

Intro to R and Bioconductor

HMS RESEARCH COMPUTING



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<https://rc.hms.harvard.edu/>

What can you do with R?

- R is a statistical language
- R is free!
- Bioconductor - thousands of packages for your workflow
- Heavy duty statistics!
- Make nice plots!



Course Objectives

- Learn to run R on O2
- Gain familiarity with R language
- Learn to import/export data
- Simple Statistics/Plotting



Notation

O2 Bash

```
[wgr4@compute-a-16-162 ~]$  
[wgr4@compute-a-16-162 ~]$ module load gcc/6.2.0  
[wgr4@compute-a-16-162 ~]$ module load R/4.0.1  
[wgr4@compute-a-16-162 ~]$ R  
  
R version 4.0.1 (2020-06-06) -- "See Things Now"  
Copyright (C) 2020 The R Foundation for Statistical Computing  
Platform: x86_64-pc-linux-gnu (64-bit)  
  
R is free software and comes with ABSOLUTELY NO WARRANTY.  
You are welcome to redistribute it under certain conditions.  
Type 'license()' or 'licence()' for distribution details.  
  
Natural language support but running in an English locale  
  
R is a collaborative project with many contributors.  
Type 'contributors()' for more information and  
'citation()' on how to cite R or R packages in publications.  
  
Type 'demo()' for some demos, 'help()' for on-line help, or  
'help.start()' for an HTML browser interface to help.  
Type 'q()' to quit R.  
  
> |
```



Notation

```
[wgr4@compute-a-16-162 ~]$  
[wgr4@compute-a-16-162 ~]$ module load gcc/6.2.0  
[wgr4@compute-a-16-162 ~]$ module load R/4.0.1  
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Type 'q()' to quit R.
```

In R

> |



Notation

Blue content: try it out!



R on O2

- Open a high-memory R session – **better than a desktop!**
- Log in to O2 with X11 enabled (important for graphics)
- Mac (XQuartz must be installed)
 - `ssh -XY user123@o2.hms.harvard.edu`
- Linux
 - `ssh -XY user123@o2.hms.harvard.edu`
- Windows: MobaXterm has X11 client built-in
 - `ssh -XY user123@o2.hms.harvard.edu`



SLURM and O2

- SLURM is how we interact with the cluster
- Simple interactive session:

```
mfk8@login01:~$ srun --pty -p interactive -t 0-2:00 --mem 8G --x11 -c 1 bash
```



SLURM and O2

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```
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```

Walltime
(DD-HH:MM)



SLURM and O2

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```
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```

Memory
(units:K|M|G|T)



SLURM and O2

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```
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```

Enables X11



SLURM and O2

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- Simple interactive session:

```
mfk8@login01:~$ srun --pty -p interactive -t 0-2:00 --mem 8G --x11 -c 1 bash
```

CPU



SLURM and O2

- SLURM is how we interact with the cluster
- Simple interactive session:

```
mfk8@login01:~$ srun --pty -p interactive -t 0-2:00 --mem 8G --x11 -c 1 bash
```

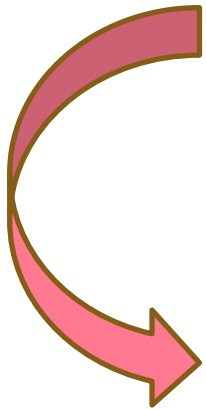
Unix shell



SLURM and O2

- SLURM is how we interact with the cluster
- Simple interactive session:

mfk8@login01:~\$ **srun --pty -p interactive -t 0-2:00 --mem 8G --x11 -c 1 bash**



mfk8@compute-a :~\$



R Versions on O2

mfk8@compute-a :~\$ module spider R

- Why does it matter what version of R you run?
- Downstream packages may only work with certain versions of R.
- How to load a version of R

mfk8@compute-a :~\$ module load gcc/6.2.0 R/version

- How to unload a version R

mfk8@compute-a :~\$ module load gcc/6.2.0 R/version

- Starting R from an interactive (not login!)

mfk8@compute-a:~\$ R



Managing your R packages on O2

- An R Personal Library is required on O2
- You must create an R Personal Library per version.
- Let's set up an R Personal Library in 3 simple steps!
 - **R/4.0.1** (most current version on O2)



Managing your R packages on O2

- Let's set up an R Personal Library in 3 simple steps!
 - **R/4.0.1** (most current version on O2)

- **1) Create an R Personal Library directory**

```
mfk8@compute-a:~$ mkdir -p ~/R-4.0.1
```

- **2) Export the R_LIBS_USER variable**

```
mfk8@compute-a:~$ export R_LIBS_USER="~/R-4.0.1"
```

- **3) Create .Renviron file**

```
mfk8@compute-a:~$ echo 'R_LIBS_USER="~/R-4.0.1"'> $HOME/.Renviron
```



Loading the latest R version on O2

Starting R from an interactive (not login!)

```
mfk8@compute-a :~$ module load gcc/6.2.0 R/4.0.1
```

```
mfk8@compute-a:~$ R
```



Installing Packages



Installing R packages from four general “bins”



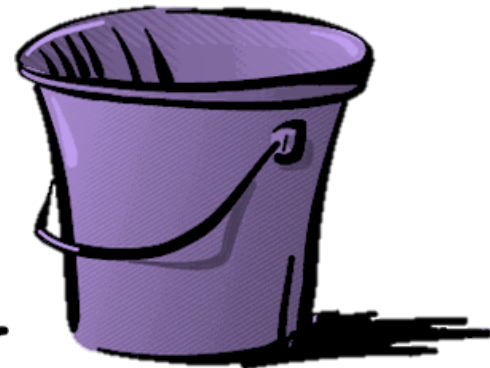
Source



Bioconductor



CRAN



GitHub



Installing packages from Source

- If you must manually download a package, you can place the package inside the R Personal Library directory (e.g., ~/R-4.0.1)
- Accessing packages manually uploaded to your O2 R library (first time)
> install.packages("name-of-your-package")



Source



Installing packages from Bioconductor

- *“Bioconductor provides tools for the analysis and comprehension of high-throughput genomic data.”*
- **1) Install BiocManager (only once)**
 - > `install.packages("BiocManager")`**
- **2) Install a package from Bioconductor using BiocManager**
 - > `BiocManager::install("PackageName")`**



Bioconductor



Installing packages through CRAN

- CRAN is the *Comprehensive R Archive Network*
- **1) First time install of a package:**
 - > install.packages("PackageName")**
- **2) Select a mirror from or near the country you're in**



CRAN



Installing packages through Github

- First, you must install the R package “devtools”
 > install.packages(“devtools”)
- Second, load the installed package
 > library(“devtools”)
- Finally, use the install_github function
 > install_github(“repo/package”)



GitHub



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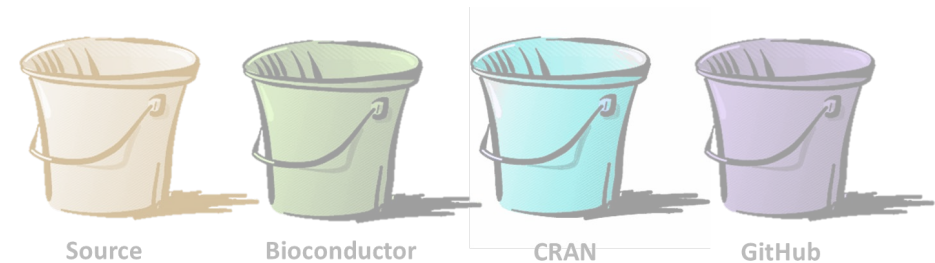
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24

Loading an R package

- After installing a package, you must use the *library* function to load it

> library("PackageName")



Exercise: Install and load the biomaRt package from Bioconductor

- Hint: the BiocManager and library functions are required!



Exercise: Install and load the biomaRt package from Bioconductor

- Hint: the BiocManager and library functions are required!
 - > **BiocManager::install("biomaRt")**
 - > **library("biomaRt")**



R documentation

- General R help on a function

?name_of_function

help(name_of_function)

- For example:

> ?t.test

Note: press “q” to exit the help view



Setting your R “working directory”

- Prints current working directory:
 > **getwd()**
- Setting your WD:
 > **setwd(“path”)**



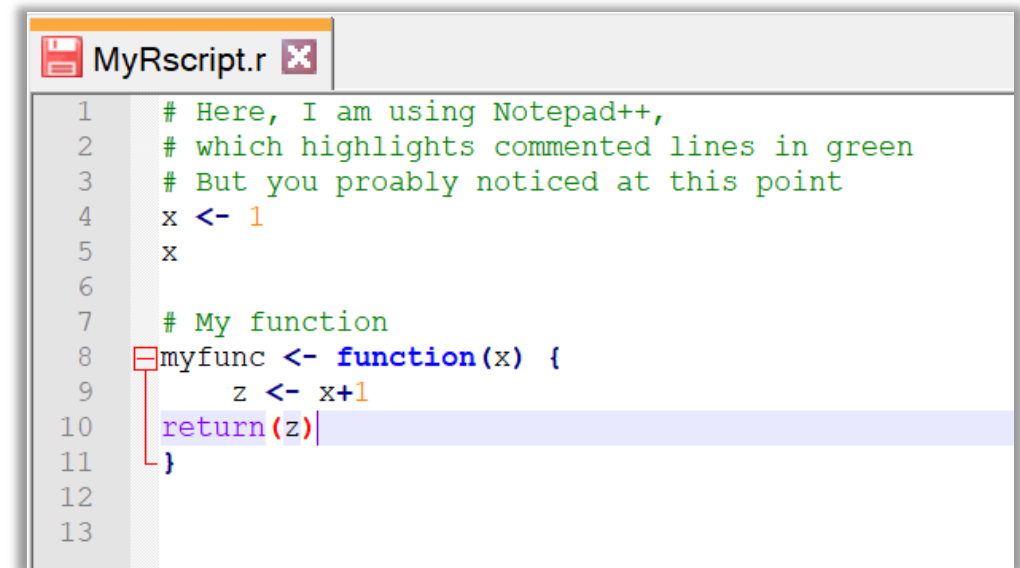
Setting your R “working directory”

- An O2 example:
 > setwd(“/home/mfk8/MyDataDirectory”)
- A Mac example:
 > setwd(“/Users/mfk8/MyDataDirectory”)
- A Windows example:
 > setwd(“C:/Users/mfk8/My Documents/MyDataDirectory”)



R Basics

- Keep a file of your commands
– any text editor works
- For example, Notepad++ have syntax highlighting
- Type a variable or object's name to “print” the value(s)
- A line starting with “#” is not passed as an an argument



```
1  # Here, I am using Notepad++,
2  # which highlights commented lines in green
3  # But you proably noticed at this point
4  x <- 1
5  x
6
7  # My function
8  myfunc <- function(x) {
9      z <- x+1
10     return(z)
11 }
12
13
```



Data Objects



Variables in R

- Assign variables with a “<-”
- A variable can be overwritten so be careful with naming
- Names can be UPPER/lowercase/.//_ mixes, but can't start with a number

```
> myX <- 5
```

```
> myX
```

```
[1] 5
```



Data Type: Vectors

- Basic way to store data
- “c” stands for concatenate
- “c” can be used to create a vector
- For example:

```
> myvector <- c(3,5,7)
```

```
> myvector
```

```
[1] 3 5 7
```



Vectors Types

- Numeric:
> mynumeric <- c(3,5,7)
- Character (single or double quotes are fine, but be consistent) :
> mycharacter <- c("bob", "nancy", "jose")
- Logical:
> mylogical <- c(TRUE, FALSE, TRUE)



Changing Your Vector Type

- General workflow:

```
> variable <- as.type(variable)
```

- For example:

```
> myvector <- as.character(myvector)
```

```
> myvector
```

```
[1] "3", "5", "7"
```



Changing Your Vector Type

- General workflow:

```
> variable <- as.type(variable)
```

- For example:

```
> myvector <- as.character(myvector)
```

```
> myvector
```

```
[1] "3", "5", "7"
```

Numbers are now in “quotes” like a character vector!



Changing Your Vector Type

- General workflow:
 - > **variable <- as.type(variable)**
- For example:
 - > **myvector <- as.character(myvector)**
 - > **myvector**
 - [1] "3", "5", "7"
 - > **class(myvector)**



Data Type: Lists

- Like vectors with mixed data types (numeric, character, logical)

```
> mylist <- c(3, "TP53", FALSE)
```

```
> mylist
```

```
[1] "3"    "TP53" "FALSE"
```



Data Type: Factors

- Makes a vector nominal (able to be ordered by integers)
- Factors represent an efficient method to store character values
- Create a variable “gender” with 2 "male" entries and 4 "female" entries

```
> gender <- c("male", "male", "female", "female", "female", "female")
```
- Convert vector as a factor

```
> gender <- factor(gender)
```

```
> gender
```

```
[1] male  male  female female female female
```

```
Levels: female male
```



Data Type: Matrices

- Data must be all the same type (numeric, character, logical)
- Columns must have the same length
- Creation:
 - > **mymatrix <- matrix(c(1:6), nrow=3, ncol=2)**
 - > **mymatrix**
- Indexing matrix elements: mymatrix[row,column]



Data Type: Matrices

- Data must be all the same type (numeric, character, logical)
- Columns must have the same length
- Creation:
 - > **mymatrix <- matrix(c(1:6), nrow=3, ncol=2)**
 - > **mymatrix**
- Indexing matrix elements: mymatrix[row,column]
 - > **mymatrix[1,1]** #returns item in row 1, column 1
 - > **mymatrix[1,]** #returns all of row 1
 - > **mymatrix[,1]** #returns all of column 1



Data Type : Data Frames

- Subset of matrices allowing mixed types
 - > **mydataframe <- as.data.frame(mymatrix)**
- You can give columns names so you can index by them
 - > **names(mydataframe) <- c("column1name", "column2name")**
- You can use unique identifiers as rownames
 - > **row.names(mydataframe) <- mydataframe[,1]**



Dataframes: Indexing/Converting

- matrix or \$ notation are acceptable
- For example (column):

> mydataframe\$column1name

-OR-

> mydataframe[,1]



Dataframes: Indexing/Converting

- For example (row):

> mydataframe[“rowname1”,]

-OR-

> mydataframe[1,]

- To make a data frame into a matrix:

> mymatrix <- as.matrix(mydataframe)



Adding and joining rows/columns

- “rbind” to add a row or another df/matrix to a pre-existing dataframe/matrix
 - > **mymatrix <- rbind(mymatrix, newrow)**
 - > **mymatrix <- rbind(mymatrix, matrixtwo)**
- “cbind” to add a column or another df/matrix to a pre-existing dataframe/matrix
 - > **mymatrix <- cbind(mymatrix, newcol)**
 - > **mymatrix <- cbind(mymatrix, matrixtwo)**



Useful functions:

- > **class(object)** #gives object class
 - > **mode(object)** #gives object type
 - > **length(vector)** #gives length
 - > **head(object)** #gives first 6 rows
 - > **tail(object)** #gives last 6 rows
 - > **summary()** #quick statistics
- > **nrow(object)** #gives number of rows
 - > **ncol(object)** #gives number of columns
 - > **str(object)** #gives object structure
 - > **dim(object)** #gives matrix/df dimensions



Missing Values

- NA: Not Available
- NaN: Not a Number
- To identify missing values:
 - > **is.na(x)** # logical test for NA/NaN
 - > **is.nan(x)** #logical test for only NaN
 - > **x[!is.na(x)]** #subsets and excludes NAs



Doing Math



Simple Arithmetic

- > **18 + 22** #addition
- > **18 – 12** #subtraction
- > **18 * 2** #multiplication
- > **18 / 2** #division
- > **18 %/% 4** #integer part of quotient
- > **18 %% 4** #modulo (remainder)
- > **18 ^ 2** #exponent



Built-in math functions

- > **log(10)** #natural log (base e)
- > **exp(2.302585)** #antilog (e raised to power)
- > **log10(100)** #log base 10
- > **sqrt(88)** #square root
- > **round(log(10), digits=3)** #round to specified digits
- > **runif(5)** #number of random numbers between 0-1
- > **rnorm(5)** #random numbers from uniform normal distribution
- > **abs(18 / -12)** #absolute value



Built-in math functions

- > **max(object)** # max
- > **min(object)** #min
- > **sum(object)** #sum
- > **mean(object)** #mean
- > **median(object)** #median

- > **range(object)** #range
- > **var(object)** #variance
- > **sd(object)** #standard deviation
- > **length(object)** #number of values



Series Shortcuts

- Series (colon or “seq”):

> **10:1**

-OR-

Format: seq(from, to, by)

> **seq(1, 10, 2)** # gives odd numbers

- Repeat

Format: rep(what, times)

> **rep(10, 10)**



Logical Operations: < > =

- Test of condition: returns logical TRUE/FALSE

```
> test1<- c(1,2,3)
```

```
> test1 > 2
```

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

```
> which(test1 >= 2)
```

```
[1] 2 3
```

```
> test1[test1 >=2] #subsetting data based on equality condition
```

```
> any(test1 >=5) #FALSE
```

```
> all(test1 >=5) #FALSE
```



Control Structures



“for” loops

- Way to iterate over data

```
> myvector <- c(1,3,5)
```

```
> j <- NULL #initialize it, good practice
```

```
> newvector <- NULL #initialize it, good practice
```

```
> for (i in myvector) {
```

```
  j <- i + 20
```

```
  newvector <- c(newvector, j)
```

```
}
```

```
> newvector
```


Functions

- Way to pack up commands into a repeatable format

```
> five <- 5
```

```
> three <- 3
```

```
> myfunction <- function(x,y) { z <- x + y  
  return(z)
```

```
}
```

```
> myfunction(five,three)
```

```
[1] 8
```



Apply

- Returns an object based on applying a function to a dataframe or matrix or list
- Format: **apply** (**to_what**, **how**, **function**)
- Where **how** accepts a “1” to apply the **function** over rows or “2” to apply over columns
- For example:
 - > **apply(mymatrix, 1, sum)** #row sums
 - > **apply(mymatrix, 2, sum)** #column sums



Variations on apply

- Format: **lapply(to_what, function)**
#returns a list

- For example

```
> lapply(1:3, function(x) x^2)
```

```
[[1]]
```

```
[1] 1
```

```
[[2]]
```

```
[1] 4
```

```
[[3]]
```

```
[1] 9
```

- Format: **sapply(to_what, function)**
#returns a vector

- For example:

```
> sapply(1:3, function(x) x^2)
```

```
[1] 1 4 9
```



Importing Data





Importing Data: text file

- Format: `mydata <- read.table(file="PathToFile/filename.csv", header=TRUE, sep=",")`
- You can specify how your data is separated (e.g., comma: “,”; tab: “\t” ; space: “ ”)
- Add “header=TRUE” to make the first row a header containing the column names
- Add “row.names=1” to make column 1 the row names (must be unique identifiers!)





Importing Data from MS Excel

- The “xlsx” package is required
 - > **install.packages(“xlsx”)**
 - > **library (“xlsx”)**
- Read in the first worksheet from the workbook myexcel.xlsx
 - Format: **mydata <- read.xlsx(“FileName.xlsx”, sheetIndex=1)**
- Read in the worksheet named mysheet
 - Format: **mydata <- read.xlsx(“FileName.xlsx”, sheetName = “mysheet”)**



Exporting Data

- Easy way to export a variable:

Format: **`write.table(x="ObjectName", file="FileName.tsv", sep="\t")`**

- More options:

`row.names=FALSE` #turn off row names

`col.names=FALSE` #turn off column names

`quote=FALSE` #turn off character string quoting



Saving and Loading your workspace

- Save and pick up where you leave off – saves variables

Format: **save.image(file="FileName.RData")**

-OR-

Format: **save(object list, file="FileName.RData")**

- Load workspace

Format: **load(file="FileName.RData")**



Class Example



Class Example – Download Data

- Download class data and R script to a folder from:
`github.com/hmsrc/user-training/tree/master/RBioconductorMaterial`
- Set your working directory to the folder where your data is located
- O2 cluster and Mac example:
`setwd("path/to/folder")`
- Windows example:
`setwd("C:/path/to/folder")`



Class Example – Import Data

- Import Rcoursetestdata1.csv as data frame, with headers and row names
 - > `mydf <- read.table("Rcoursetestdata1.csv", header=TRUE, row.names=1, sep=",")`
 - > `head(mydf)`

```
> head(mydf)
```

	TNBC1	TNBC2	TNBC3	Normal1	Normal2	Normal3
ENSG00000008988	15258	15077	144720	12095	43544	46883
ENSG00000009307	14660	20767	8678	13774	23030	18917
ENSG00000019582	50866	55775	15089	6696	13754	86319
ENSG00000026025	21174	47966	26682	6068	21126	12728
ENSG00000034510	25645	31574	56403	29590	25216	37199
ENSG00000044574	23910	27200	13757	13364	10852	12378



Class Example – Basic Statistics

- Get basic statistics on mydf

> **summary(mydf)**

```
> summary(mydf)
      TNBC1      TNBC2      TNBC3      Normal1
Min.   :    0  Min.   :   65  Min.   :   31  Min.   :   22
1st Qu.: 7888  1st Qu.: 9538  1st Qu.: 9324  1st Qu.: 5074
Median :13034  Median :16568  Median :19108 Median :10869
Mean   :18596  Mean   :26036  Mean   :25646  Mean   :14746
3rd Qu.:23850  3rd Qu.:28194  3rd Qu.:30389  3rd Qu.:18866
Max.   :103007 Max.   :351603  Max.   :272582  Max.   :89837

      Normal2      Normal3
Min.   :   208  Min.   :   15
1st Qu.: 7124  1st Qu.: 8944
Median :14005  Median :17710
Mean   :19425  Mean   :25481
3rd Qu.:21576  3rd Qu.:32191
Max.   :212582  Max.   :244692
```



Class Example – Transposing Data

- Need your data to read the other way? Turn it into a matrix, and transpose!
- For example:

```
> mymatrix <- as.matrix(mydf)
```

```
> myTmatrix<- t(mymatrix) #t = transpose
```

```
> myTdf <- as.data.frame(myTmatrix) #makes a data frame again
```

```
> myTdf
```



Class Example: Basic Statistics

- Get basic statistics on transposed data frame
 > **summary(myTdf)**



Class Example: Simple t-test

- Do a t-test on TNBC and Normal from mydf
 > **t.test(mydf[,1:3], mydf[,4:6])**



Class Example: Simple Wilcoxon

- Do a Wilcoxon test on TNBC and Normal from mydf
 > `wilcox.test(mymatrix[,1:3], mymatrix[,4:6])`



Useful R packages

- “RColorBrewer” and “Viridis” – define colors and palettes
- “ggplot2” – great for plotting
- “genefilter” – useful to apply filters over matrices
- “plyr” and “dplyr” – advanced matrix/df operations
- “edgeR” and “DESeq2” – RNAseq differential analysis alternatives using count data as input
- “biomaRt” – cross-annotate samples



Plotting



Plotting: short example

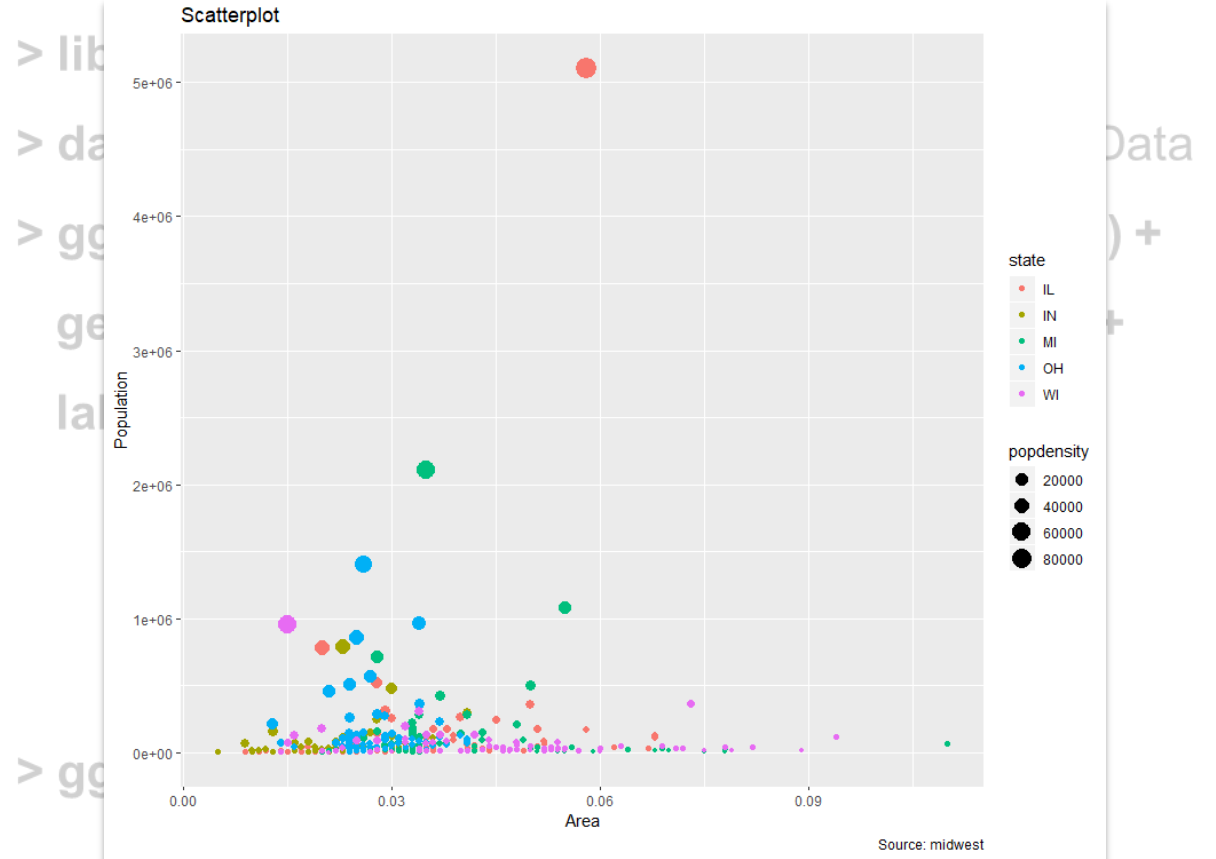
- “ggplot2” is an R package for building graphs
- Three general components
 - Data set
 - Coordinate system
 - Geoms

```
> library("ggplot2") # Load R package
> data("midwest", package = "ggplot2") # Load Data
> gg <- ggplot(midwest, aes(x=area, y=poptotal)) +
  geom_point(aes(col=state, size=popdensity)) +
  labs(y="Population",
        x="Area",
        title="Scatterplot",
        caption = "Source: midwest")
> gg
```



Plotting: short example

- “ggplot2” is an R package for building graphs
- Three general components
 - Data set
 - Coordinate system
 - Geoms
- For more information:
 - Chan Bioinformatics Core
https://hbctraining.github.io/Intro-to-R-flipped/lessons/11_ggplot2.html



RStudio

- Feature-rich GUI for R, works on top of version of R installed
- RMarkdown
- Not optimized for multithreading
- Now offered through HMS-RC on O2 via X11
- Coming soon as a standalone service via Open OnDemand





Contact information


 **Email:** rchelp@hms.harvard.edu

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Wiki: <https://wiki.rc.hms.harvard.edu/display/O2>

 **Phone:** 617-432-2000 (HMS IT Service Desk, 8a-5p)

 **Twitter:** @hms_rc

 **Location:** Gordon Hall 500, 5th Floor, 25 Shattuck Street

- <https://rc.hms.harvard.edu/office-hours/> for Zoom web conferencing during remote work

 **Office hours:** Wednesdays 1-3p for pressing needs, but appointments encouraged.



Please fill out the survey

- Accessible through the Harvard Training Portal
- <https://trainingportal.harvard.edu/>
- Click on “Me” then “Intro to O2”
- Scroll to “Evaluations” and click on the survey
- We appreciate any feedback or comments!

