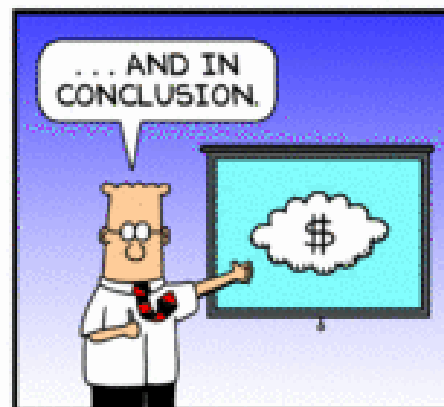
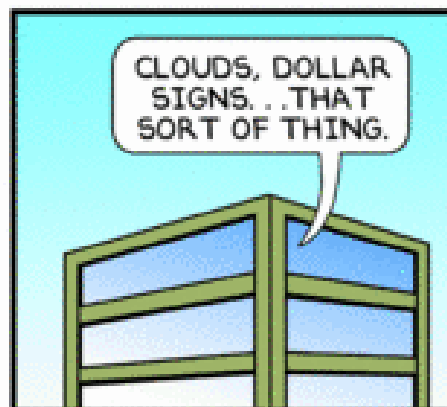
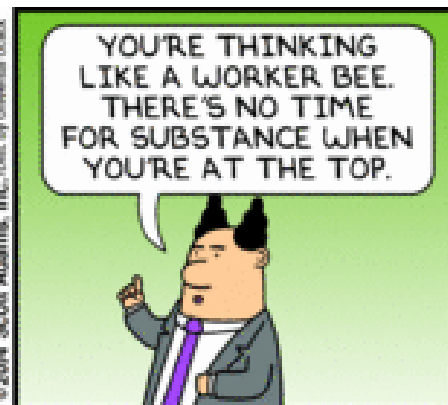




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DCMB BioComputing BootCamp

Day 3, Lecture 3:

Data Exploration and Visualization in R

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Now What?

- Scenario:
 - You understand the fundamentals of R
 - You've read your data into an R data frame
- During this session we will talk about
 1. Basic data summarization
 2. Visualize data with plots



Overview

1. Summarizing Data in R
2. Creating plots in R Using ggplot2

Example Data Set:

Cancer Research

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Molecular and Cellular Pathobiology

Activation of Wnt/ β -Catenin in Ewing Sarcoma Cells Antagonizes EWS/ETS Function and Promotes Phenotypic Transition to More Metastatic Cell States

Elisabeth A. Pedersen, Rajasree Menon, Kelly M. Bailey, Dafydd G. Thomas, Raelene A. Van Noord, Jenny Tran, Hongwei Wang, Ping Ping Qu, Antje Hoering, Eric R. Fearon, Rashmi Chugh, and Elizabeth R. Lawlor

DOI: 10.1158/0008-5472.CAN-15-3422 Published September 2016

- Ewing's sarcoma: rare bone and soft tissue cancer occurring in children and teenagers
 - 70-80% survival
- *In vitro* CHLA25-7TGP ES cells stimulated to over-express WNT3A

Exercise: Write a Script to Read Pedersen Gene Expression Data into a Data Frame

1. `options(stringsAsFactors=F)`
2. Use the `read.delim()` function to read in “pedersenLog2RPKM20180817.txt” file into a data frame called “data1”
3. Use the `head()` and `dim()` function to find out about the structure of this data file

How many rows? What are the columns?

Quickly Calculate Simple Statistics

- R has many built in statistical functions that use fast vector and matrix operations
- No need to write a for loop, sum, and then divide by n
- Just provide a vector of data to the mean() function:
 - `mean(data1$log2RPKM)`
- With one line of code you have take the mean of 97,000 values!

Exercise: Use mean(), median(), max(), min(), summary() functions on the Pedersen data

Using the table() Function

- When exploring new datasets it is often useful to count the number of values
- table() can be used to build a contingency table of the counts of each value
 - For one column:

```
> table(data1$tx)  
  
control    WNT3A  
  48585    48585
```

- For multiple columns:

```
> table(data1$tx,data1$rep)  
  
      1      2      3  
control 16195 16195 16195  
WNT3A   16195 16195 16195
```


Using the aggregate() Function

- Often times we want to perform a function on subsets of our data
 - example question: What is the mean expression for each sample?
- aggregate() splits data into subsets, computes summary statistics for each and returns the result
- aggregate() takes several arguments:

formula input data

```

> aggregate(log2RPKM ~ sample, data1, FUN='mean')
      sample log2RPKM
1 control_rep1 2.598991
2 control_rep2 2.583161
3 control_rep3 2.579987
4 WNT3A_rep1 2.593578
5 WNT3A_rep2 2.583571
6 WNT3A_rep3 2.598349
  
```

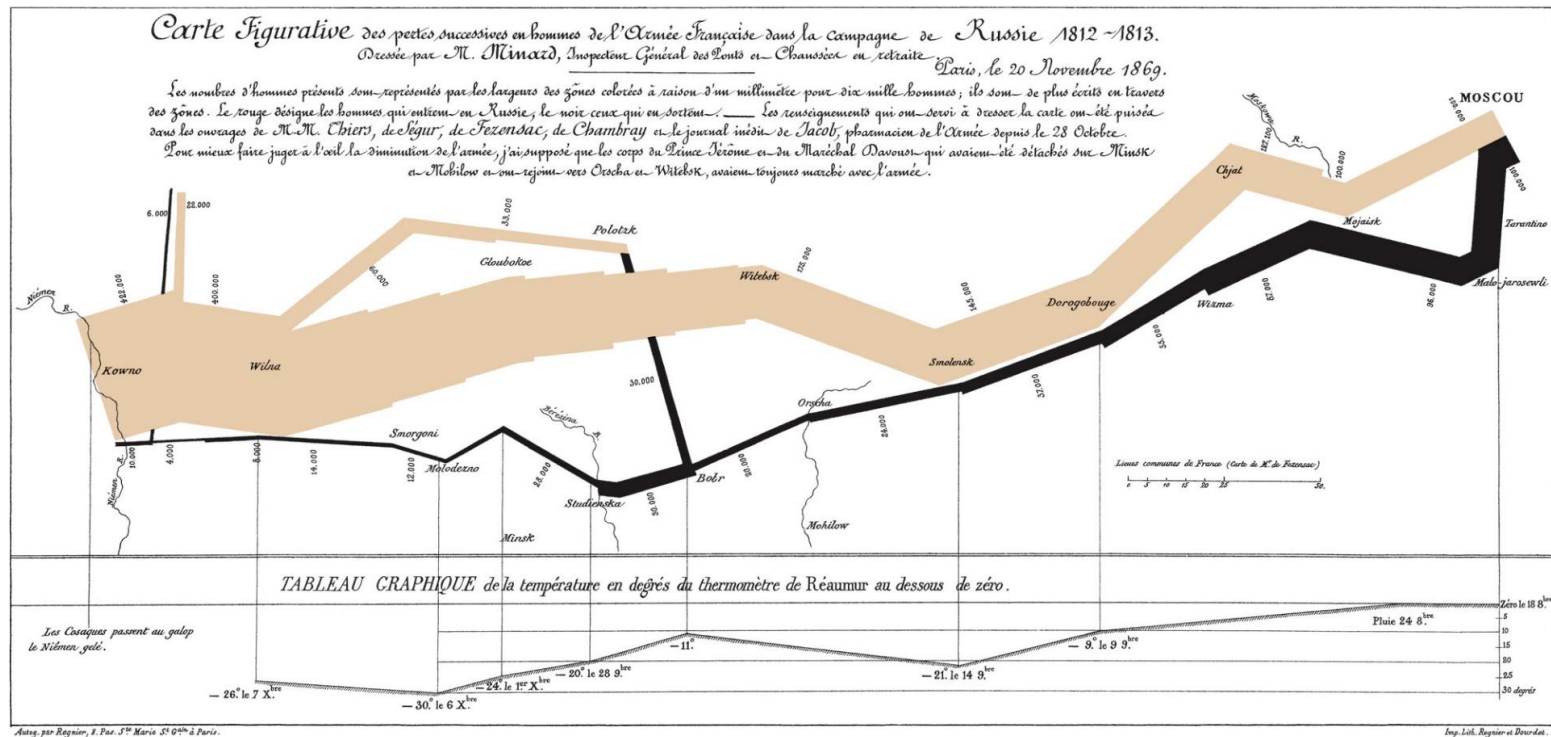
summary statistic

Exercise: Use aggregate() to calculate the maximum log2RPKM value per sample.

formula format: $y \sim x1$

- y is a numeric value
- $x1$ is a grouping variable
- possible to specify multiple groups as $x1 + x2 + \dots$

Data Visualization Allows Researchers to Visually Present Data



- Minard's 1869 diagram of Napoleonic France's invasion of Russia
 - Line width indicates size of army
 - Color indicates army's course to and from Russia

Data Visualization Allows Researchers to Visually Present Data

- Data visualizations should:
 - Show the data
 - Avoid distorting the data
 - Present many numbers in a small space
 - Make large data sets coherent
 - Serve a reasonably clear purpose
 - Be closely integrated with the statistical and verbal descriptions of a data set

R Base Graphics Versus ggplot2

- R comes with “base graphics” built in to support commonly used data visualizations
- Today we will focus on using an alternative data visualization framework called ggplot2
- ggplot2 is an external package that must be downloaded, installed, and loaded with the library command
- A common practice is to use ggplot2 to construct publication quality graphs but still use base graphics to quickly visualize data

ggplot2

- ggplot2 is an R data visualization package created by Hadley Wickham
 - One of the most popular R packages
 - an alternative to R base graphics which are built into R
 - Breaks up graphs construction into additive functions
- ggplot2 documentation and cheat sheet:
<https://www.rdocumentation.org/packages/ggplot2/versions/3.0.0>

Creating a Visualization with ggplot2()

- ggplot2 visualization function calls consist of several basic components:

1. `ggplot()`
2. `geom_XXX()`
3. optional layers
4. `ggsave()`

- Multiple function calls are combined together using “layers”
- `aes()` functions are used to map input data to plot features (e.g. x axis, y axis, colors)

```
options(stringsAsFactors=F)
```

```
library(ggplot2)
```

```
inFile = 'data.txt'
```

```
data1 = read.delim(inFile)
```

```
ggplot(data1, aes(x = log2RPKM)) +
```

```
  geom_histogram()
```

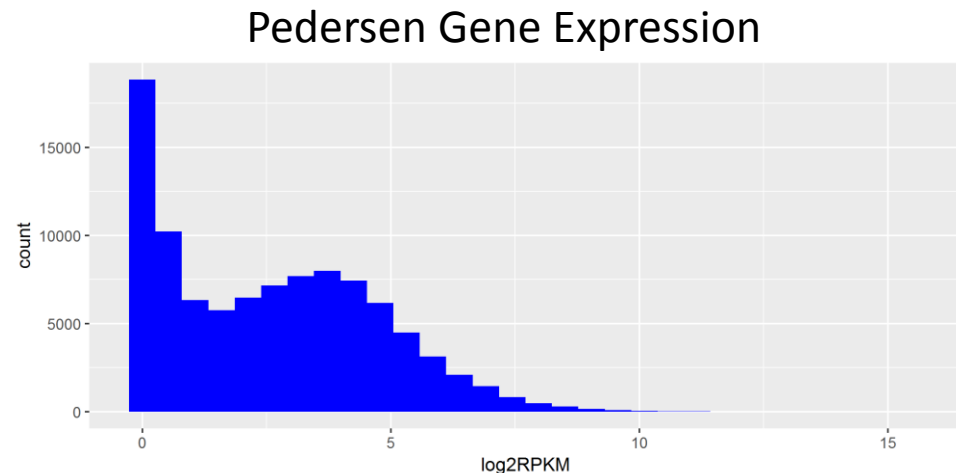
```
ggsave('histogram.png')
```

input data frame

aesthetic

Visualizing Data Using Histograms

- histogram: a type of bar graph visualization in which data measurements are counted based on value
 - For **discrete** measures it shows the frequency of values in each category
 - For **continuous** measure it shows the frequency of values occurring in small intervals covering the whole range



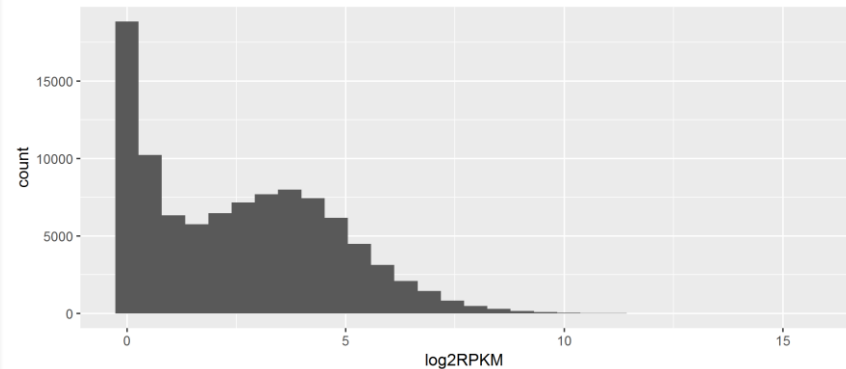
How to Create a Basic Histogram Using ggplot2

```
options(stringsAsFactors=F)

library(ggplot2)

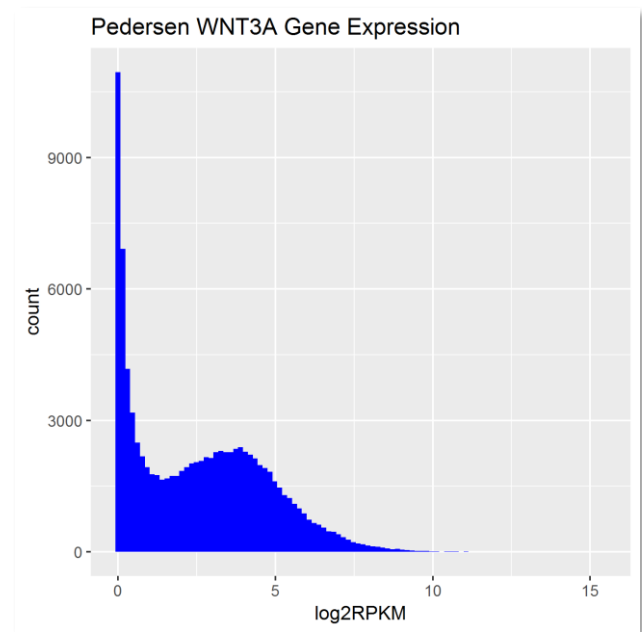
inFile1 = 'pedersenLog2RPKM20180817.txt'
data1 = read.delim(inFile1)

ggplot(data1,aes(x = log2RPKM)) +
  geom_histogram()
ggsave('histogram1.png')
```



Exercise: Create a histogram using the code from the previous page and update your visualization to have the following features

1. Change the color
 - HINT: `?geom_histogram`
 - HINT: `fill = "blue"`
2. Set the image width and height to be 5 inches
 - HINT: `?ggsave`
 - HINT: `height = 5`
3. Adjust the number of bins to 100
4. Add a title
 - HINT: `?labs`

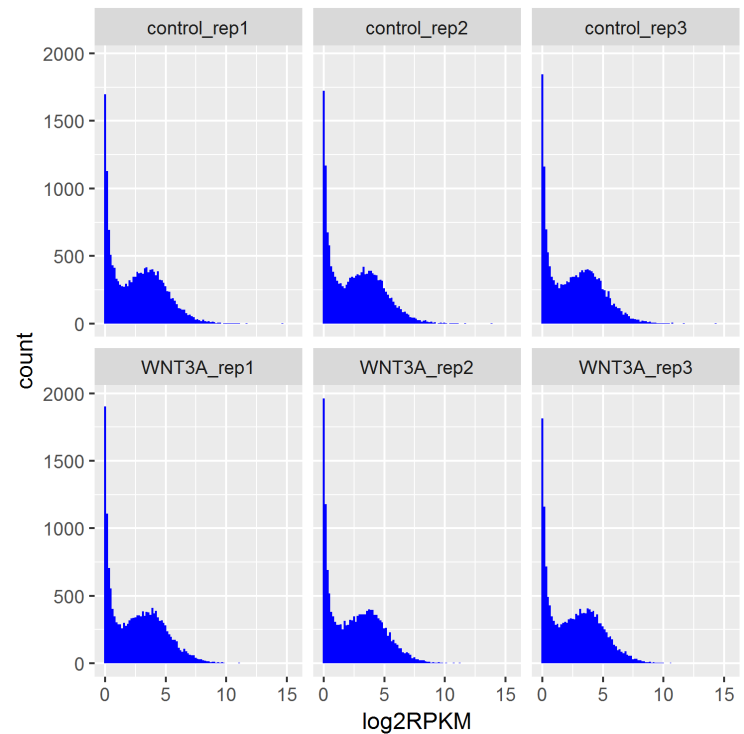


Create Sub-plots Using Facets

- Sub-plots can easily be created using facet layers:
 - `facet_wrap()`
 - `facet_grid()`

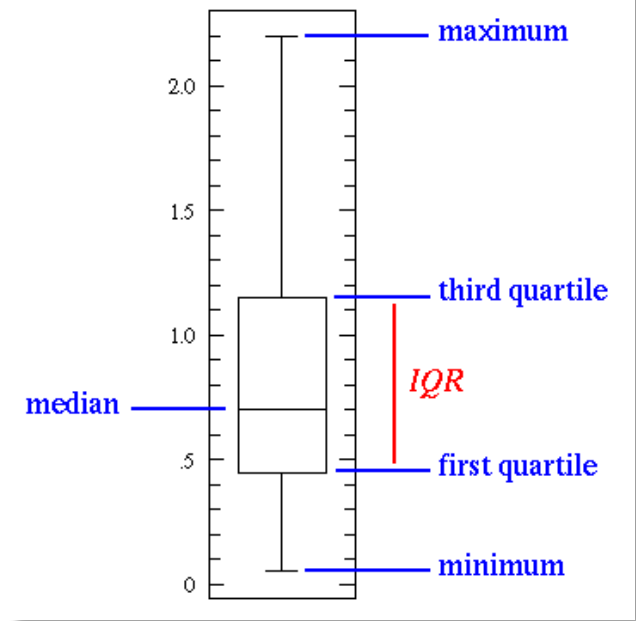
Exercise: Update your histogram visualization to facet on sample

1. Add a `facet_wrap()` layer
 - HINT: `+ facet_wrap(~sample)`



Visualizing Data Using Boxplots

- boxplot: graphically represents data distributions using quartiles
- box-and-whisker plot: includes boxplots with lines extending from boxes to indicate variability outside the upper and lower quartiles
- Why it is useful?
 - Summarize the main characteristics of the data: Mean/median, quartile, spread, symmetry and outliers.
 - Efficient – less complicated than histogram
 - Allows us to represent multiple data distributions in the same graph



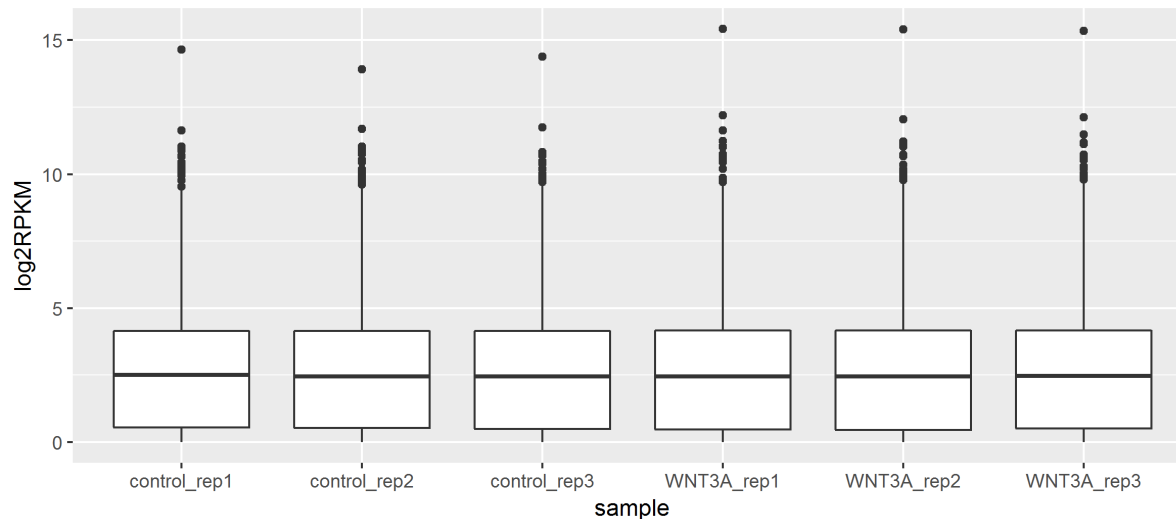
How to Create a Boxplot Using ggplot2

```
options(stringsAsFactors=F)

library(ggplot2)

inFile1 = 'pedersenLog2RPKM20180817.txt'
data1 = read.delim(inFile1)

ggplot(data1,aes(x = log2RPKM)) +
  geom_boxplot()
ggsave('boxplot1.png')
```



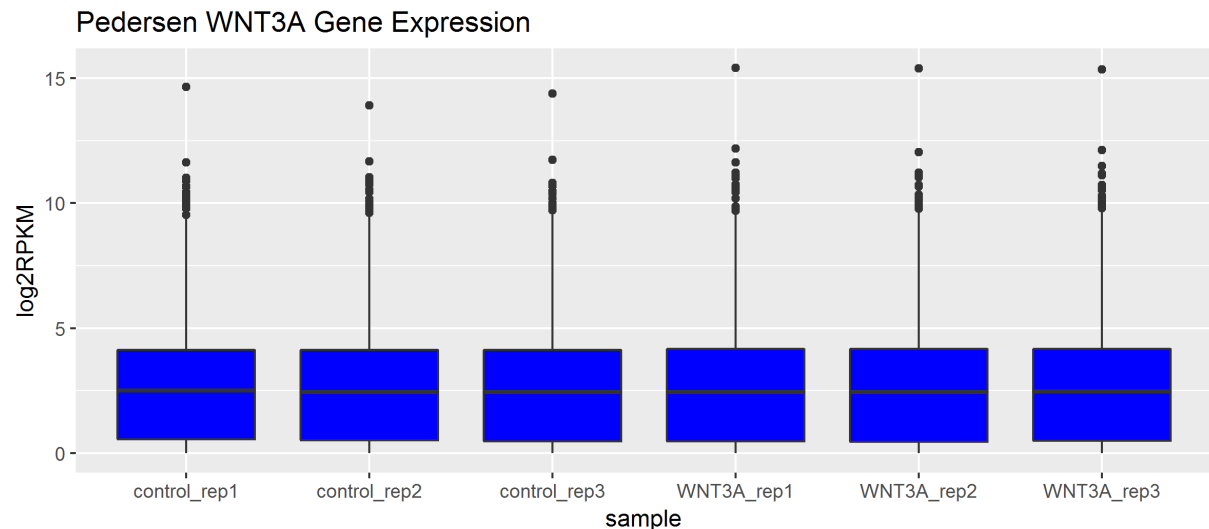
Exercise: Create a boxplot using the code from the previous page and update your visualization to have the following features:

1. Change the fill color

- HINT: `fill = 'blue'`

2. Add a title

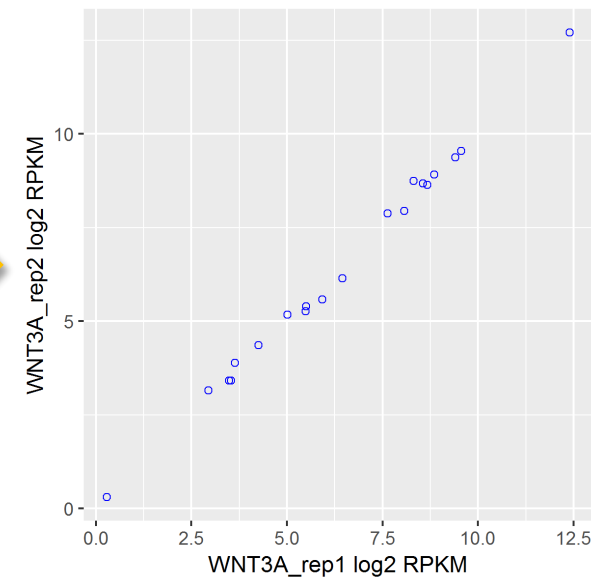
- HINT: `?labs`



Visualizing Data Using Scatter Plots

- Scatter Plots are visualizations that display two data values for the same measurement
 - example: two sample replicates expression values for each gene
- Data points that are not on the diagonal indicate disagreement
- We expect strong agreement between sample replicates

gene	WNT3A_rep1	WNT3A_rep2
A1BG	2.38	1.64
A1BG-AS1	0.83	0.58
A1CF	0.02	0.00
A2M	0.30	0.67
A2M-AS1	0.09	0.10
A2ML1	0.33	0.73
A2MP1	0.00	0.00
A4GALT	4.20	4.82
A4GNT	0.00	0.00
AAAS	5.57	5.53
AACS	2.70	2.52
AACSP1	0.13	0.15
AADAC	0.12	0.00
AADACL2	0.00	0.00
AADACL4	2.30	2.03
AADAT	0.94	1.19
AAED1	1.20	1.51
AAGAB	4.36	4.29
AAK1	1.68	1.97
AAMDC	4.41	3.78



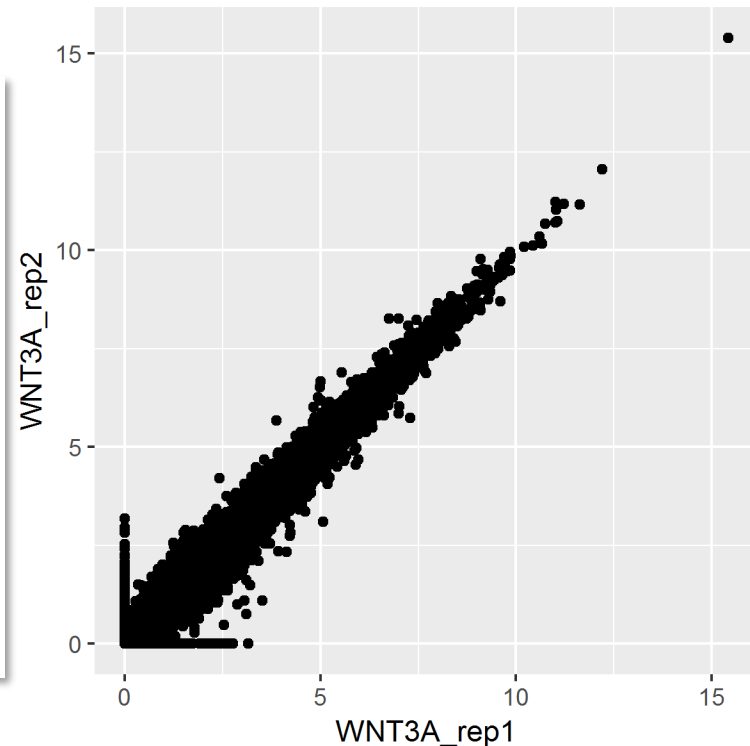
How to Create a Scatter Plot Using ggplot2

```
options(stringsAsFactors=F)

library(ggplot2)

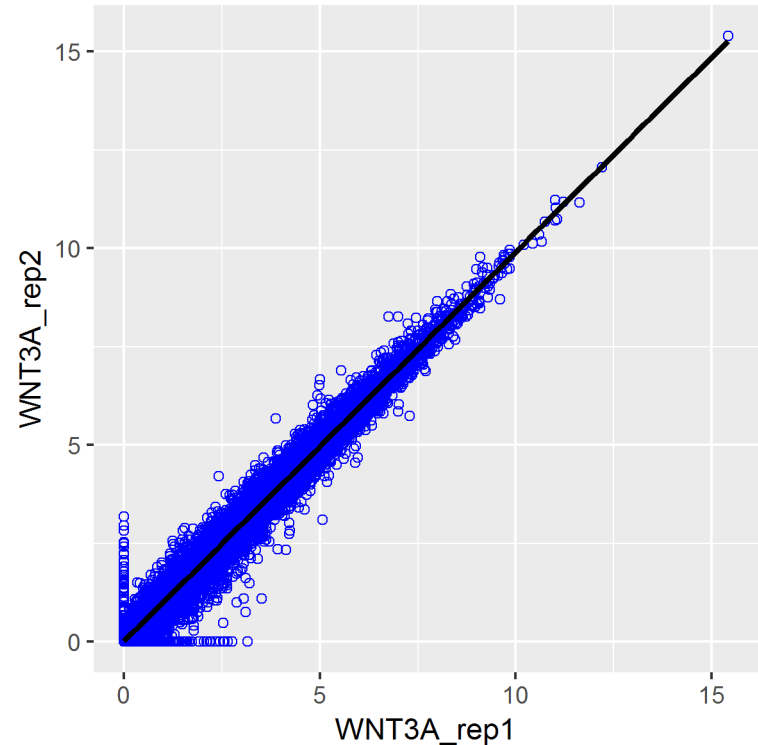
inFile2 = 'pedersenLog2RPKM_matrix20180817.txt'
data2 = read.delim(inFile2)

ggplot(data2,aes(x = WNT3A_rep1, y = WNT3A_rep2)) +
  geom_point()
ggsave('scatter2.png')
```



Exercise: Create a boxplot using the code from the previous page and update your visualization to have the following features:

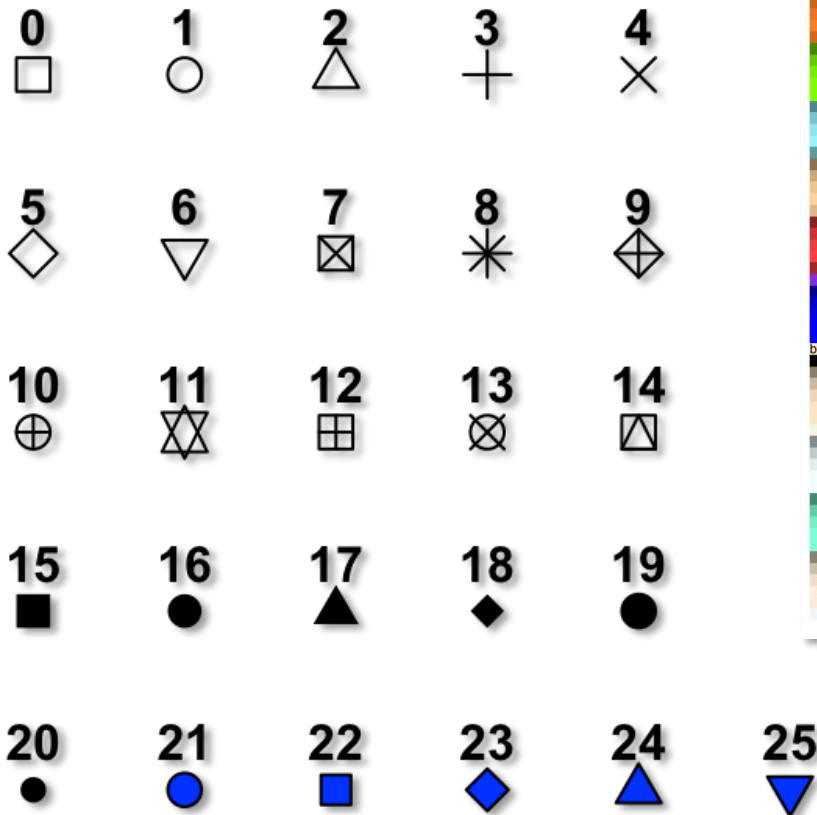
1. Change the color and shape of scatter plot points
 - HINT: color = 'blue'
 - HINT: shape = 1
2. Add a black linear regression line using a `geom_smooth` layer
 - HINT: `geom_smooth(method = lm)`



References

- Gentleman, Robert. R Programming for Bioinformatics. CRC Press, 2009.
- Slides Partially Sourced from Jacob Kitzman and Barry Grant

R Graphics Shapes



R Graphics Colors

grDevices::colors																			
coral3	deeppink4	gray27	gray87	gray98	lightpink1	mistyrose1	pink4	slategray1											
coral2	deeppink3	gray26	gray86	gray97	lightpink2	mistyrose2	pink3	slategray2											
coral1	deeppink2	gray25	gray85	gray96	lightpink3	mistyrose3	pink2	slategray3											
coral	deeppink1	gray24	gray84	gray95	lightpink4	mistyrose4	pink1	slategray4											
chocolate4	darkviolet	gray23	gray83	gray94	lightgreen	mediumslateblue	peru	slateblue1	yellowgreen										
chocolate3	darkturquoise	gray22	gray82	gray93	lightgoldenrod1	mediumspringgreen	peachpuff4	slateblue2	yellow2										
chocolate2	darkslategray4	gray21	gray81	gray92	lightgoldenrod2	mediumseagreen	peachpuff3	slateblue3	yellow3										
chocolate1	darkslategray3	gray20	gray80	gray91	lightgoldenrod3	mediumseagreen	peachpuff2	slateblue4	yellow4										
chocolate	darkslategray2	gray19	gray79	gray90	lightgoldenrod4	mediumslateblue	peachpuff1	slateblue5	yellow5										
chartreuse4	darkslategray1	gray18	gray78	gray89	lightcyan4	mediumslateblue	peachpuff	slateblue6	whitesmoke										
chartreuse3	darkslategray2	gray17	gray77	gray88	lightcyan3	mediumslateblue	peachpuff	slateblue7	wheat4										
chartreuse2	darkslategray1	gray16	gray76	gray87	lightcyan2	mediumslateblue	peachpuff	slateblue8	wheat3										
chartreuse1	darkslategray	gray15	gray75	gray86	lightcyan1	mediumslateblue	peachpuff	slateblue9	wheat2										
cadetblue4	darkseagreen4	gray14	gray74	gray85	lightcyan2	mediumslateblue	peachpuff	slateblue10	wheat1										
cadetblue3	darkseagreen3	gray13	gray73	gray84	lightcyan1	mediumslateblue	peachpuff	slateblue11	wheat										
cadetblue2	darkseagreen2	gray12	gray72	gray83	lightcyan2	mediumslateblue	peachpuff	slateblue12											
cadetblue1	darkseagreen1	gray11	gray71	gray82	lightcyan1	mediumslateblue	peachpuff	slateblue13											
cadetblue	darkseagreen	gray10	gray70	gray81	lightcyan2	mediumslateblue	peachpuff	slateblue14											
burlywood4	darksalmon	gray9	gray69	gray80	lightcyan1	mediumslateblue	peachpuff	slateblue15											
burlywood3	darkred	gray8	gray68	gray79	lightblue	mediumslateblue	peachpuff	slateblue16											
burlywood2	darkorchid4	gray7	gray67	gray78	lightblue	mediumslateblue	peachpuff	slateblue17											
burlywood1	darkorchid3	gray6	gray66	gray77	lightblue	mediumslateblue	peachpuff	slateblue18											
burlywood	darkorchid2	gray5	gray65	gray76	lightblue	mediumslateblue	peachpuff	slateblue19											
brown4	darkorchid1	gray4	gray64	gray75	lightblue	mediumslateblue	peachpuff	slateblue20											
brown3	darkorchid	gray3	gray63	gray74	lightblue	mediumslateblue	peachpuff	slateblue21											
brown2	darkorange4	gray2	gray62	gray73	lightblue	mediumslateblue	peachpuff	slateblue22											
brown1	darkorange3	gray1	gray61	gray72	lightblue	mediumslateblue	peachpuff	slateblue23											
brown	darkorange2	gray0	gray60	gray71	lightblue	mediumslateblue	peachpuff	slateblue24											
blues4	darkorange1	gray	gray59	gray70	lightblue	mediumslateblue	peachpuff	slateblue25											
blues3	darkolivegreen4	goldenrod4	gray58	gray69	lightblue	mediumslateblue	peachpuff	slateblue26											
blues2	darkolivegreen3	goldenrod3	gray57	gray68	lightblue	mediumslateblue	peachpuff	slateblue27											
blues1	darkolivegreen2	goldenrod2	gray56	gray67	lightblue	mediumslateblue	peachpuff	slateblue28											
blues	darkolivegreen1	goldenrod1	gray55	gray66	lightblue	mediumslateblue	peachpuff	slateblue29											
blanchedalmond	darkolivegreen	goldenrod	gray54	gray65	lightblue	mediumslateblue	peachpuff	slateblue30											
black	darkviolet	gold4	gray53	gray64	lightblue	mediumslateblue	peachpuff	slateblue31											
bisque4	darkviolet	gold3	gray52	gray63	lightblue	mediumslateblue	peachpuff	slateblue32											
bisque3	darkkhaki	gold2	gray51	gray62	lightblue	mediumslateblue	peachpuff	slateblue33											
bisque2	darkgray	gold1	gray50	gray61	lightblue	mediumslateblue	peachpuff	slateblue34											
bisque1	darkgray	ghostwhite	gray49	gray60	lightblue	mediumslateblue	peachpuff	slateblue35											
beige	darkgray	gainsboro	gray48	gray59	lightblue	mediumslateblue	peachpuff	slateblue36											
azure4	darkgoldenrod4	forestgreen	gray47	gray58	lightblue	mediumslateblue	peachpuff	slateblue37											
azure3	darkgoldenrod3	forestgreen	gray46	gray57	lightblue	mediumslateblue	peachpuff	slateblue38											
azure2	darkgoldenrod2	forestgreen	gray45	gray56	lightblue	mediumslateblue	peachpuff	slateblue39											
azure1	darkgoldenrod1	forestgreen	gray44	gray55	lightblue	mediumslateblue	peachpuff	slateblue40											
azure	darkcyan	forestgreen	gray43	gray54	lightblue	mediumslateblue	peachpuff	slateblue41											
aquamarine4	darkcyan	forestgreen	gray42	gray53	lightblue	mediumslateblue	peachpuff	slateblue42											
aquamarine3	cyan4	dodgerblue4	gray41	gray52	lightblue	mediumslateblue	peachpuff	slateblue43											
aquamarine2	cyan3	dodgerblue3	gray40	gray51	lightblue	mediumslateblue	peachpuff	slateblue44											
aquamarine1	cyan2	dodgerblue2	gray39	gray50	lightblue	mediumslateblue	peachpuff	slateblue45											
aquamarine	cyan1	dodgerblue1	gray38	gray49	lightblue	mediumslateblue	peachpuff	slateblue46											
antiquewhite4	cornsilk4	dimgrey	gray37	gray48	lightblue	mediumslateblue	peachpuff	slateblue47											
antiquewhite3	cornsilk3	dimgrey	gray36	gray47	lightblue	mediumslateblue	peachpuff	slateblue48											
antiquewhite2	cornsilk2	deeppink4	gray35	gray46	lightblue	mediumslateblue	peachpuff	slateblue49											
antiquewhite1	cornsilk1	deeppink3	gray34	gray45	lightblue	mediumslateblue	peachpuff	slateblue50											
antiquewhite	cornsilk	deeppink2	gray33	gray44	lightblue	mediumslateblue	peachpuff	slateblue51											
aliceblue	cornflowerblue	deeppink1	gray32	gray43	lightblue	mediumslateblue	peachpuff	slateblue52											
white	coral4	deeppink	gray31	gray42	lightblue	mediumslateblue	peachpuff	slateblue53											
	coral3	deeppink3	gray30	gray41	lightblue	mediumslateblue	peachpuff	slateblue54											
	coral2	deeppink2	gray29	gray40	lightblue	mediumslateblue	peachpuff	slateblue55											
	coral1	deeppink1	gray28	gray39	lightblue	mediumslateblue	peachpuff	slateblue56											

<http://bc.bojanorama.pl/wp-content/uploads/2013/04/rcolorsheet.pdf>