## Introduction to programming in R: Day 3

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## **Learning objectives**

- Functions
  - User defined functions
  - using anonymous function in apply()
- Introduction to Bioconductor
  - Bioconductor in genomic research
  - Common packages
- Data wrangling
  - aggregate()
  - other functions()
- Plotting using ggplot
- From here to where?

#### **User defined functions**

- $\bullet$  One of the great strengths of R is the user's ability to add functions.
- many of the built-in functions in R are actually functions of functions.

```
myfunction <- function(arg1, arg2, ...){
   statement 1
   statement 2
   statements ...
   return(object)
}</pre>
```

- Objects in the function are local to the function
- can return only one object
  - object returned can be any data type.

## **Example:** to calculate area of the circle

 Create a function that accepts radius of a circle and returns area of the circle.

```
# area of a circle = pi * r^2
area_circle <- function(r){
   area <- pi * r^2;
   return(area);
}
area_circle(1)</pre>
```

```
## [1] 3.141593
```

contd...

## **Example**

```
## Using the function with a vector
x <- c(1,0.5,12)
area_circle(x)

## [1] 3.1415927 0.7853982 452.3893421
## using with sapply()
sapply( x, area_circle )</pre>
```

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## [1] 3.1415927 0.7853982 452.3893421

# **Example:** function for calculating area of a traingle

 Create a function that accepts height, width and base of a triangle and returns area.

```
# area of a traingle = height * base * width
area_triangle <- function(h ,b ,w){
   area <- h*b*w;
   return(area);
}
area_triangle(12,4,9)</pre>
```

## [1] 432

contd..

# **Example:** function for calculating area of a traingle

```
## equal length vectors can be used as input
h < -c(3,4,10,9)
b < c(5,6,10,19)
w \leftarrow c(1,10,12,8)
area_triangle(h=h, b=b, w=w)
```

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## [1] 15 240 1200 1368

# **User defined functions: return()**

- the return() state ment returns the final processed value
  - multiple values can be packages as
  - Named vector
  - Named List
  - Data frame

# Return different values: a named vector

circumference <- 2 \* pi \* r; # 2 x pi x r

## return as a named vector

area <- pi \* r^2; # pi x r^2

circle <- function(r){

return(result);

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```
##Use the function to calculate for r=1
circle(r=1)

## area circumference
## 3.141593 6.283185

##Use the function to calculate for r= a vector
circle(r=c(1,0.5))

## area1 area2 circumference1 circumference2
```

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result <- c(area=area, circumference=circumference)

### Return different values: as a named list

```
## return as a named vector
circle <- function(r){
  area <- pi * r^2; # pi x r^2
  circumference <- 2 * pi * r; # 2 x pi x r
  result <- list(
    area=area,
    circumference=circumference
  return(result);
}
##Use the function to calculate for r=1
circle(r=1);
```

```
## $area
## [1] 3.141593
##
```

## Return different values: As a named data frame

```
## return as a named vector
circle <- function(r){
 area <- pi * r^2; # pi x r^2
 circumference <- 2 * pi * r; # 2 x pi x r
 result <- data.frame(
   area=area,
    circumference=circumference
 return(result);
}
# Use the function
circle(r=1);
## area circumference
```

circle(c(1,0.5))

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## 1 3.141593 6.283185

## **Anonymous functions**

- anonymous functions are not saved with a name.
- we do not want to reuse the code
- Usually written inline and used with apply functions

```
marks <- matrix(sample(1:100, 20,replace=T), nrow=4)
colnames(marks) <- c('Physics','Chemistry','Math','Geography'
rownames(marks) <- c('foo','bar','baz','qux')
marks <- as.data.frame(marks)
head(marks)</pre>
```

##	Physics	Chemistry	Math	Geography	Literature	
## foo	40	89	63	52	14	
## bar	7	70	12	50	71	
## baz	34	53	78	37	62	
## aux	7	4	45	87	14	

## **Anonymous functions**

using with apply() to calulate percentage

```
marks$Percentage <- apply(marks, 1,
      function(x){
       return(100*sum(x)/500)
       }
head(marks)
```

```
##
       Physics Chemistry Math Geography Literature Percentage
## foo
            40
                       89
                            63
                                       52
                                                  14
                                                            51.6
                       70
                            12
                                       50
                                                  71
                                                            42.0
## bar
```

53 78 37 62 52.8 45 87 14 31.4

## baz

## qux

34

# Anonymous functions using paste()

```
genes <- c('AT04G1245', 'AT01G9987', 'AT01G9861',
          'AT01G8761', 'ATCh12301')
## Add a Prefix 'Ath/' to all genes
pre_genes <- sapply(genes, function(x){</pre>
  return(paste('Ath',x,sep='|'))
 } )
pre_genes
##
        AT04G1245 AT01G9987 AT01G9861
                                                       AT010
```

## "Ath|AT04G1245" "Ath|AT01G9987" "Ath|AT01G9861" "Ath|AT01G

ATCh12301 ## "Ath|ATCh12301"

##

# **Anonymous functions using strsplit()**

```
## Split Ath from the pre_genes elements
sapply(pre_genes, function(x){
   return(strsplit(x,split="|", fixed=TRUE)[[1]][2] )
} )
```

## AT04G1245 AT01G9987 AT01G9861 AT01G8761 ATCh12301

# **Data Wrangling**

- Manipulating raw data tables in to more structured format.
- Subletting tables
- 2 Sorting tables
- Merging two tables
- Aggregating Data
- Seshaping tables

# **Sorting tables**

- To sort a vector/dataframe in R, use the order() function.
  - returns the vector indexes
  - Default: sorting is ASCENDING.
  - With minus sign (-) Pre-fixed to a variable indicates DESCENDING order.

```
x <- c(10,8,6,8,14)
x
## [1] 10 8 6 8 14
```

```
# Returns increasing indexes
order(x)
```

```
## [1] 3 2 4 1 5
x[order(x)]
```

## [1] 6 8 8 10 14

# **Sorting tables**

```
df <- data.frame(
    A=c(10,2,18,50),
    B=c(4,2,12,10),
    C=c(1,3,9,5)
)
df
## A B C
## 1 10 4 1</pre>
```

## 3 18 12 9 ## 4 50 10 5

# **Sorting tables**

```
df[order(df$A, decreasing=TRUE), ]
```

```
## 4 50 10 5
## 3 18 12 9
## 1 10 4 1
## 2 2 2 3
```

## A B C

# **Compute Summary Statistics of Data Subsets**

 aggregate(): computes summary statistics for each category women\_data <- read.csv('Data/women.tsv', sep="\t")</pre> # head(women data, 10) ### Calculate mean Heights for each Age category aggregate( x=women\_data[, c('Height','Weight')] , by=list(Women age=women data\$Age ), mean)

```
## Women_age Height Weight
## 1 adult 63.49032 165.84553
## 2 child 53.90876 92.57694
```

### **Merging Data tables**

• merge (): merge two data frames (datasets) horizontally by one or more common key variables (i.e., an inner join).

```
## Gene TPM
## 1 FLC 0.50
## 2 FT 2.30
## 3 AG 12.00
## 4 LFY 9.10
## 5 CO 21.03
## 6 AP1 14.00
```

## **Merging Data tables**

```
## Gene descriptions
desc <- data.frame(Gene=c('FLC', 'FT', 'AG', 'LFY',</pre>
                           'CO', 'AP1', 'EIN2', 'ABI1', 'ABI3'),
                    AA len= c(143,144,51,291,
                               101.50.33.90.105).
                    Description=c('Repressor of flowers',
                       'Mobile floral stimulus protein',
                       'Regulator of floral identity',
                        'Regulator of flowering',
                        'Regulator of flowering',
                        'Regulator of floral organ identity',
                        'Ethylene receptor',
                        'Abscisic acid signaling repressor',
                        'Abscisic acid response regulator')
```

head(desc)

## **Merging Data tables**

#### Merged table

```
head(merge(exprs,desc, by="Gene"))
##
     Gene
            TPM AA len
                                               Description
                              Regulator of floral identity
## 1
       AG 12.00
                    51
     AP1 14.00
                       Regulator of floral organ identity
## 2
      CO 21.03
                   101
                                    Regulator of flowering
## 3
     FI.C
         0.50
                   143
## 4
                                      Repressor of flowers
## 5
     FT 2.30
                   144
                            Mobile floral stimulus protein
     I.FY 9.10
                   291
## 6
                                    Regulator of flowering
```

# Subsetting using %in% operator

- %in% is a very handy operator to match two vectors.
  - $\bullet$  x %in% y: compares each elements of x in its left vector y
  - returns a logical vector if a value from x is found in y

```
x <- c('a','c','b','p','o','x')
y <- c('a','b','x','c','d','e')
x %in% y</pre>
```

```
## [1] TRUE TRUE TRUE FALSE FALSE TRUE
```

```
# y %in% x ## try
## Subset a named dataframe
exprs[exprs$Gene %in% c('AP1','FT','LFY'), ]
```

```
## Gene TPM
## 2 FT 2.3
## 4 LFY 9.1
## 6 AP1 14.0
```

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### **Data arrangements**

#### Wide and Two-Dimensional: The Spreadsheet

## 3 Gene3 7.581 2.1 0.0 ## 4 Gene4 9.747 4.2 9.6

## 5 Gene5 9.747 4.2

```
df <- data.frame(</pre>
 Genes=c('Gene1','Gene2','Gene3','Gene4','Gene5'),
 Sample1=sample(seq(0,10, by=1.083), size = 5, replace = T)
 Sample2=sample(seq(0,10, by=2.1), size = 5, replace = T),
 Sample3=sample(seq(0,10, by=3.2), size = 5, replace = T)
df
##
    Genes Sample1 Sample2 Sample3
## 1 Gene1 6.498
                      4.2
                              0.0
## 2 Gene2 1.083 8.4 3.2
```

6.4

### **Data arrangements**

#### Long and Skinny: The Database

## Using Genes as id variables

#### head(df2)

```
## Genes Sample Expression
## 1 Gene1 Sample1 6.498
## 2 Gene2 Sample1 1.083
## 3 Gene3 Sample1 7.581
## 4 Gene4 Sample1 9.747
## 5 Gene5 Sample1 9.747
## 6 Gene1 Sample2 4.200
```

### **Data arrangements**

- Wide format is more human readable and easy to interpret
- Long format is best for data analysis
  - suitable for ggplot2 plots

```
https://eagereyes.org/basics/
spreadsheet-thinking-vs-database-thinking
```

# Package: reshape2

- to convert Spreadsheet tables to long Database format and vice versa.
  - melt(): wide => long
  - cast() : long => wide

install.packages('reshape2')

# **Melting and Casting DFs**

### melt()

```
library(reshape2)
## use melt function
melt(df. id="Genes")
     Genes variable value
##
## 1 Gene1 Sample1 6.498
## 2 Gene2 Sample1 1.083
## 3 Gene3 Sample1 7.581
            Sample 1 9.747
## 4 Gene4
## 5 Gene5
            Sample 1 9.747
## 6
    Gene1
            Sample2 4.200
## 7 Gene2
             Sample2 8.400
## 8
    Gene3
            Sample2 2.100
## 9
     Gene4
            Sample2 4.200
  10 Gene5
            Sample2 4.200
             Sample 3 0 000
     Canal
```

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# **Melting and Casting DFs**

### cast()

```
## Genes ~ Sample: Describe Genes vs Sample
dcast(df2, formula=Genes ~ Sample)

## Using Expression as value column: use value.var to override
## Genes Sample1 Sample2 Sample3

## 1 Gene1 6.498 4.2 0.0

## 2 Gene2 1.083 8.4 3.2

## 3 Gene3 7.581 2.1 0.0

## 4 Gene4 9.747 4.2 9.6

## 5 Gene5 9.747 4.2 6.4
```

# Data visualization using 'ggplot2'

- de facto standard for data visualization.
- Design publication quality plots with less typing .
- Gives fine control over graphical elements using functions ('grammar') to build final image
- Create images as layers; easy to reuse previous codes.

## The graphical grammar and its components

Every graphical plot has following components

- Data

The raw data table you want to plot

- Layer

The plots (points, text, lines )

- Scale

Maps data to the graphical output.

- Coordinates

Visualization perspective (rotation, angle)

- Faceting

Sub grouping the plots by categories.

- Themes

Display details (Font, background color, font size)

# Working with ggplo2 grammar

- Data: Raw data in Database format.
  - required
- Aesthetics: Mapping your data to the visualization
  - required
- Layers: Creates the real visualization using the data and aesthetics provided.
  - at least one layer is required.
  - usually have a pre-fix geom\_<layer name>

## Create a simple XY scatter plot

### Scatter plot using base R

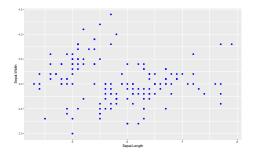
```
plot(
   x=iris$Sepal.Length,
   y=iris$Sepal.Width,
   col='blue'
                        ris$Sepal.Wdth
                                     5.0
                                                                 7.5
                                               iris$Sepal.Length
```

## Create a simple XY scatter plot

#### Scatter plot using ggplot2 lib

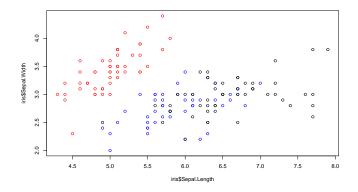
```
# import library
library(ggplot2)
# Data and define aesthetics
p <- ggplot(data=iris,</pre>
            aes(x=Sepal.Length, y=Sepal.Width )
# add (+) scatterplot layer to visualize
p + geom point(color='blue')
```

# Create a simple XY scatter plot



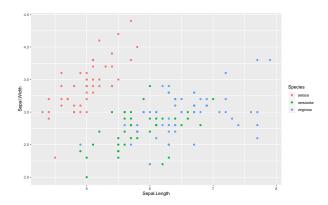
#### Base R plot

```
# color to each species
levels(iris$Species)
## [1] "setosa" "versicolor" "virginica"
color_point <- function(x){</pre>
  ifelse(x=="setosa", 'red',
         ifelse(x=="versicolor",'blue','black')
plot(
  x=iris$Sepal.Length,
  y=iris$Sepal.Width,
  col= sapply(iris$Species,color_point )
```



#### Using ggplot2

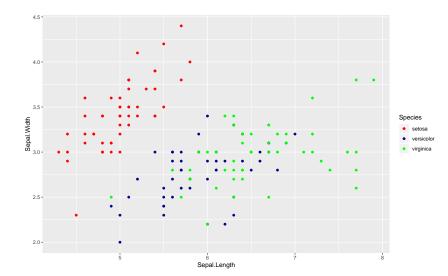
```
# import library
library(ggplot2)
# Data and define aesthetics (x, y, color)
p <- ggplot(data=iris,</pre>
            aes(x=Sepal.Length, y=Sepal.Width, color=Species)
# add (+) scatterplot layer to visualize
p + geom point()
```



### Add user-defined colors

```
# define colors for each category as a vector
species_color <- c('red', 'green', 'darkblue')</pre>
## Define category names to each color
names(species color) <- c("setosa", "virginica", "versicolor")</pre>
species color
##
       setosa virginica versicolor
        "red"
              "green" "darkblue"
##
  use this vector to fill color
# Use this mappings to scale color manual() layer
p + geom point() +
  scale_color_manual(values =species_color)
```

### Add user-defined colors

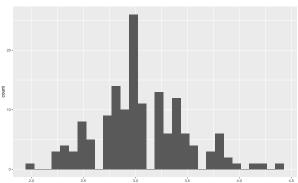


# Plot histogram geom\_histogram()

```
# import library
library(ggplot2)
# Data and define aesthetics
p <- ggplot(data=iris,</pre>
            aes (x=Sepal.Width)
# add (+) histogram layer to visualize
p + geom_histogram()
```

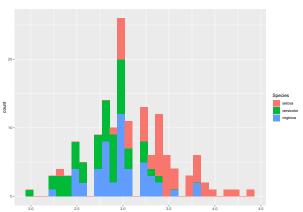
# Plot histogram geom\_histogram()

## `stat\_bin()` using `bins = 30`. Pick better value with `bin

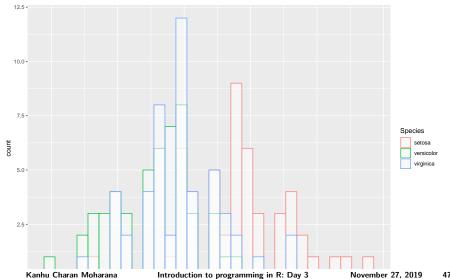


```
# add (+) histogram layer define color category
p + geom_histogram(aes(fill=Species))
```

## `stat\_bin()` using `bins = 30`. Pick better value with `bin

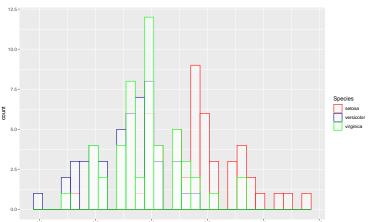


## `stat\_bin()` using `bins = 30`. Pick better value with `bin



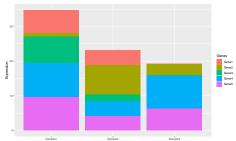
```
##
       setosa virginica versicolor
##
        "red"
                 "green" "darkblue"
# add (+) histogram layer + user defied color scale + theme
p + geom histogram(
  aes(color=Species),
  fill='white'.
  alpha=0.5,
  position="identity"
  ) +
  scale_color_manual(values =species_color)
```

## `stat\_bin()` using `bins = 30`. Pick better value with `bin



Sepal.Width

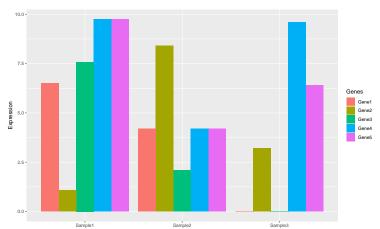
## Multiple category bar plots



## Multiple category bar plots

with deafult options ggplot created a stacked bar.

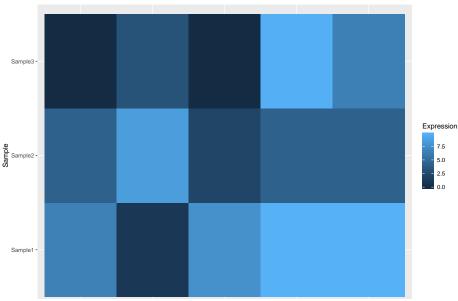
```
# Transform the position of the categories.
p + geom_bar(stat="identity", position="dodge")
```



# **Heatmaps using geom\_tile()**

• Use the values as Fill asthestics

# **Heatmaps using geom\_tile()**

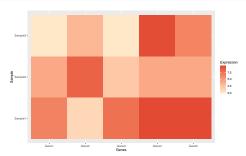


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# **Heatmaps using geom\_tile()**

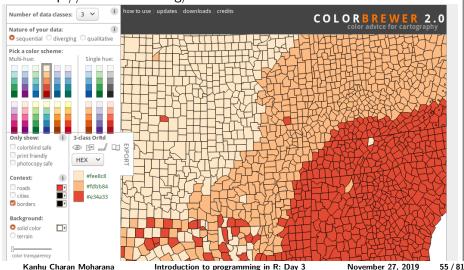
- Use the values as Fill asthestics
- Use specific color range

```
## Add a color gradient scale
p + geom_tile() +
    scale_fill_gradient(low = "#fee8c8", high = "#e34a33")
```



Online: ColorBrewer2.0

URL: http://colorbrewer2.org/



Online: ColorBrewer2.0

Select color from three group

- 1. Sequential: Light colors for low data, dark for high data
- 2. **Diverging**: Light colors for mid-range data, low and high contrasting dark colors
- 3. Qualitative: Co lours designed to give maximum visual difference

between classes

#### R Packages

#### **RColorBrewer**

R packages that uses the scheme from ColorBrewer2.0

```
# load package
library(RColorBrewer)

# get the colors as a vector
brewer.pal(n = 3,name = 'YlOrRd')

## [1] "#FFEDAO" "#FEB24C" "#F03B2O"
brewer.pal(n = 5, name = 'Blues')

## [1] "#EFF3FF" "#BDD7E7" "#6BAED6" "#3182BD" "#08519C"
```

# **Choosing Color Palettes: RColorBrewer**

#### **Example of Pallates**

brewer.pal.info

	maxcolors	category	colorblind
BrBG	11	div	TRUE
RdBu	11	div	TRUE
Accent	8	qual	FALSE
Set2	8	qual	TRUE
Greens	9	seq	TRUE
YIOrBr	9	seq	TRUE

# **Choosing Color Palettes: RColorBrewer**

### To view the color palette

```
## display a divergent palette
display.brewer.pal(7,"BrBG")
```



## display a qualitative palette
display.brewer.pal(7,"Accent")



## display a Sequential palette
display.brewer.pal(name="Y10rRd", n=8)



### R Packages

### ggsci

 color palettes inspired by plots in scientific journals, data visualization libraries, science fiction movies, and TV shows.

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URI · https://cran.r-project.org/web/packages/ggsci/vignettes/ggsci.html

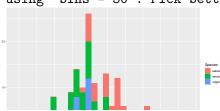
```
# Install package
install.packages('ggsci')
```

- ## load library
- library(ggsci) # NPG: Nature Publishing Group pal\_npg()(5)
- ## [1] "#E64B35FF" "#4DBBD5FF" "#00A087FF" "#3C5488FF" "#F39B he New England Journal of Medicine Kanhu Charan Moharana

### Default ggplot2 color scheme

```
# import library
library(ggplot2)
# Data and define aesthetics
p <- ggplot(data=iris,</pre>
            aes(x=Sepal.Width, fill=Species)
# add (+) histogram layer to visualize
p + geom histogram()
```

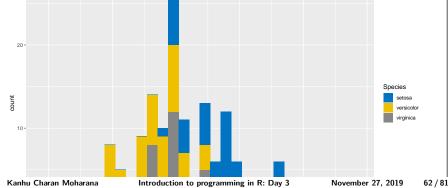
## `stat\_bin()` using `bins = 30`. Pick better value with `bin



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```
ggsci: JCO color scheme
```

```
# Journal of Clinical Oncology
p + geom_histogram()+
    scale_fill_jco()
## `stat_bin()` using `bins = 30`. Pick better value with `bin
```

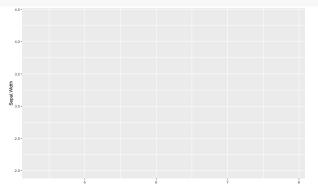


# ggsci: Lancet color scheme

```
# Lancet Oncology
p + geom_histogram()+
  scale_fill_lancet()
## `stat_bin()` using `bins = 30`. Pick better value with `bin
     20 -
                                                                 Species
                                                                   setosa
                                                                   versicolor
                                                                   virginica
     10-
```

#### Default theme

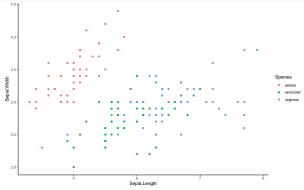




### theme\_classic()

A classic-looking theme, with  $\boldsymbol{x}$  and  $\boldsymbol{y}$  axis lines and no gridlines.

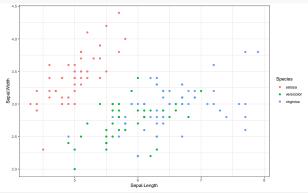
```
# add (+) scatterplot layer + black-white theme
p + geom_point() + theme_classic()
```



### theme\_bw()

The classic dark-on-light ggplot2 theme.

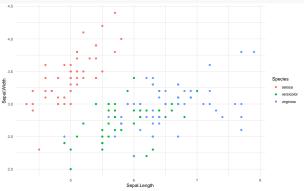
```
# add (+) scatterplot layer + black-white theme
p + geom_point() + theme_bw()
```



### theme\_minimal():

A minimalistic theme with no background annotations.

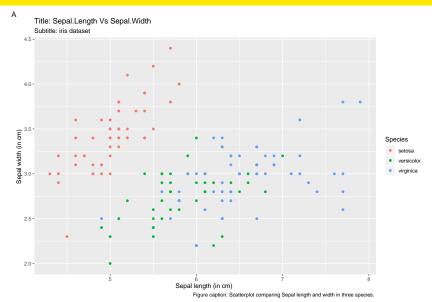
```
# add (+) scatterplot layer + minimalistic theme
p + geom_point() + theme_minimal()
```



# Modify axis, legend, and plot labels

```
p + geom_point()+
  labs(
    title="Title: Sepal.Length Vs Sepal.Width",
       subtitle="Subtitle: iris dataset",
       tag="A",
       caption="Figure caption: Scatterplot comparing Sepal le
  ylab("Sepal width (in cm) ")+
  xlab("Sepal length (in cm) ")
```

# Modify axis, legend, and plot labels



### Multiple plots

#### Create figures and save in a variable

```
library(ggplot2)

p1 <- ggplot(data=iris, aes(x=Sepal.Width,y=Sepal.Length, col
    geom_point()+theme(legend.position="top")+
    labs(tag='A')
p1</pre>
```



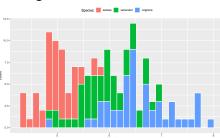
### Multiple plots

#### Create figures and save in a variable

```
p2 <- ggplot(data=iris, aes(x=Sepal.Length,fill=Species)) +
   geom_histogram(color='white')+
   theme(legend.position="top")+
   labs(tag='B')</pre>
```

p2

## `stat\_bin()` using `bins = 30`. Pick better value with `bin

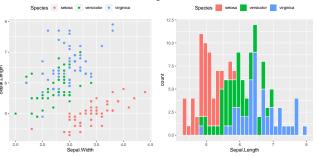


### Multiple plots

#### Create joint figures

```
library(gridExtra)
grid.arrange(p1,p2, nrow=1, respect=TRUE, clip=TRUE)
```

##  $\operatorname{stat\_bin}()$  using  $\operatorname{bins} = 30$ . Pick better value with  $\operatorname{bin}$ 



# Add tables with figures

```
## Table as a data frame
df <- data.frame(Genes=c('PHYB', 'PHYA', 'CRY1', 'COP1', 'HY5')</pre>
                  Sample_a=seq(5,20, length=5),
                  Sample b=sample(seq(1,20), 5)
row.names(df) <- df$Genes
exprs <- melt(df)
## Using Genes as id variables
```

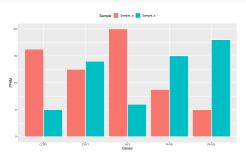
Introduction to programming in R: Day 3

names(exprs) <- c('Genes', 'Sample', 'FPKM')</pre>

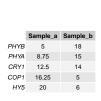
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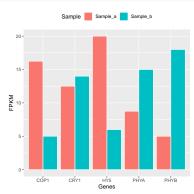
## Add tables with figures

```
## Graphical object
p1 <- ggplot(data=exprs, aes(x=Genes, y=FPKM, fill=Sample))+
   geom_bar(stat="identity", position="dodge", color='white')+
   theme(legend.position="top")
p1</pre>
```



## Add tables with figures





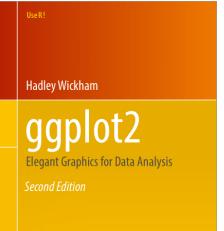
## Save plot to file

## More on ggplot2

ggplot2: Elegant Graphics for Data Analysis

Hadley Wickham

Free e-book: https://ggplot2-book.org/



# **Bio-conductor: Bioinformatics repository**

- Bioconductor (www.bioconductor.org)is a project within R.
- Bioconductor works with a high throughput genomic data from DNA sequence, micro-array, proteomics, imaging and a number of other data types.
- The current available version of Bioconductor (Version 3.6) consists of 1477 software packages.

### Why

- dependencies: It makes very easy to resolve library dependencies.
- compatibility: Correct and compatible versions of all the dependencies are installed.
- Reliability: Packages in Bioconductor are (reasonably) reliable.
   Compiled every day by someone around the world. So any error will be reported.

## **Bio-conductor: Bioinformatics repository**

#### Install core packages

```
install.packages("BiocManager")
```

#### Install a package from Bioconductor

```
BiocManager::install('ape')
```

#### Update all packages

```
BiocManager::install()
```

# Where to go from here?

- Take your data, import in R
- Search Internet
  - How to do ... use keyword R
  - Stack overflow, R-blogger
- Follow YouTube channels, blog sites
  - twitter hash tags:
  - #rstats
  - one R tip per day (@RLangTip)
- Print a cheatsheet
- Each day practice a little.

# Thank you for participating

- Email kcm.eid@gmail.com
- Twitter @kc\_moharana

