data=brca1)
(results <- broom::tidy(fit_cox))
```

```
  # A tibble: 3 x 7
    term                      estimate std.error statistic p.value conf.low conf.high
    <chr>                        <dbl>     <dbl>     <dbl>   <dbl>    <dbl>     <dbl>
  1 ER.StatusPositive          -0.0428     0.909   -0.0471   0.962    -1.82     1.74
  2 PR.StatusPositive          -1.00       0.923   -1.08     0.278    -2.81     0.808
  3 HER2.Final.StatusPositive  -1.55       1.12    -1.39     0.166    -3.75     0.644
```

Notice that a Cox regression does not have an intercept term

# Cox regression

```
results <- results %>%
  mutate(HR = exp(estimate),
         LCB = exp(estimate - 2*std.error),
         UCB = exp(estimate + 2*std.error))
ggplot(results)+
  geom_pointrange(aes(x = term, y = HR,
                      ymin = LCB, ymax = UCB)) +
  geom_hline(yintercept = 1, linetype=2)+
  coord_flip()+
  theme_439
```




These results may seem counter-intuitive

One may need to do some model building to see what model fits the data well, i.e. what variables and transformed variables should be included in the model.

# Creating a function for these plots

We've basically used the same code to create the graphs in all three cases, albeit with some modest data transformations.

A general rule is that if you're using the same code more than twice, you should probably make it into a function, so you avoid copy-and-paste of code and the mistakes that engenders

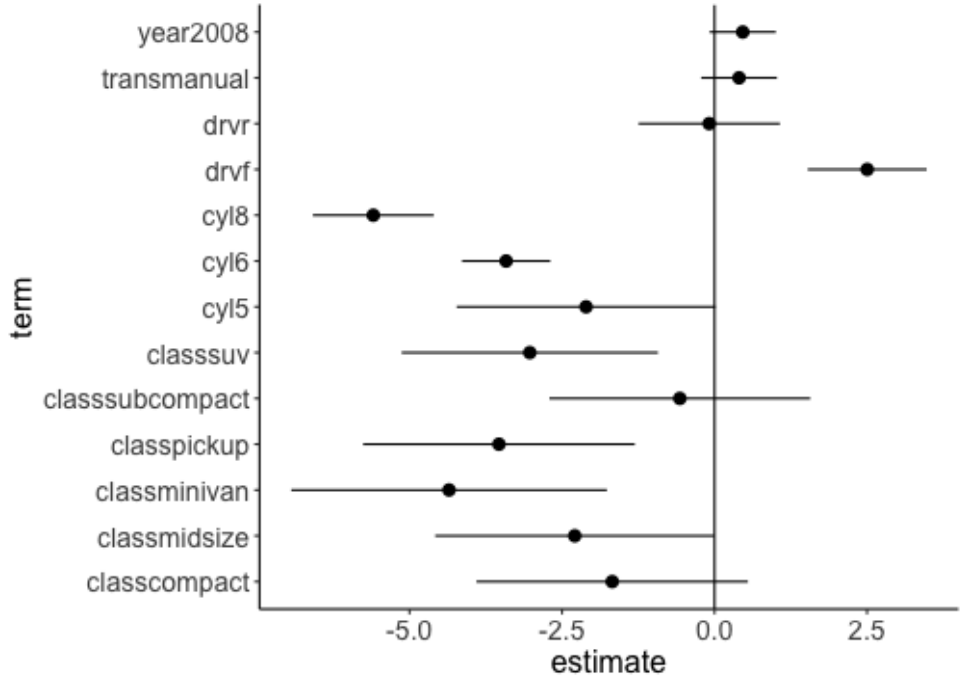
```
plt_reg_results <- function(results, reflevel=0){
  require(ggplot2)
  plt <- ggplot(results)+
    geom_pointrange(aes(x = term, y = estimate,
                        ymin = LCB, ymax = UCB))+
    geom_hline(yintercept = reflevel)+
    coord_flip()
  return(plt)
}
```

You should document what format `results` should be in, and what `reflevel` means using comments

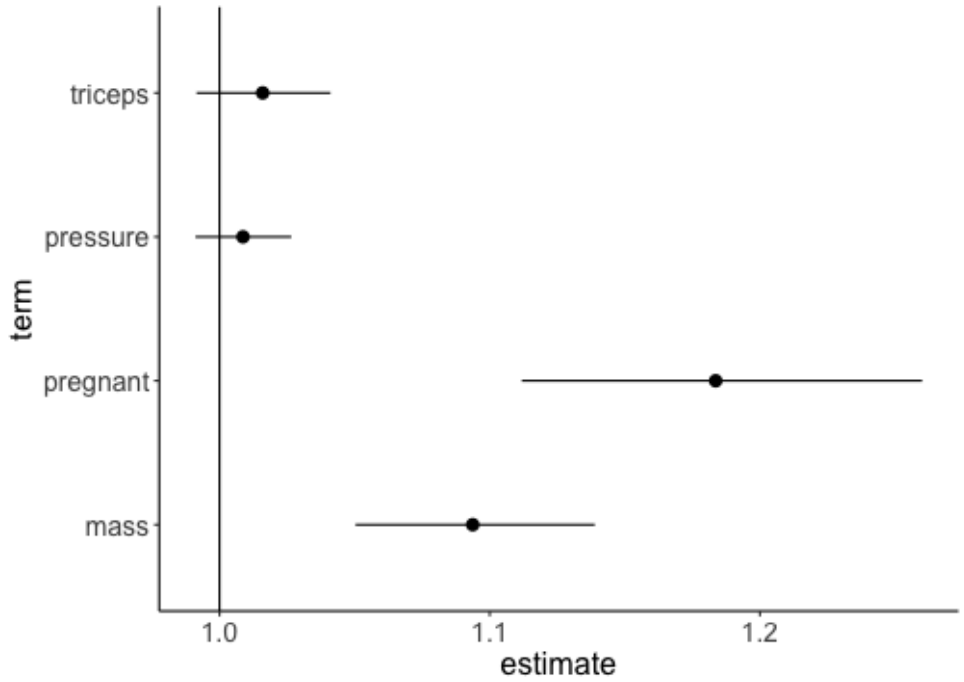
With such a function, you might need to do a bit more data munging, but creating the plots becomes a unified process.

# Creating a function for these plots

```
broom::tidy(fit) %>%
  mutate(LCB = estimate - 2*std.error,
         UCB = estimate + 2*std.error) %>%
  filter(term!='(Intercept)') %>%
  plt_reg_results()
```




```
broom::tidy(fit_logistic) %>%
  mutate(LCB = exp(estimate - 2*std.error),
         UCB = exp(estimate + 2*std.error),
         estimate = exp(estimate)) %>%
  filter(term != '(Intercept)') %>%
  plt_reg_results(reflevel=1)
```



# Maps and spatial data

Abhijit Dasgupta, PhD

# Maps

## Toolsets

Visualizing spatial and geographic data is a specialized area on its own

R has increasing capabilities in this regard, and its increasingly mature. Some of the packages that we might need are

### Data

- `sf`
- `sp`
- `raster`
- `spData`
- `rnaturalearthdata`

### Visualization

- `ggplot` + `ggmap` + `geom_sf`
- `tmap`
- `leaflet`

> Several parts of this lecture are inspired by Chapter 8 of Geocomputation with R by Lovelace, Nowosad and Muenchow (2019), also available on Amazon

## Toolset

We'll start of loading the following packages

```
library(ggplot2)
library(sf)
library(spData)
```

The **sf** package provides simple features access for R, and helps to store and process geographic data within the tidyverse framework, while linking to several geospatial packages that are standard in the geography world.

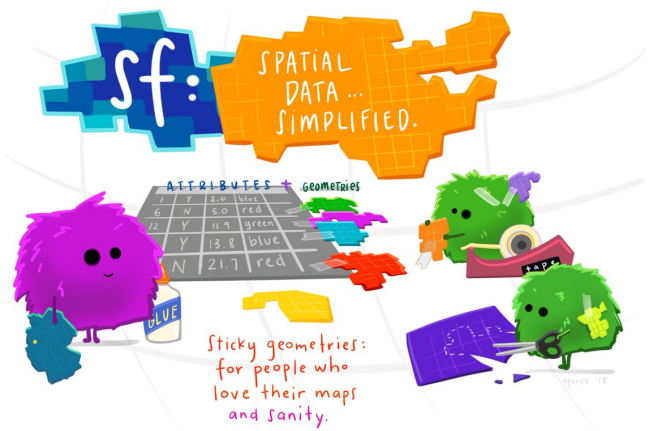


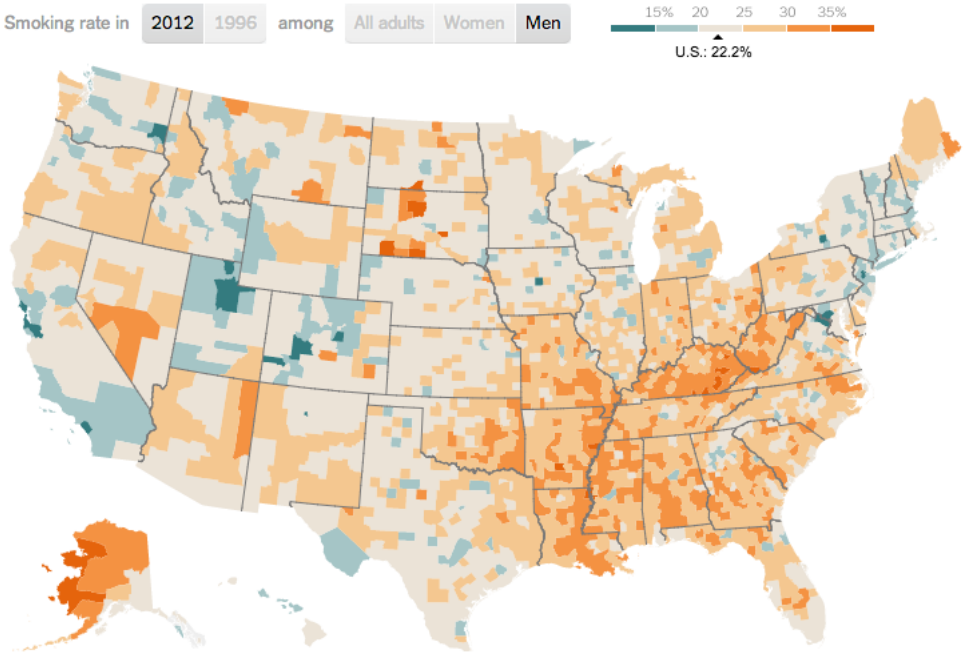

Illustration by Allison Horst

To use **sf** you may need to install some additional software. At the very least you will need to install the R packages **rgdal** and **rgeos**.

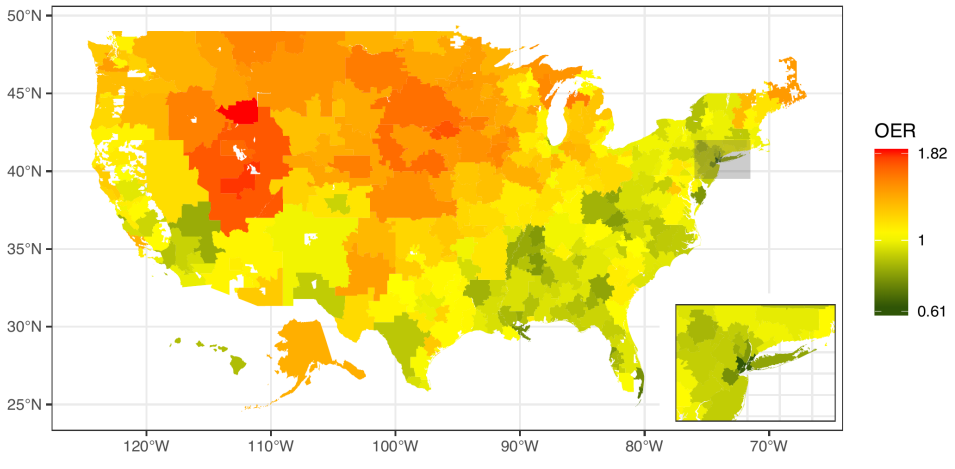
Additional information is available here

# Chloropleth maps

Chloropleth maps are maps with some geometries filled in to signify levels of some variable.




Smoking rates in USA in 2012 (NY Times, March 24, 2014)




Observed to Expected Ratios (OERs) for Rates of Primary Total Knee Arthroplasty Among White Medicare Beneficiaries by Health Referral Region (Ward & Dasgupta, 2020)

# A chloropleth of life expectancy

We'll start off with a world map

```
library(sf); library(spData)

ggplot(data = world) +
  geom_sf() # a special geometry for plotting maps
```

There are several ways of getting map geometries, which are specifications of polygons.

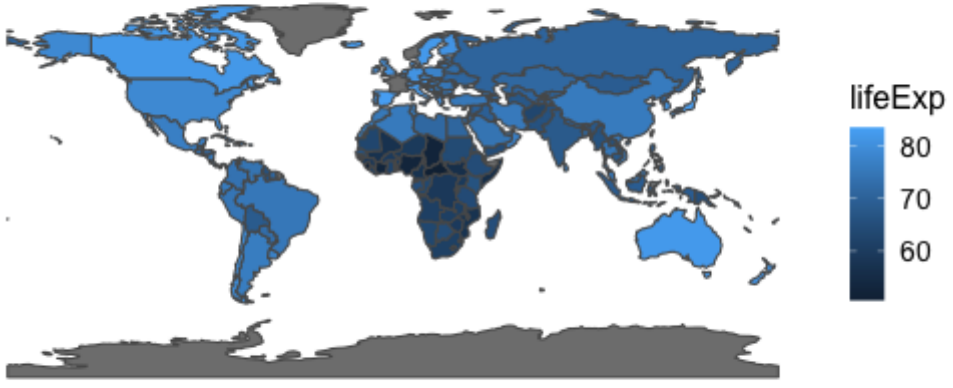
If you look at `world`, you'll see it's a data.frame, with one column named `geometry`. This column provides the shapes of the polygons, and what `geom_sf` looks for

# A chloropleth of life expectancy

If you look at `world`, it also provides life expectancy estimates from 2014 (World Bank). The data set is tidy, with one row corresponding to one country. We'll use our known **ggplot2** way of filling things in.

```
ggplot(data = spData::world) +
  geom_sf(aes(fill = lifeExp)) # a special geometry for plotting maps
```

We need a more distinctive color palette.

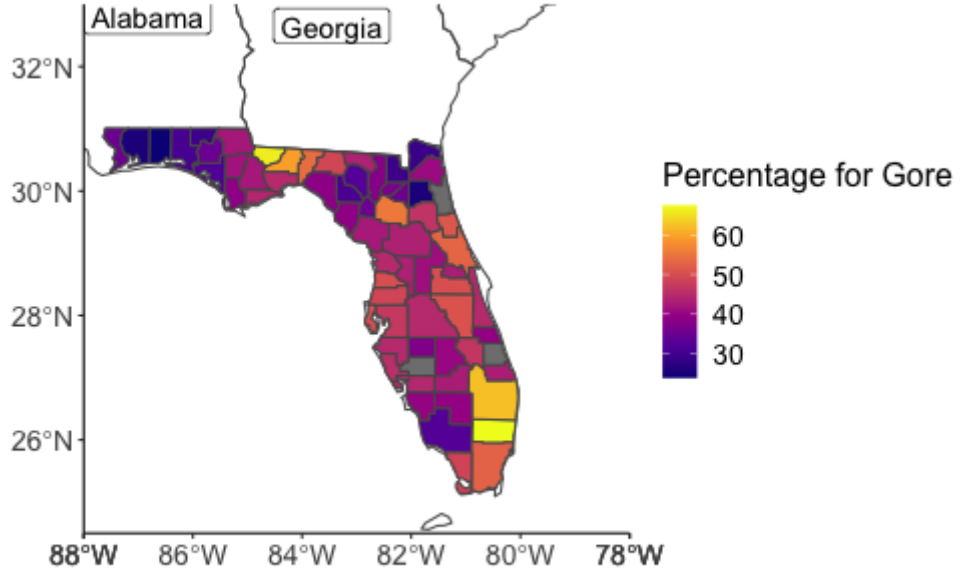


# A chloropleth of life expectancy

If you look at `world`, it also provides life expectancy estimates from 2014 (World Bank). The data set is tidy, with one row corresponding to one country. We'll use our known **ggplot2** way of filling things in.

```
ggplot(data = spData::world) +
  geom_sf(aes(fill = lifeExp)) + # a special geometry for plotting maps
  viridis::scale_fill_viridis('Life Exp', option='plasma',
                              trans='sqrt', labels = scales::label_number_si())
```

# The electoral picture in Florida, 2000



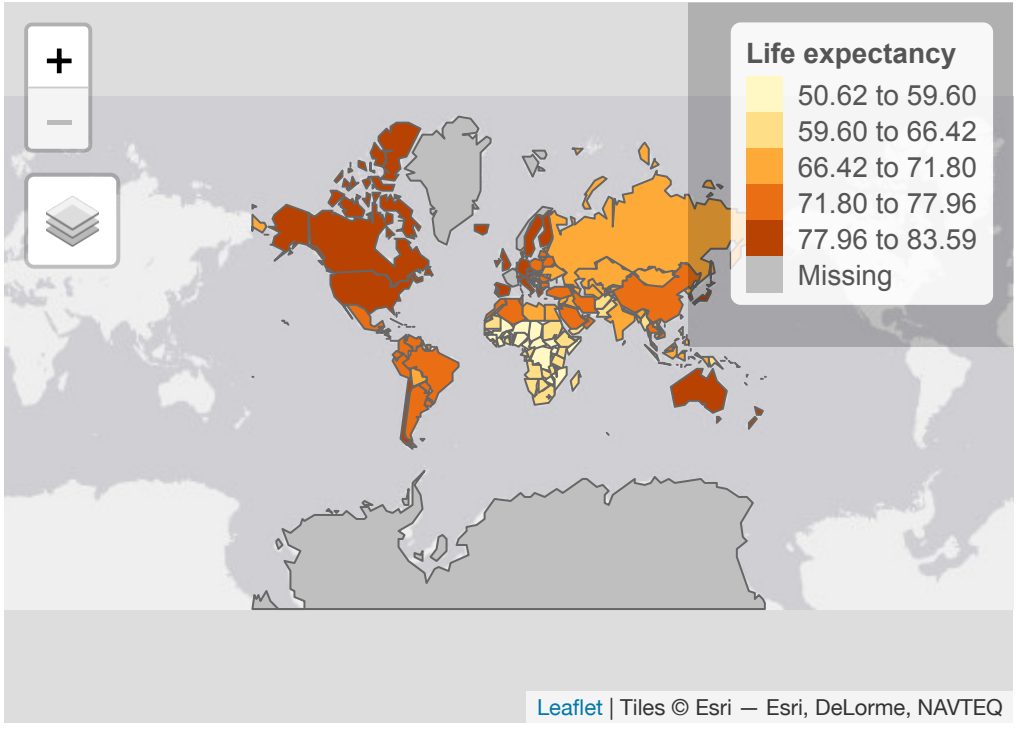

We'll develop this map in a tutorial

# Using tmap

# Using tmap

The **tmap** package uses many synactical structures similar to **ggplot2**, but can be nicer in some ways

```
library(tmap)
tm_shape(spData::world) + tm_polygons(col = "lifeExp", style='jenks',
                                      title = 'Life expectancy')
```

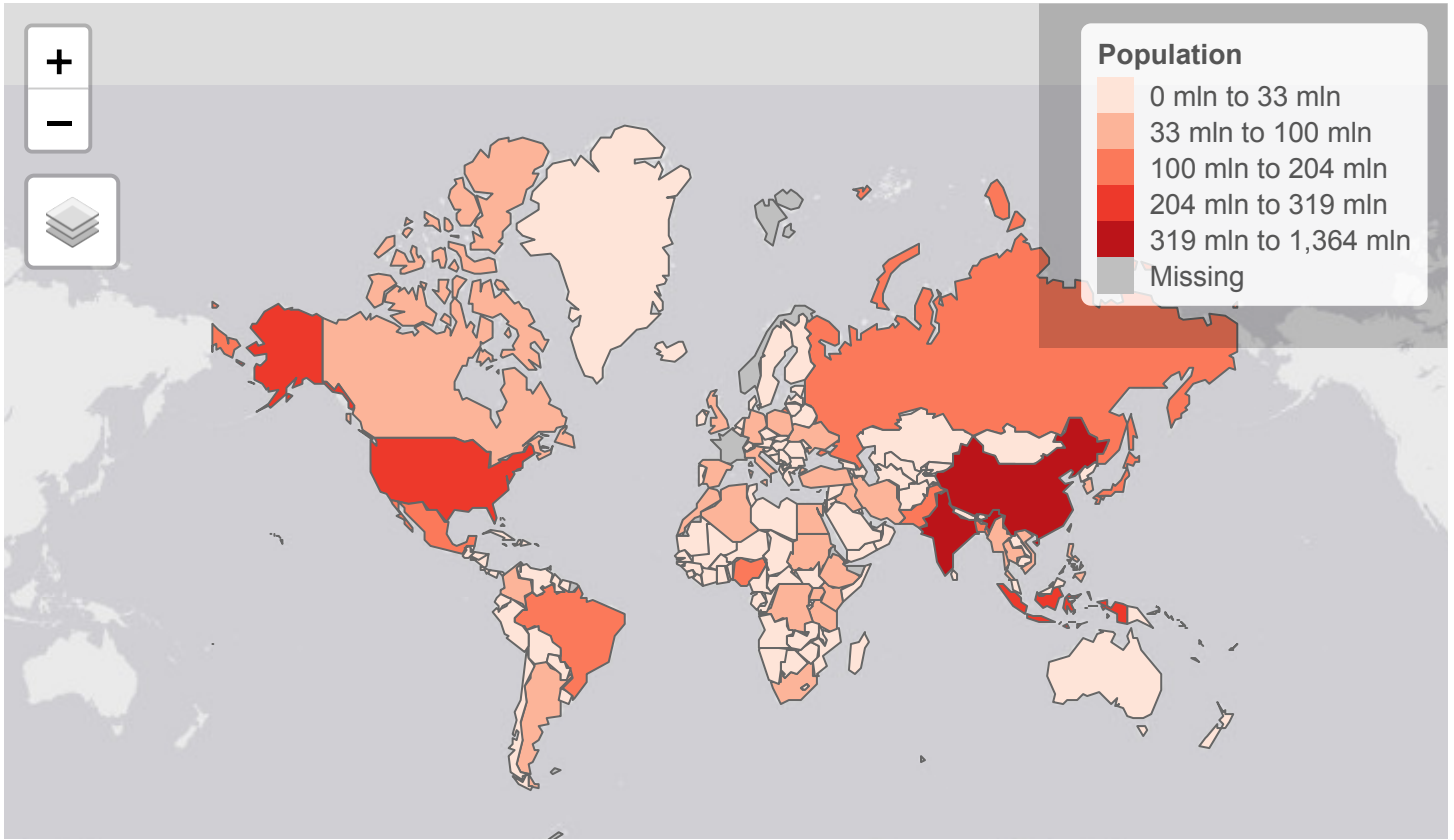



It's more "publication-ready" by default

It makes some nice choices

# tmap (interactive)

```
tmap_mode('view')
tm_shape(spData::world) + tm_polygons(col = 'pop', style='jenks',
                                      palette='Reds', title='Population',
                                      popup.vars = c('pop'), id='name_long')
```



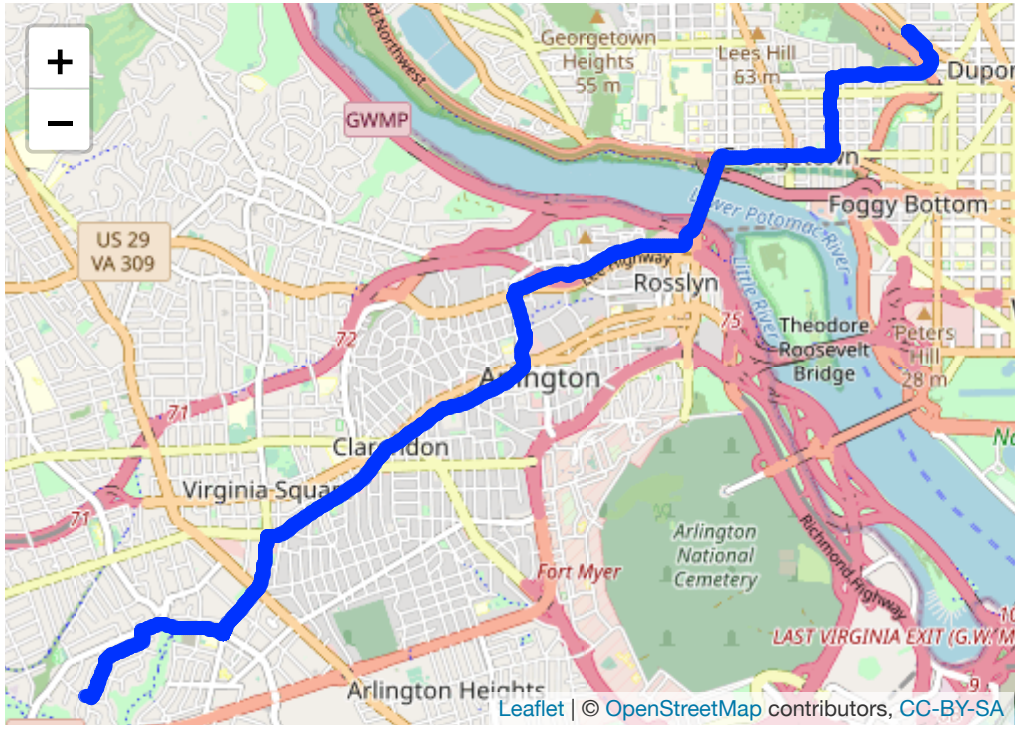

Just need one line of code for interactivity!

It gains interactivity using **leaflet**, which we'll see in a bit

# Street maps

The easiest ways to overlay data on street maps is with the **leaflet** package.

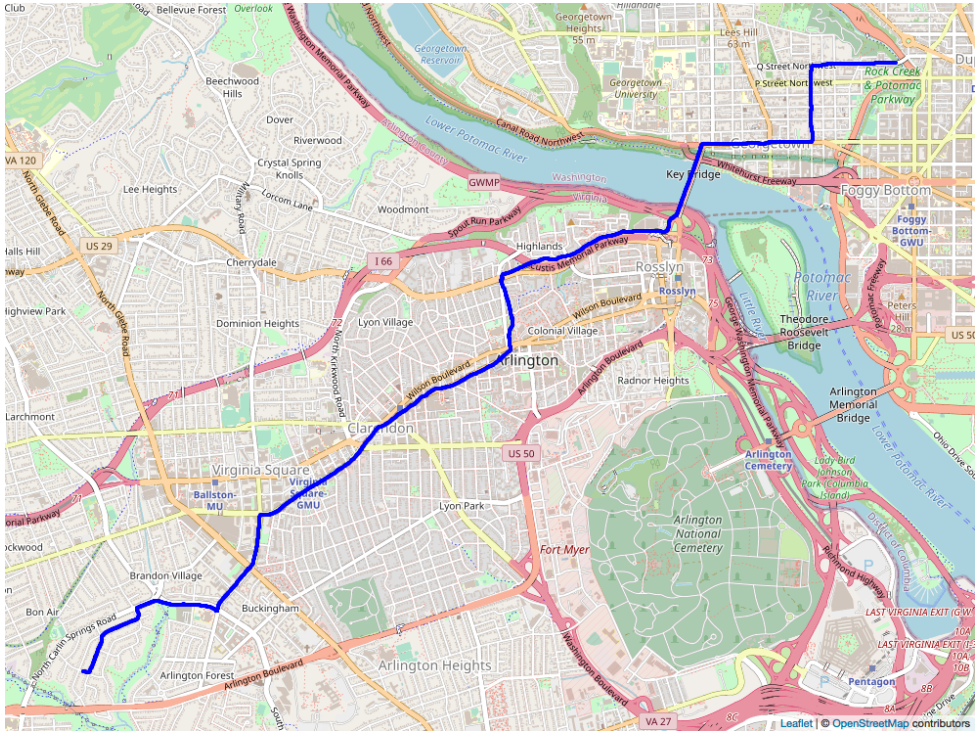
```
library(leaflet)
library(leaflet.providers)
load(file.path(here::here('slides','lectures','data', 'exdata.rda')))
leaflet(gpx) %>% addTiles() %>% addCircleMarkers(~Longitude, ~Latitude, radius=1)
```



# Street maps

You can also use the **mapview** package, which calls **leaflet** and has a bit more compact syntax

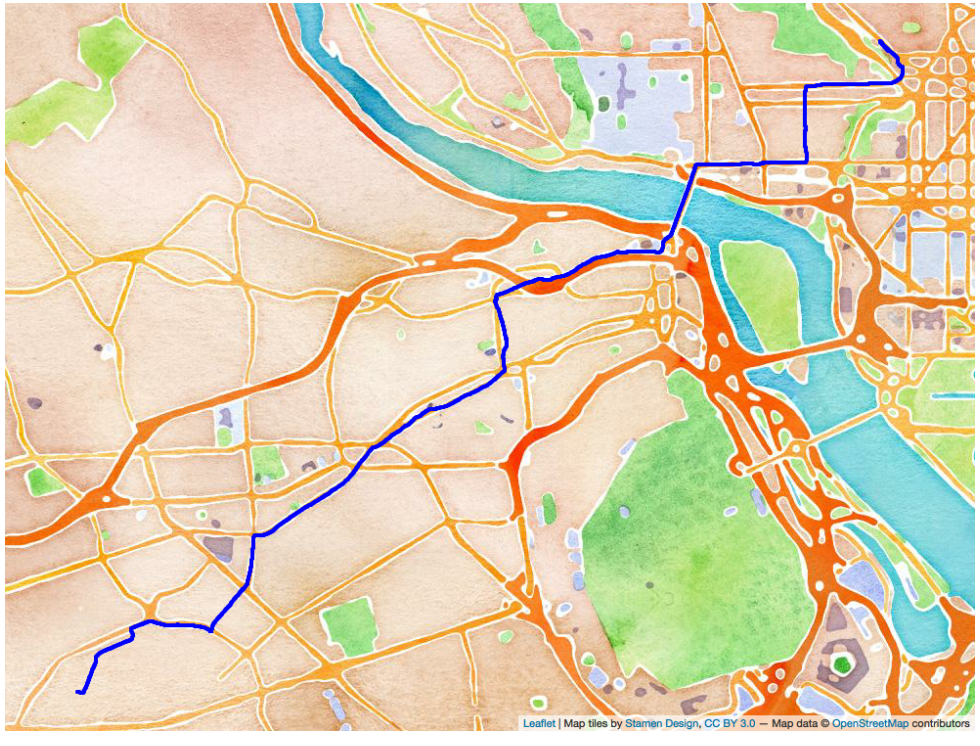
```
load(file.path(here::here('slides','lectures','data',
gpx <- sf::st_as_sf(gpx,
                    coords=c("Longitude", "Latitude"),
                    crs = 4267) # Need to make sf object
mapview::mapview(gpx, color='blue',
                     map.type = 'OpenStreetMap.Mapnik',
                     cex = 0.2, # size of points
                     legend=FALSE)
```



# Street maps

You can also use the **mapview** package, which calls **leaflet** and has a bit more compact syntax

```
load(file.path(here::here('slides','lectures','data',
gpx <- st_as_sf(gpx,
                coords=c("Longitude", "Latitude"),
                crs = 4267) # Need to make sf object
mapview::mapview(gpx, color='blue',
                 map.type = 'Stamen.Watercolor',
                 cex = 0.2, # size of points
                 legend=FALSE)
```

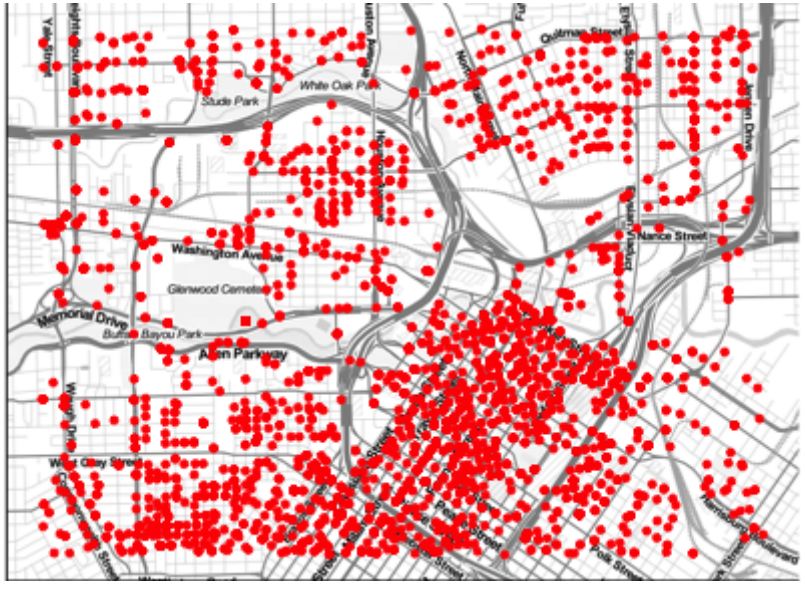



You can also have some stylistic fun with maps.

More possibilities at http://leaflet-extras.github.io/leaflet-providers/preview/

# Dot density maps

```
library(ggmap)
crime1 <- crime %>%
  filter(between(crime$lon, -95.4, -95.34) &
           between(crime$lat, 29.746, 29.784))

qmplot(lon, lat, data=crime1, maptype='toner-lite',
       color = I('red'))
```

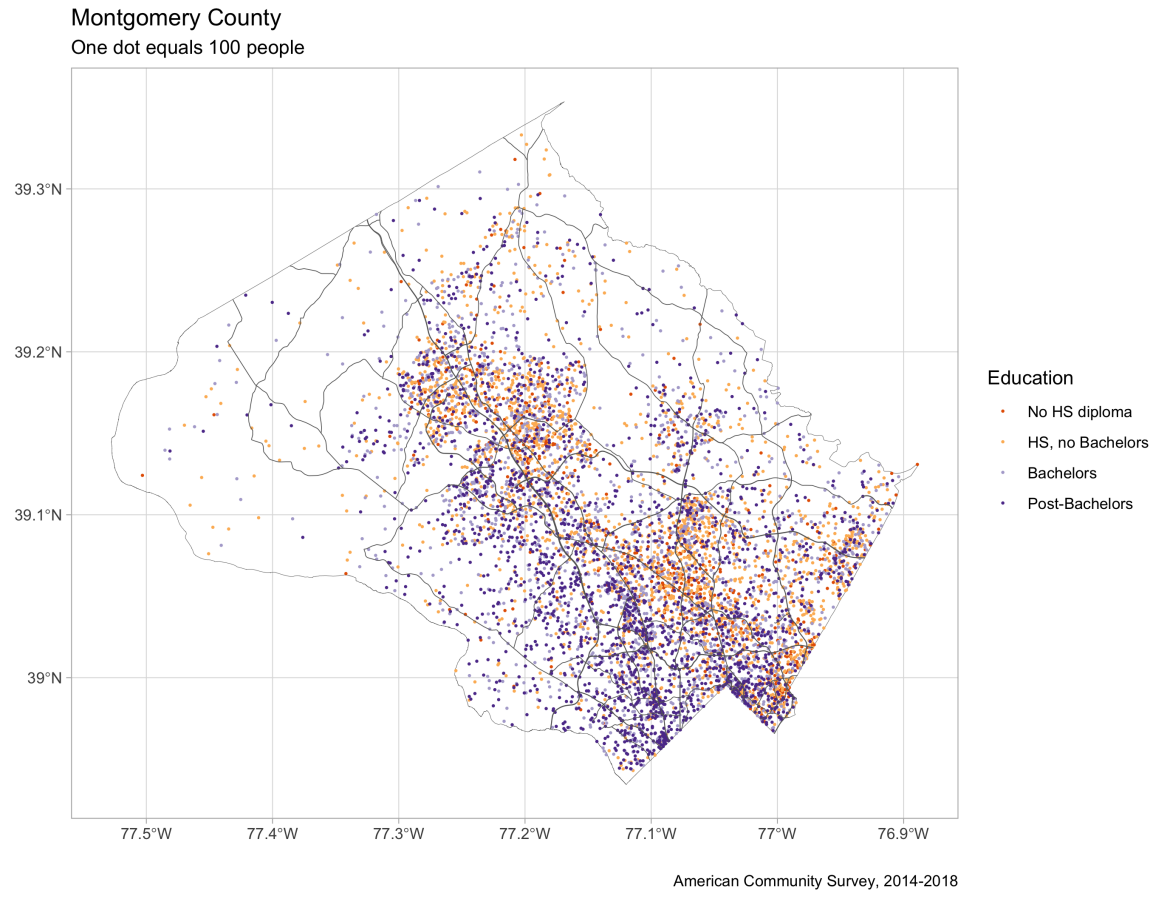



**ggmap** was built for Google Maps

Google Maps requires a credit card now

Better option is Stamen Maps, which uses OpenStreetMap data

# Dot density maps

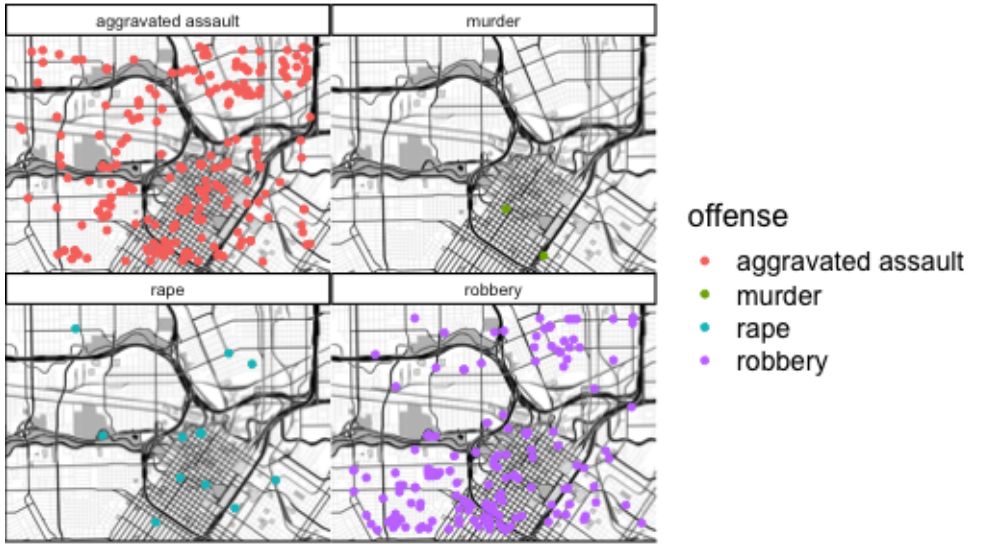



Code is available here

Based on this blog by Tarak Shah

# Facetted maps

```
library(ggmap)
crime1 <- crime1 %>%
  filter(!(offense %in% c('auto theft','theft',
                          'burglary')))
qmplot(lon, lat, data=crime1,
       maptype='toner-background',
       color = offense)+
  facet_wrap(~offense)
```



# Visualization in bioinformatics

Abhijit Dasgupta, PhD

# Networks

# Visualizing a proteomic network

We read a dataset that contains the network relationships between different proteins

```
library(ggnetwork)
datf <- rio::import('data/string_graph.txt')
head(datf)
```

```
     node1  node2 node1_string_id node2_string_id node1_external_id
1   CXCR3   CCR7         1855969         1843829   ENSP00000362795
2   ITGA4    EED         1858446         1845338   ENSP00000380227
3    SMC3  CENPK         1854200         1843648   ENSP00000354720
4 HNRNPA1 LUC7L3         1852510         1843556   ENSP00000341826
5    SMC2    RB1         1847012         1845924   ENSP00000286398
6   RBBP4  CENPK         1855919         1843648   ENSP00000362592
  node2_external_id neighborhood fusion cooccurence homology coexpression
1   ENSP00000246657            0      0           0    0.847        0.000
2   ENSP00000263360            0      0           0    0.000        0.000
3   ENSP00000242872            0      0           0    0.000        0.000
4   ENSP00000240304            0      0           0    0.000        0.000
5   ENSP00000267163            0      0           0    0.000        0.136
6   ENSP00000242872            0      0           0    0.000        0.000
  experimental knowledge textmining combined_score
1        0.000       0.9      0.878          0.913
2        0.566       0.0      0.312          0.688
3        0.000       0.9      0.081          0.904
```

# Visualizing a proteomic network

The **igraph** package allows the creation of network graphs.

However, here, we're only using it for data ingestion

```
pacman::p_load(igraph)
grs <- graph_from_data_frame(datf[,c('node1','node2')],
                             directed = F)
grs
```

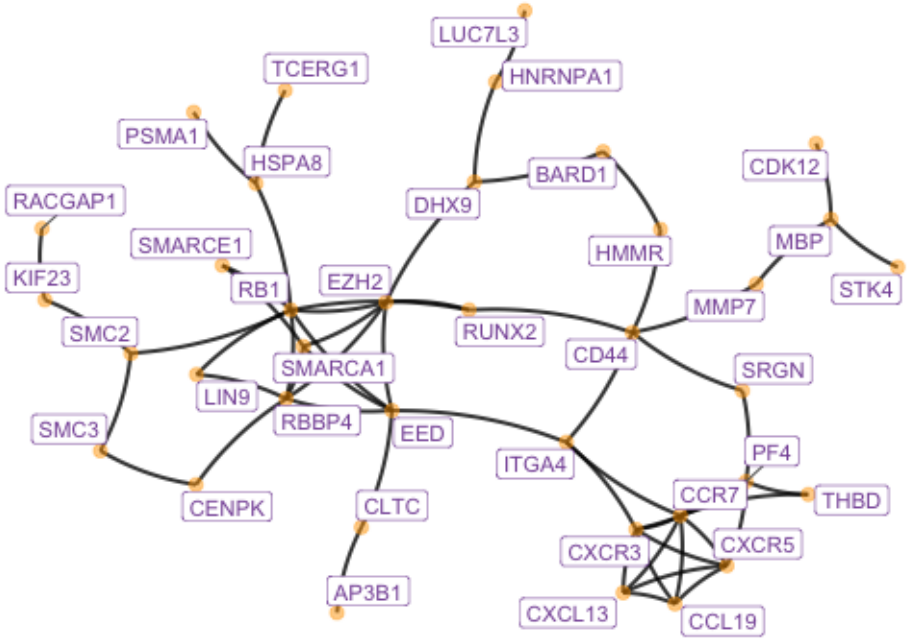
```
  IGRAPH 473d753 UN-- 37 58 --
  + attr: name (v/c)
  + edges from 473d753 (vertex names):
   [1] CXCR3  --CCR7    ITGA4  --EED     SMC3   --CENPK   HNRNPA1--LUC7L3
   [5] SMC2   --RB1     RBBP4  --CENPK   CXCR5  --CXCL13  CD44   --RUNX2
   [9] CXCR5  --PF4     PF4    --THBD    SMARCA1--EZH2    HMMR   --BARD1
  [13] MBP    --MMP7    CCL19  --CCR7    RBBP4  --EZH2    RUNX2  --RB1
  [17] RB1    --HSPA8   DHX9   --BARD1   CXCL13 --CCR7    SMC2   --KIF23
  [21] CD44   --HMMR    ITGA4  --CD44    RB1    --SMARCE1 ITGA4  --CCR7
  [25] MBP    --STK4    RBBP4  --LIN9    RB1    --EED     CXCR5  --CCR7
  [29] PSMA1  --HSPA8   RBBP4  --SMARCA1 CXCR3  --ITGA4   MBP    --CDK12
  + ... omitted several edges
```

We see that this object holds the different connections.

# Visualizing a proteomic network

We can then transform this data into ggplot-friendly data, to use ggplot for the plotting

```
library(intergraph)
ggdf <- ggnetwork(asNetwork(grs),
                  layout='fruchtermanreingold')
ggplot(ggdf, aes(x = x, y = y,
                 xend = xend, yend = yend)) +
  geom_edges(color = "black",
             curvature = 0.1,
             size = 0.95, alpha = 0.8)+
  geom_nodes(aes(x = x, y = y),
             size = 3,
             alpha = 0.5,
             color = "orange") +
  geom_nodelabel_repel(aes(label = vertex.names),
                       size=4, color="#8856a7") +
  theme_blank() + theme(legend.position = "none")
```



# Composing different genomic data into tracks

# The ggbio package

The **ggbio** package has several functions that allow graphical representations of different genomic entities.

You will see a lot of use of `autoplot`, which is a software technique to create default visualizations based on the type of entry.

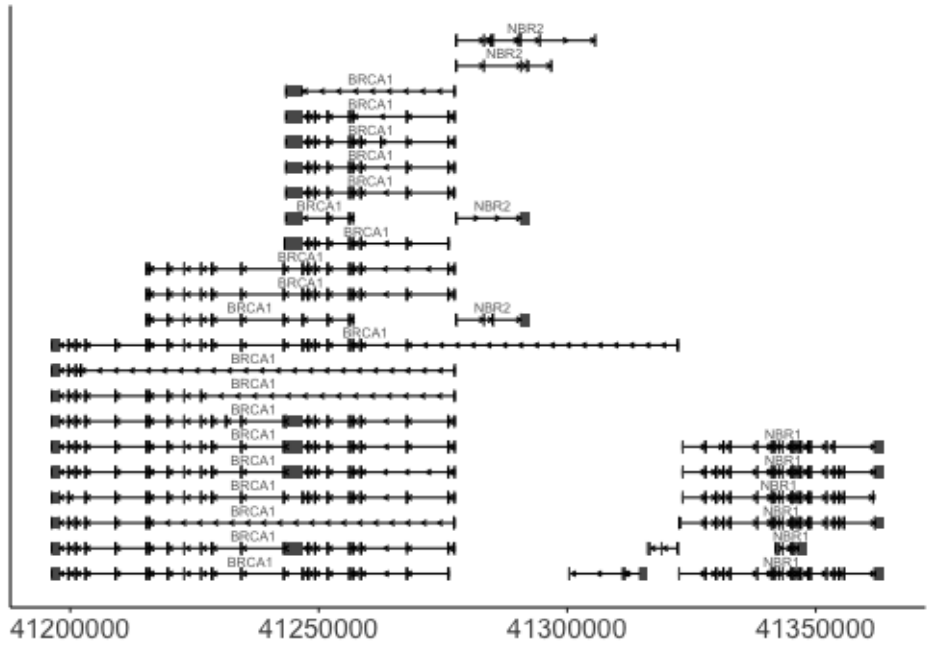
An ideogram

```
library(ggbio) # p_install('ggbio', try.bioconductor=
p.ideo <- Ideogram(genome = 'hg19')
p.ideo
```

# The ggbio package

## Visualizing the gene model

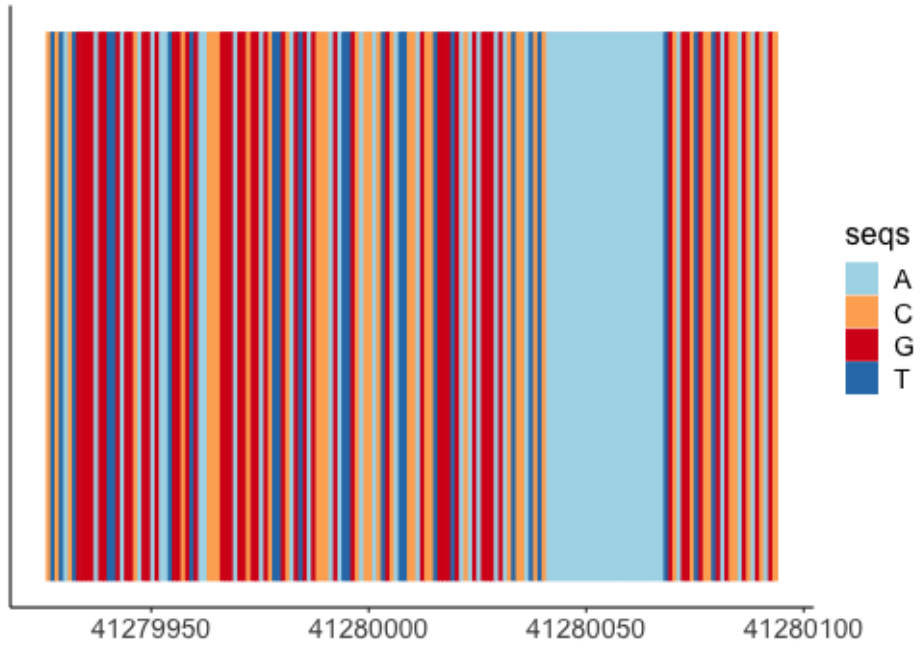
```
pacman::p_load(Homo.sapiens)
data(genesymbol, package='biovizBase')
wh <- genesymbol[c('BRCA1','NBR1')]
wh <- range(wh, ignore.strand=T)
p.txdb <- autoplot(Homo.sapiens, which = wh)
p.txdb
```



# The ggbio package

A reference track

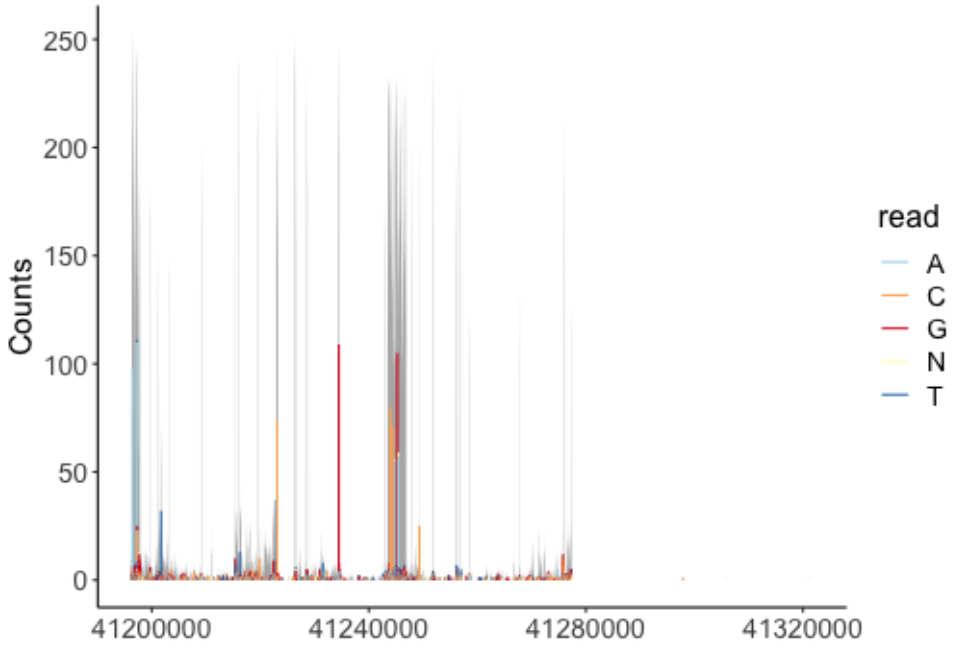
```
library(BSgenome.Hsapiens.UCSC.hg19)
bg <- BSgenome.Hsapiens.UCSC.hg19
p.bg <- autoplot(bg, which=wh)
p.bg + zoom(1/1000)
```



# The ggbio package

An alignment track with mismatch proportions

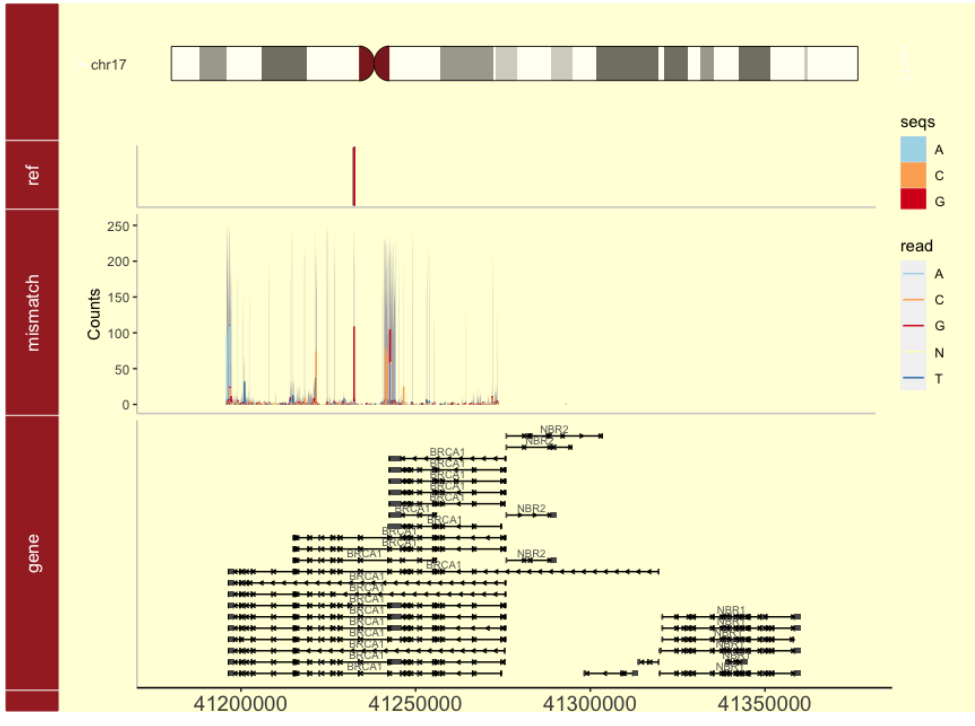
```
library(BSgenome.Hsapiens.UCSC.hg19)
fl.bam <- system.file("extdata", "wg-brca1.sorted.bam
wh <- keepSeqlevels(wh, "chr17")
bg <- BSgenome.Hsapiens.UCSC.hg19
p.mis <- autoplot(fl.bam, bsgenome = bg, which = wh,
p.mis
```



# The ggbio package

## Putting it into tracks

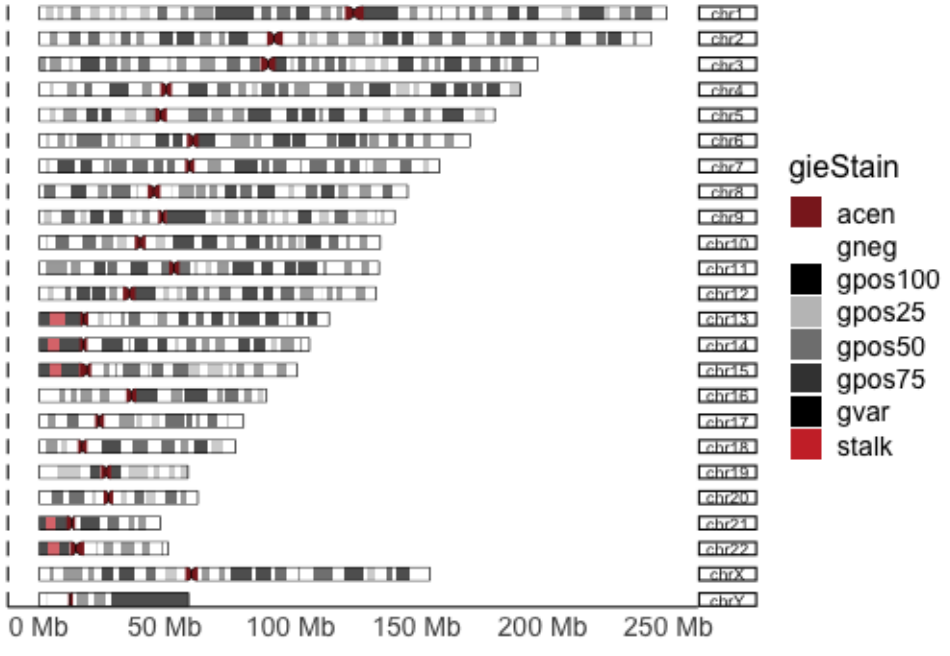
```
pacman::p_load(GenomicRanges)
gr17 <- GRanges("chr17", IRanges(41234415, 41234569))
tks <-  tracks(p.ideo,
               ref=p.bg,
               mismatch=p.mis,
               gene=p.txdb,
               heights=c(2,1,3,4))+
  xlim(gr17) +
  theme_tracks_sunset()
print(tks)
```



# The ggbio package

A karyogram

```
data(ideoCyto, package = "biovizBase")
autoplot(ideoCyto$hg19, layout = "karyogram",
         cytobands = TRUE)
```



# P-values and Manhattan plots

# A very simple example

```
library(tidyverse)
clinical <- rio::import('data/BreastCancer_Clinical.xlsx') %>% janitor::clean_names()
proteome <- rio::import('data/BreastCancer_Expression.xlsx') %>% janitor::clean_names()
final_data <- clinical %>%
    inner_join(proteome, by = c('complete_tcga_id' = 'tcga_id')) %>%
    dplyr::filter(gender == 'FEMALE') %>%
    dplyr::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  np_958782  np_958785  np_958786  np_000436
1     TCGA-A2-A0CM                                   40  Negative  0.6834035  0.6944241  0.6980976  0.6870771
2     TCGA-BH-A18Q                                   56  Negative  0.1953407  0.2154129  0.2154129  0.2053768
3     TCGA-A7-A0CE                                   57  Negative -1.1231731 -1.1231731 -1.1168605 -1.1294857
4     TCGA-D8-A142                                   74  Negative  0.5385958  0.5422105  0.5422105  0.5349810
5     TCGA-AO-A0J6                                   61  Negative  0.8311317  0.8565398  0.8565398  0.8367780
6     TCGA-A2-A0YM                                   67  Negative  0.6558497  0.6581426  0.6558497  0.6558497
   np_958781  np_958780  np_958783  np_958784  np_112598  np_001611
1  0.6870771  0.6980976  0.6980976  0.6980976 -2.6521501 -0.9843733
2  0.2154129  0.2154129  0.2154129  0.2154129 -1.0357599 -0.5172257
3 -1.1294857 -1.1200168 -1.1231731 -1.1231731  2.2445844 -2.5750648
4  0.5422105  0.5422105  0.5422105  0.5422105 -0.1482049  0.2674902
5  0.8650092  0.8565398  0.8508936  0.8508936 -0.9671961  2.8383705
6  0.6512639  0.6581426  0.6558497  0.6558497 -1.9695337  1.3070365
```

# 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 np_958784 np_112598    np_001611
  1 0.6988415 0.6910103 0.6832121 0.6910103 0.6832121 0.6910103 0.6910103 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

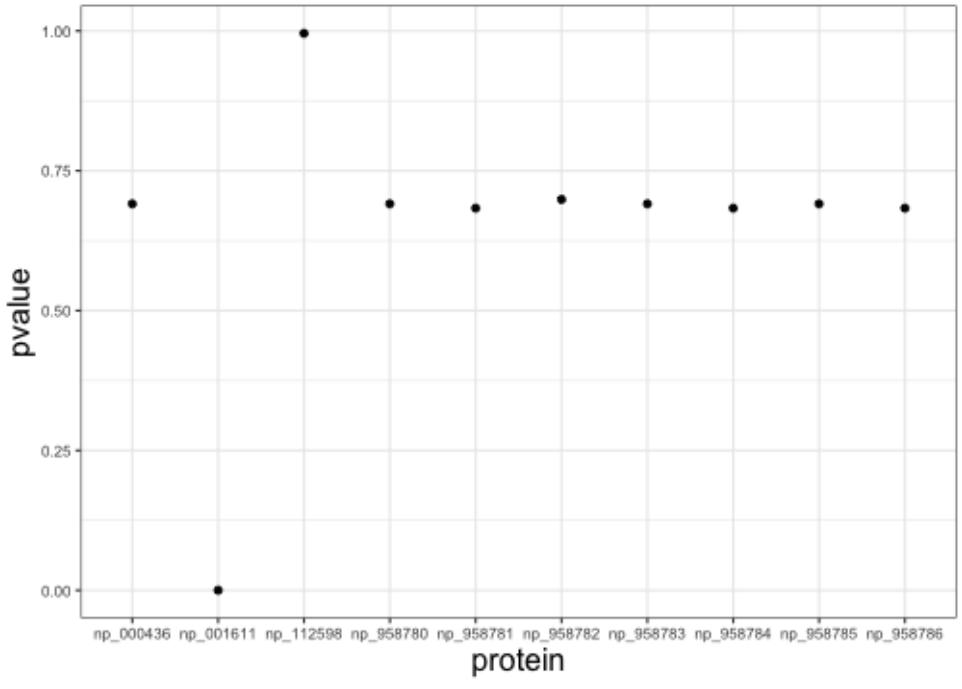
# A very simple example

```
results %>% tidyr::pivot_longer(cols=everything(),
                                names_to='protein',
                                values_to='pvalue')
```

```
# A tibble: 10 x 2
   protein     pvalue
   <chr>        <dbl>
 1 np_958782 0.699
 2 np_958785 0.691
 3 np_958786 0.683
 4 np_000436 0.691
 5 np_958781 0.683
 6 np_958780 0.691
 7 np_958783 0.691
 8 np_958784 0.683
 9 np_112598 0.996
10 np_001611 0.000122
```

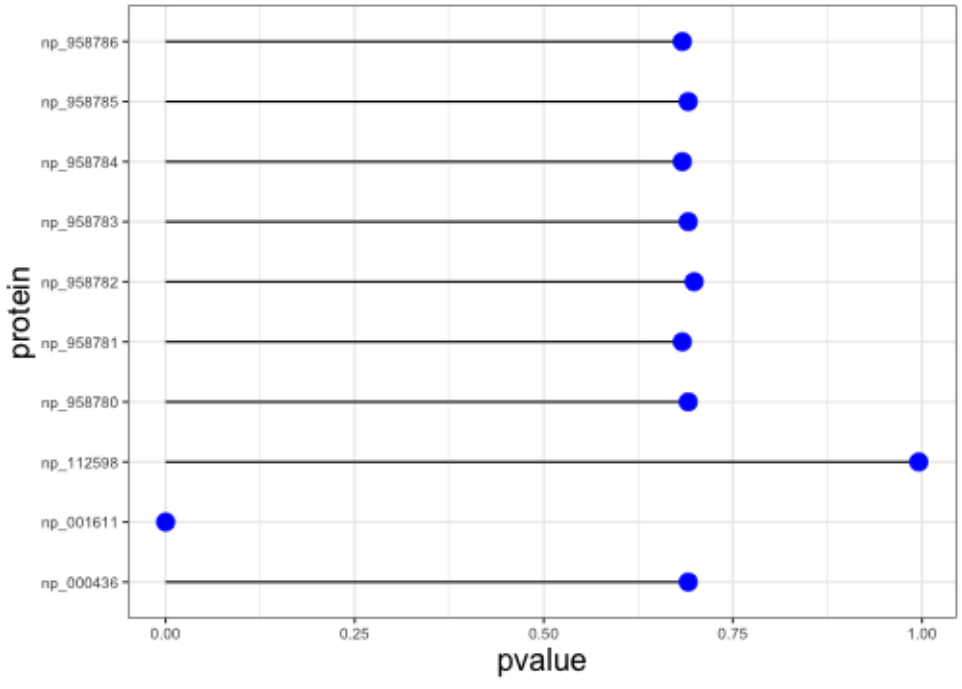
# A very simple example

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


results %>% pivot_longer(
  cols=everything(),
  names_to='protein',
  values_to='pvalue') %>%
  ggplot(aes(x = protein, y = pvalue)) +
  geom_point() +
  theme_439
```



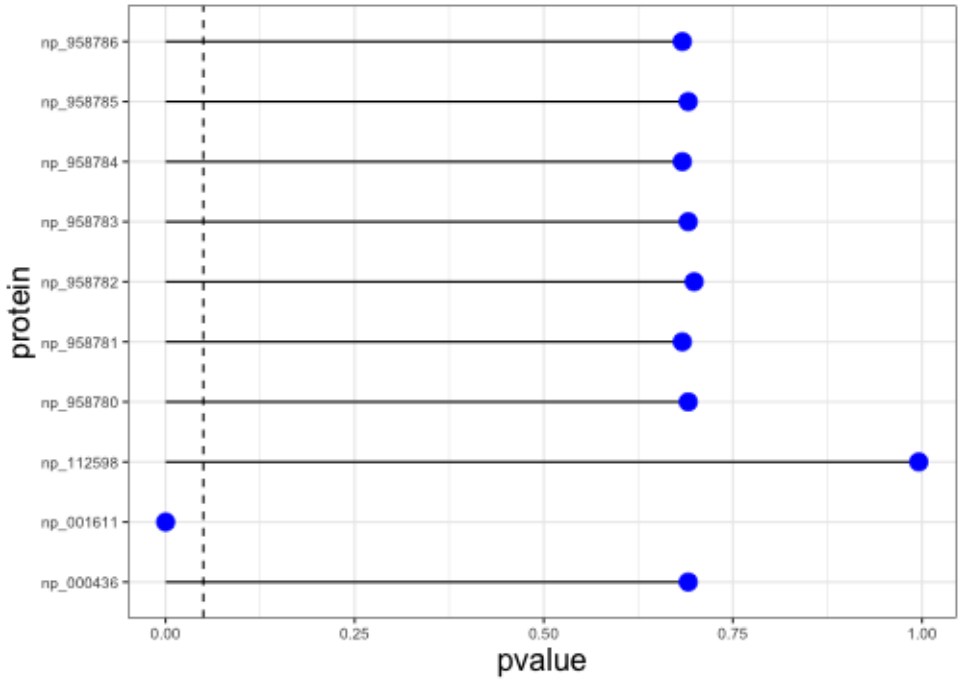
# A very simple example

```
pacman::p_load('ggalt')
results %>% pivot_longer(
  cols=everything(),
  names_to = 'protein',
  values_to = 'pvalue') %>%
  ggplot(aes(x = protein, y = pvalue)) +
  geom_point() +
  geom_lollipop(point.colour='blue', point.size=4)+
  coord_flip()+
  theme_439
```



# A very simple example

```
results %>% pivot_longer(
  cols=everything(),
  names_to = 'protein',
  values_to = 'pvalue') %>%
  ggplot(aes(x = protein, y = pvalue)) +
      geom_point() +
      geom_lollipop(point.colour='blue', point.size=4
      geom_hline(yintercept = 0.05, linetype=2)+
      coord_flip() +
      theme_439
```

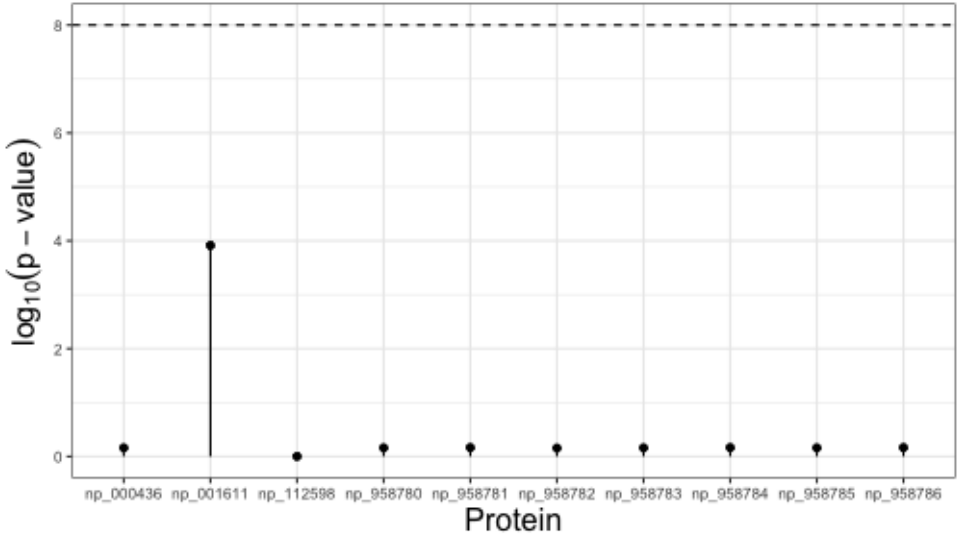


# Manhattan plot

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 %>% pivot_longer(
  cols=everything(),
  names_to = 'protein',
  values_to = 'pvalue') %>%
  ggplot(aes(x = protein, y = -log10(pvalue))) +
    geom_point() +
    geom_lollipop() +
    geom_hline(yintercept = 8, linetype=2)+
    labs(x = 'Protein',
         y = expression(log[10](p-value))) +
    theme_439
```



# Manhattan plot

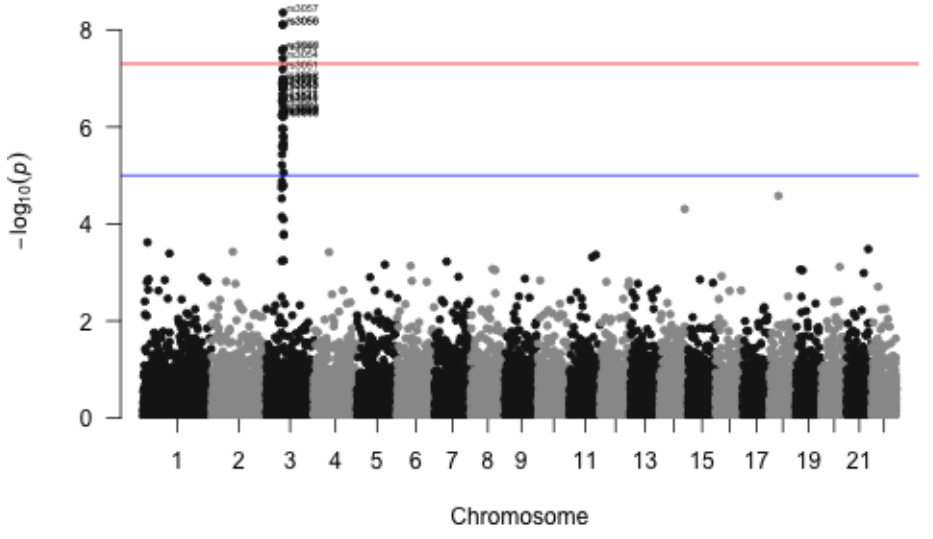
There is a specialized package for doing Manhattan plots and quantile plots for GWAS data

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

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

# Manhattan plot

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



# Heatmaps

# Let us count the ways

There are several ways of doing heatmaps in R:

- https://jokergoo.github.io/ComplexHeatmap-reference/book/
- 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/

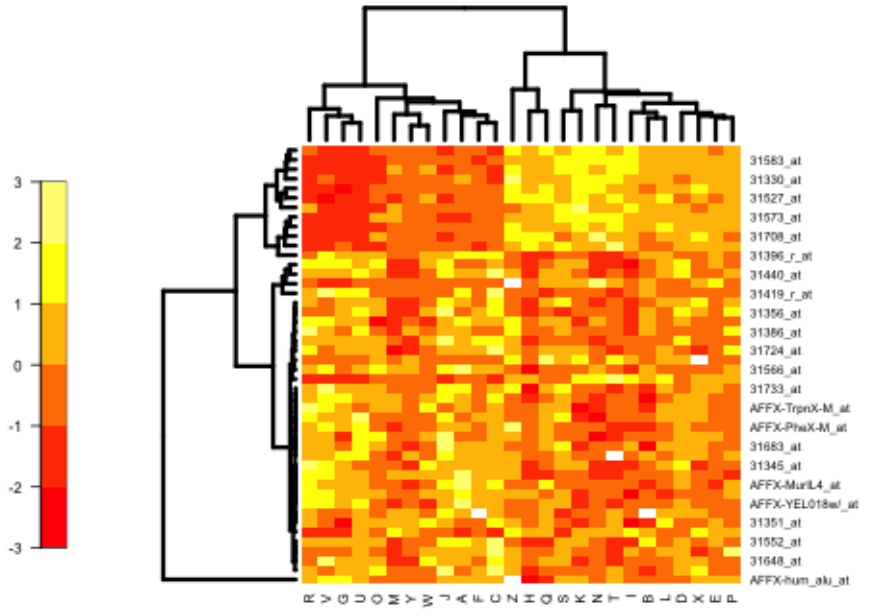
# Some example data

```
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
```

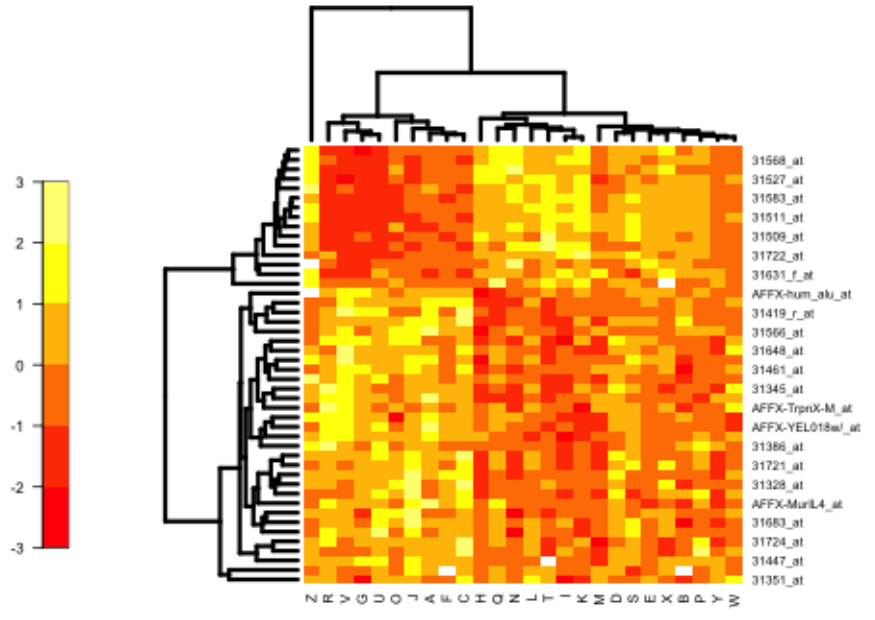
# Using Heatplus

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



# Using Heatplus

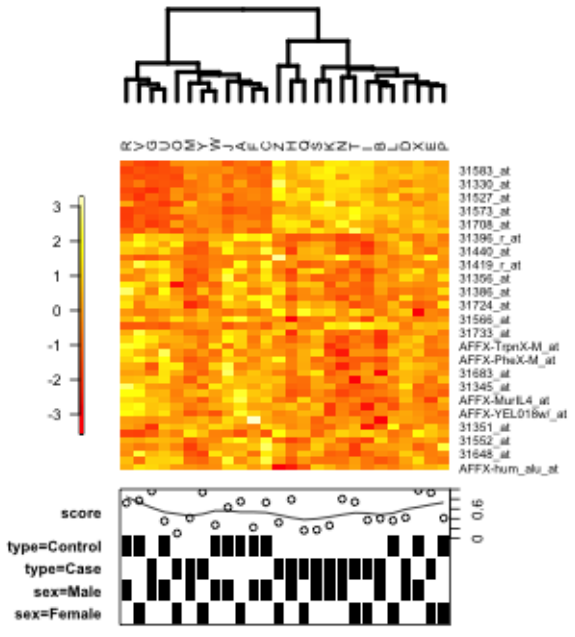
```
corrdist <- function(x) as.dist(1-cor(t(x)))
hclust.avl <- function(x) hclust(x, method='average')
reg2 <- regHeatmap(exprs(exdat2), legend=2,
                   col=heat.colors,
                   breaks=-3:3,
                   dendrogram =
                       list(clustfun=hclust.avl,
    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



# Using Heatplus

## Adding annotations

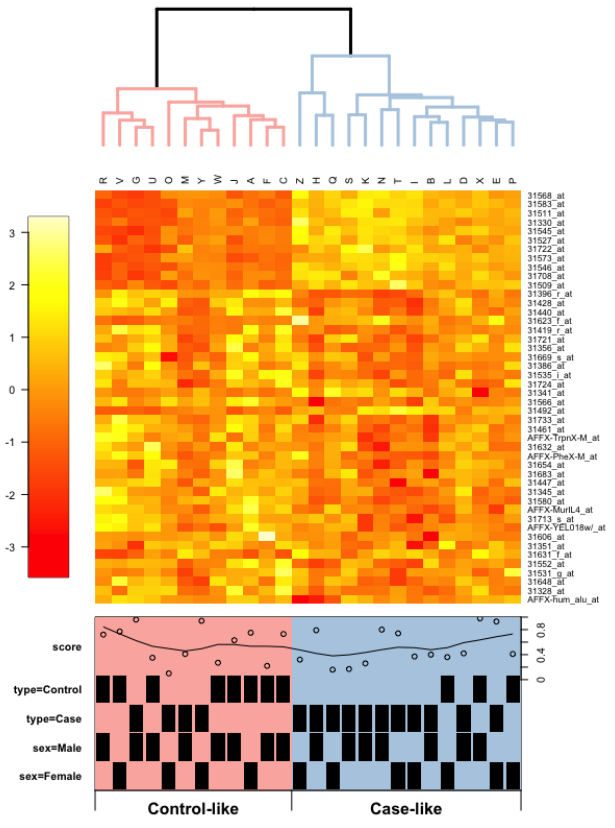
```
ann1 <- annHeatmap(exprs(exdat2),
                   ann=pData(exdat2),
                   col = heat.colors)
plot(ann1)
```



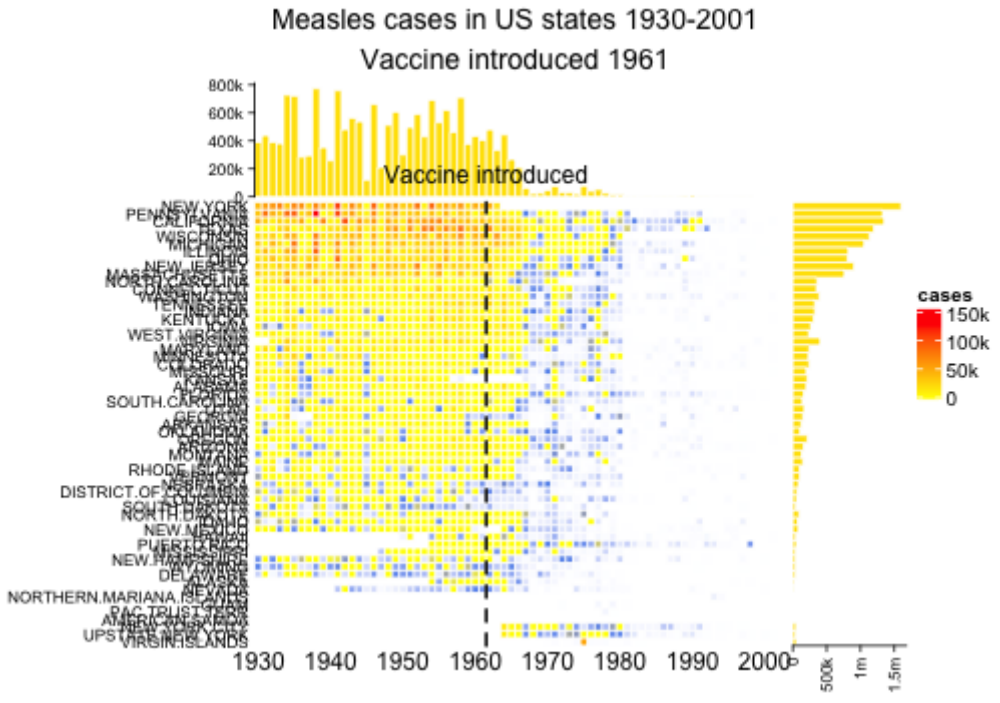
# Using Heatplus

## Adding annotations

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



# Using ComplexHeatmap

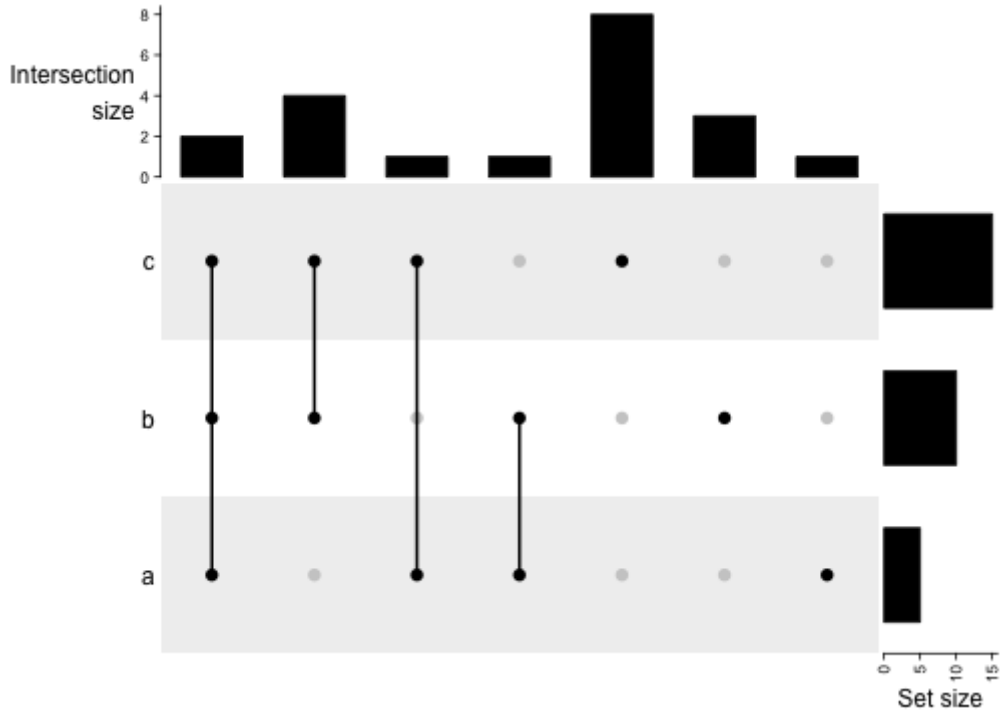



Source code here

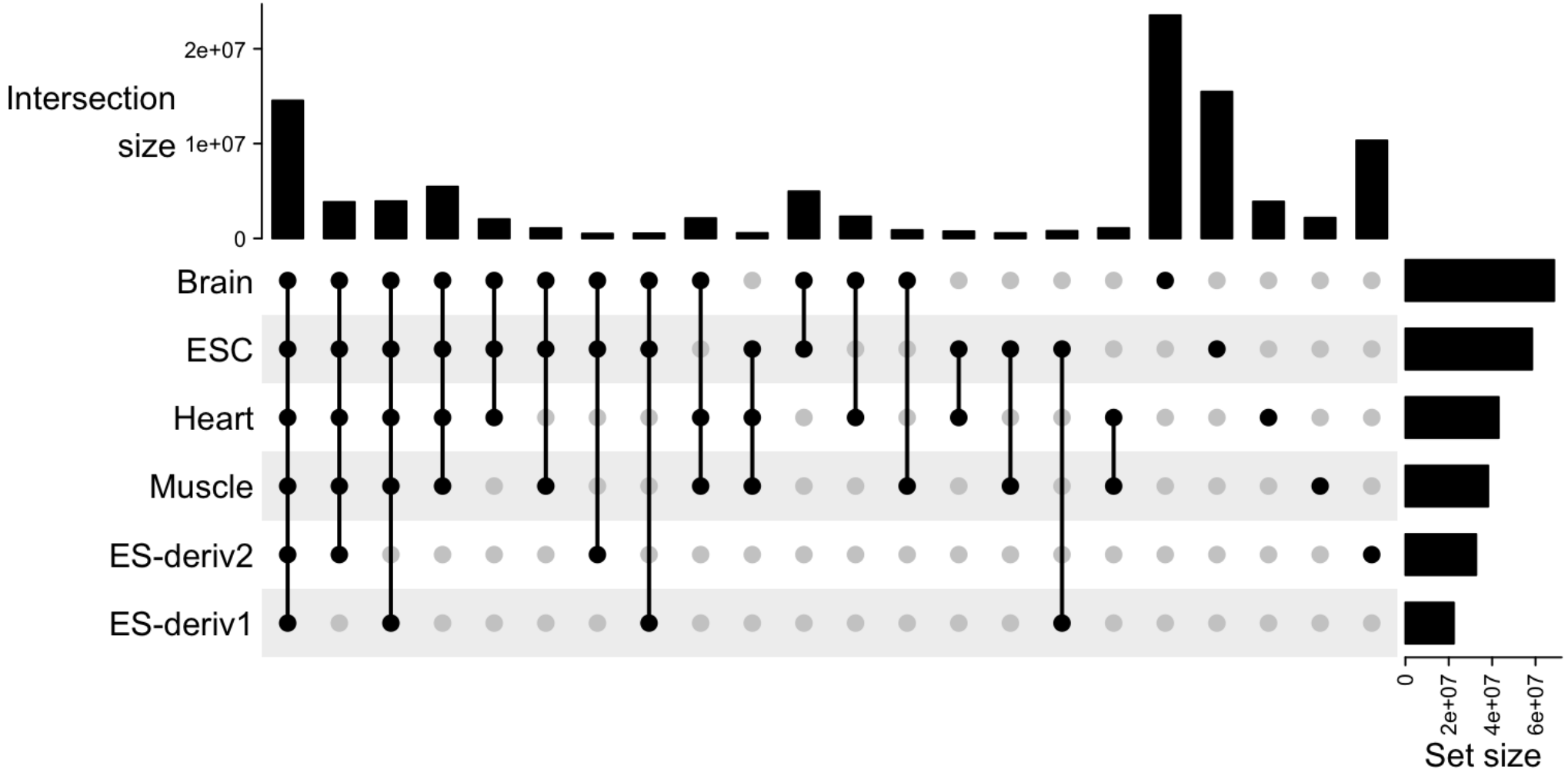
# UpSet plots

UpSet plots are nice visualizations for looking at commonalities (complex intersections) between sets of objects.

We used UpSet plots to look at missing value patterns

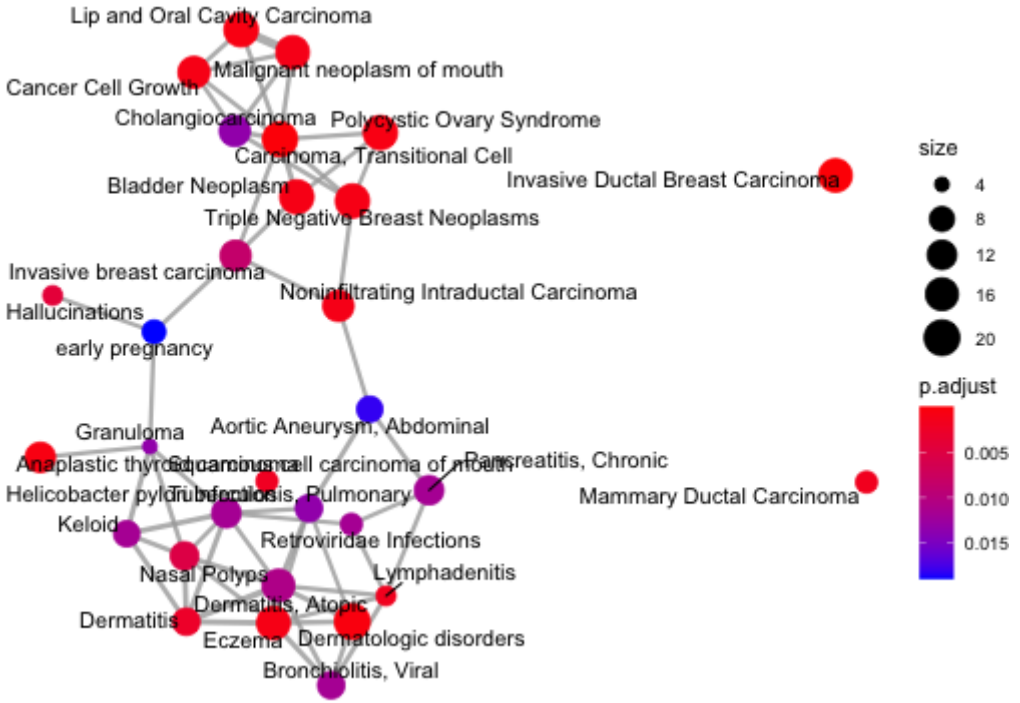


# UpSet plots


Intersection size
2e+07
1e+07
0
Brain
ESC
Heart
Muscle
ES-deriv2
ES-deriv1
0
2e+07
4e+07
6e+07
Set size

# clusterProfiler

Enrichment network based on GSEA



# 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, 0 variable features)
```

```
names(pbmc)
```

```
  [1] "RNA"
```

```
slotNames(pbmc)
```

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

# Adding QC metrics and plotting

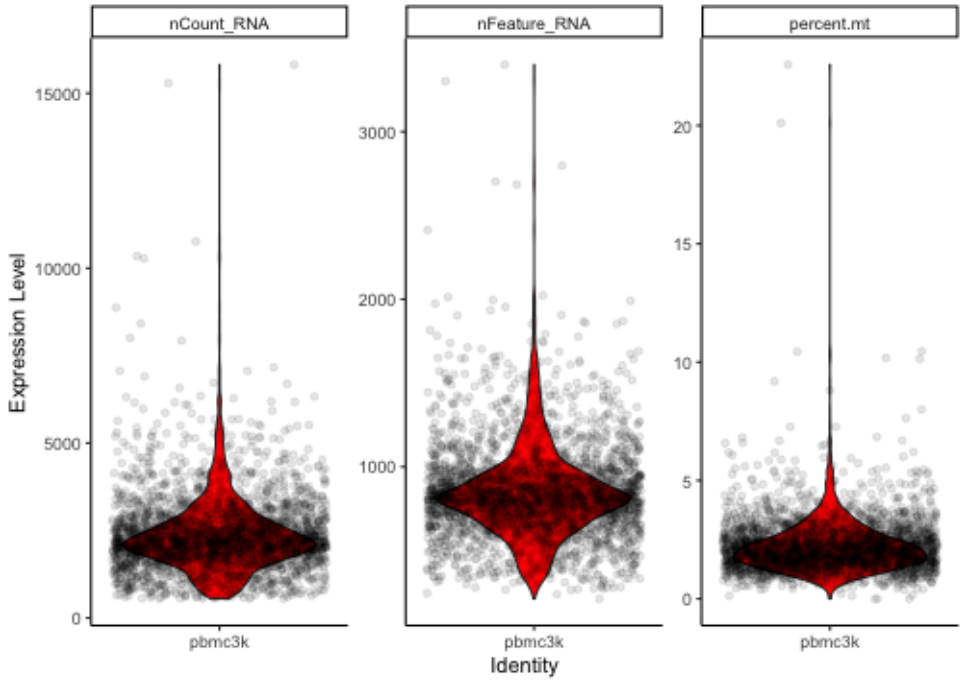
We'll calculate mitochondrial QC metrics (percentage counts originating from mitochondrial genes)

```
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
```

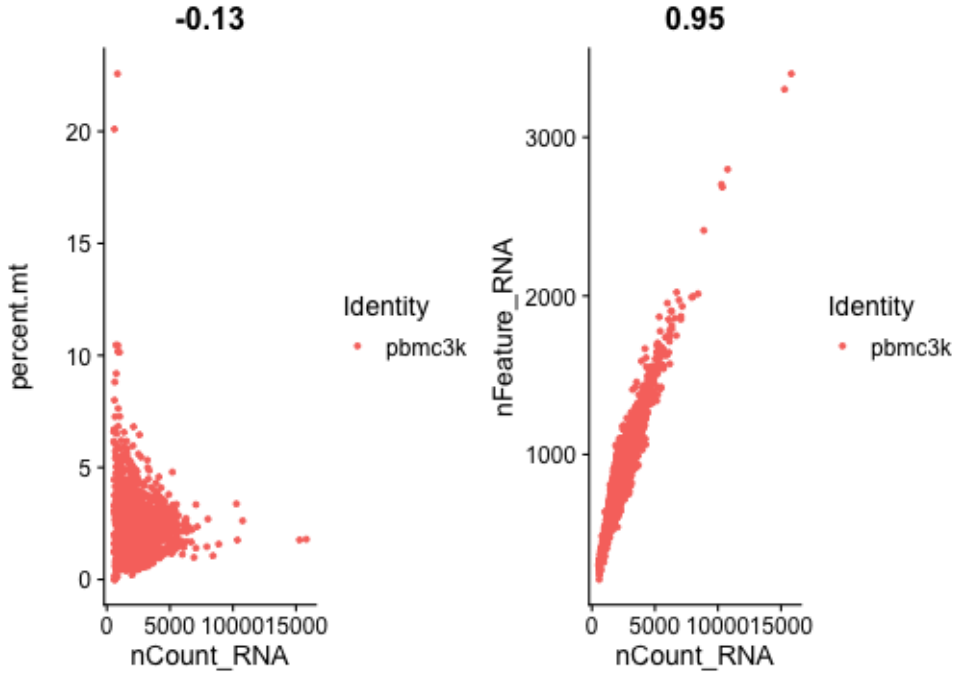
# Visualizing metrics

```
# 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()
```



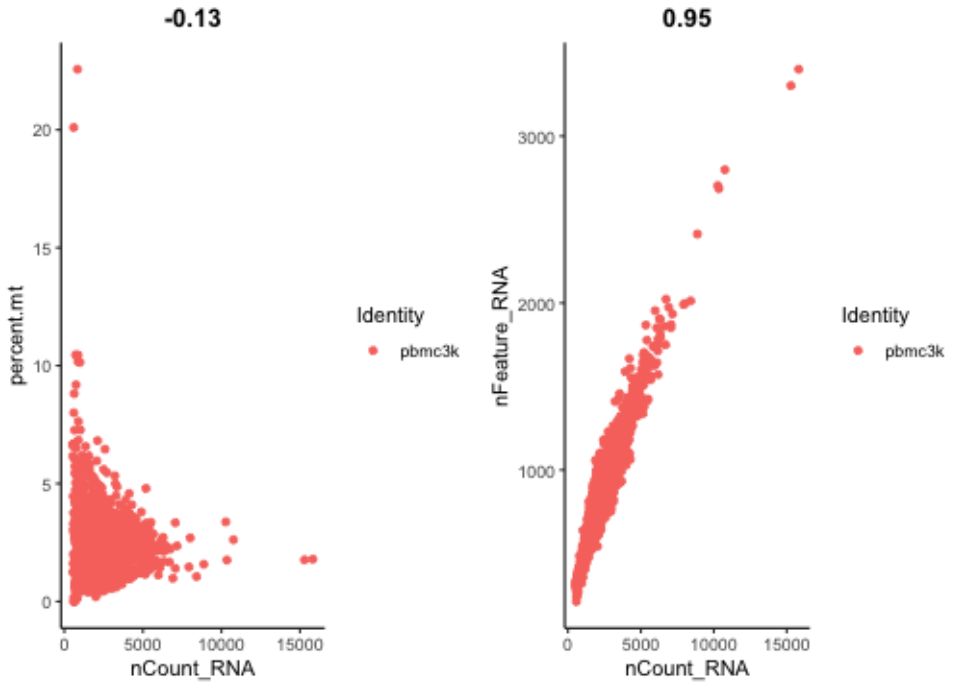
# Visualizing feature-feature relationships

```
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))
```



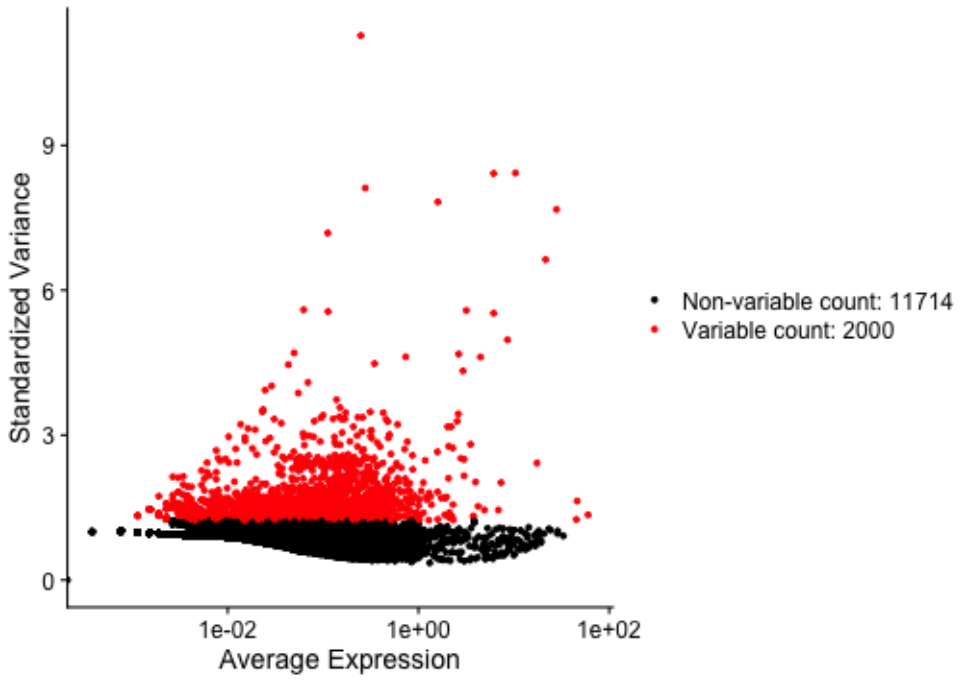
# Visualizing feature-feature relationships

```
cormatrix <- cor(pbmc@meta.data %>% dplyr::select(-or
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)
```



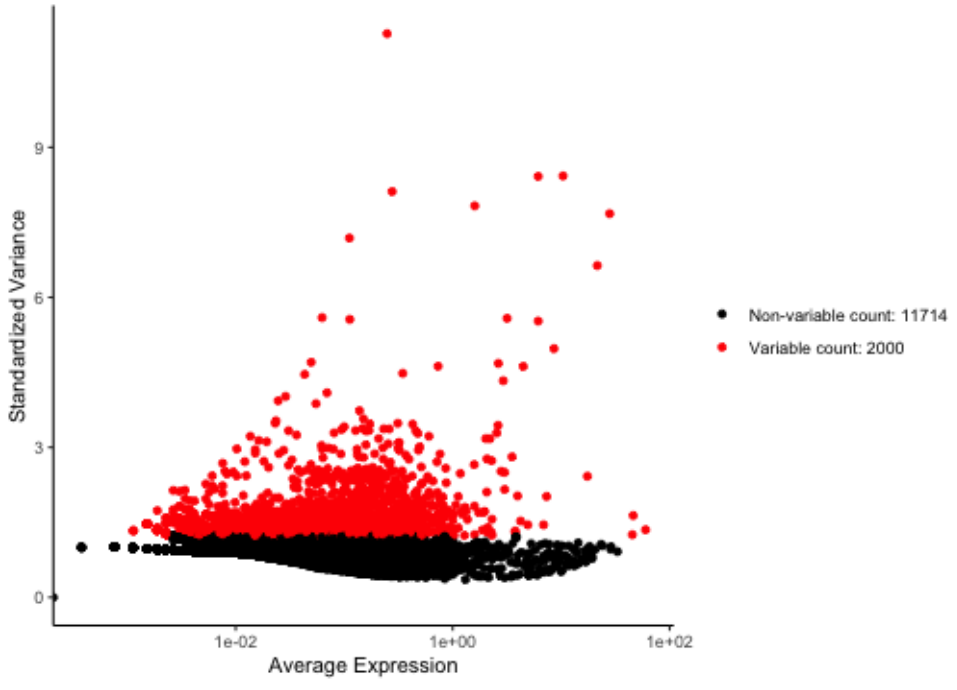
# Feature selection

```
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
```



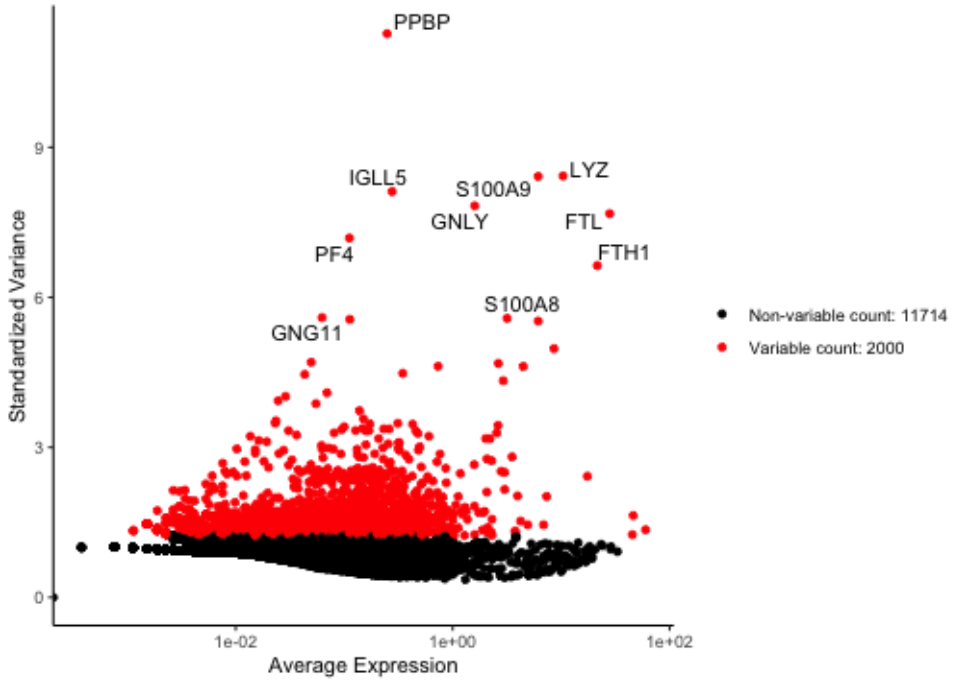
# Feature selection

```
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 %>%
    dplyr::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 = vst.mean,
                    y = vst.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

# Facetted maps

```
library(tmap)
world1 <- world %>%
  filter(continent %in% c('Europe','Asia',
                          'North America',
                          'South America')) %>%
  mutate(continent = fct_reorder(continent,
                                 lifeExp, na.rm=T))

tm_shape(world1)+tm_polygons(col='lifeExp') +
  tm_facets(by='continent', ncol=2)
```