

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
- Basic data summarization
- 2. Visualize data with plots



Overview

- 1. Summarizing Data in R
- 2. Creating Plots in R Using ggplot2
 - a. Histograms
 - b. Boxplots
 - c. Scatter plots

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
- RNA-Seq profiling used to quantify gene expression

Download pedersenLog2RPKM_v1.txt and pedersenLog2_matrix_v1.txt from the Day3 course website.

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

- 1. Download both Pedersen data files
- 2. Use setwd() to move to the data file folder
- 3. options(stringsAsFactors=F)
- Use the read.delim() function to read in "pedersenLog2RPKM_v1.txt" file into a data frame called "data1"
- 5. 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:



• 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	summary statistic
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	

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

formula format: y ~ x1

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

In Class Exercise.

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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

https://en.wikipedia.org/wiki/Data_visualization

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



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In Class Exercise.

To install ggplot2:

- 1. select "packages" tab
- 2. select "Install"
- 3. Type in ggplot2
- 4. Select "Install"

ggplot2





- ggplot2 is an R data visualization package created by Hadley Wickham
 - One of the most popular R packages
 - Breaks up graph construction into additive functions called layers
- ggplot2 documentation and cheat sheet: <u>https://www.rdocumentation.org/packages/ggplot</u> <u>2/versions/3.0.0</u>







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(thetic) 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)
```

```
input data frame
ggplot(data1, aes(x = log2RPKM)) +
```

```
→ geom histogram()
```

```
>ggsave(`histogram.png'
```

aesthetic

Visualizing Data Using Histograms

- <u>histogram</u>: 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 =
'pedersenLog2RPKM_v1.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:

- 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



In Class Exercise.

S In Class Exercise.

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)



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Visualizing Data Using Boxplots

- <u>boxplot</u>: graphically represents data distributions using quartiles
- <u>box-and-whisker plot</u>: 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 gplot2

```
options(stringsAsFactors=F)
library(ggplot2)
inFile1 = 'pedersenLog2RPKM_v1.txt'
data1 = read.delim(inFile1)
ggplot(data1,aes(x = sample, y = log2RPKM)) +
   geom_boxplot()
ggsave('boxplot1.png')
```



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

- 1. Change the fill color
 - HINT: fill = 'blue'
- 2. Add a title
 - HINT: ?labs





In Class Exercise.

Visualizing Data Using Scatter Plots

- <u>Scatter Plots</u> are visualizations that display two data values for the same measurement
 - <u>example</u>: 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_matrix_v1.txt'
data2 = read.delim(inFile2)
ggplot(data2,aes(x = WNT3A_rep1, y =
WNT3A_rep2)) +
    geom_point()
ggsave('scatter1.png')
```



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

- 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)



In class Exercise.

ggplot2



- We covered ~3 types of ggplot2 visualizations today
- There are many more!
- Check out <u>https://www.r-graph-</u> gallery.com/portfolio/ggplot2-package/ for further



References

- Gentleman, Robert. R Programming for Bioinformatics. CRC Press, 2009.
- Slides sourced in part from Jacob Kitzman and Barry Grant

R Graphics Shapes

R Graphics Colors

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

http://www.sthda.com/english/wiki/r-plot-pchsymbols-the-different-point-shapes-available-in-r 25

geom_hex() x, y, alpha, colour, fill size **Continuous Function** < ggplot(economics, aes(date, unemploy))</pre> geom_area() x, y, alpha, color, fill, linetype, size geom line() x, y, alpha, color, linetype, size geom step(direction = "hv") x, y, alpha, color, linetype, size Visualizing error df <- data.frame(grp = c("A", "B"), fit = 4:5, se = 1:2) k <- ggplot(df, aes(grp, fit, ymin = fit-se, ymax = fit+se)) 888 geom_crossbar(fatten = 2) x, y, ymax, ymin, alpha, color, fill, linetype, geom_errorbar() x, ymax, ymin, alpha, color, linetype, size, width (also geom_errorbarh()) geom_linerange() x, ymin, ymax, alpha, color, linetype, size geom_pointrange() x, y, ymin, ymax, alpha, color, fill, linetype, shape, size Maps data <- data frame(murder = USArrestsSMurder, state = tolower(rownames(USArrests))) map <- map_data("state") 1 < ggplot(data, aes(fill = murder))</pre> geom_map(aes(map_id = state), map = map) + expand_limits(x = mapSlong, y = mapSlat)

Learn more at docs.ggplot2.org • ggplot2 0.9.3.1 • Updated 3/15

m + geom_tile(aes(fill = z))

map_id, alpha, color, fill, linetype, size

vjust=0.5, interpolate=FALSE)

x, y, alpha, color, fill, linetype, size

x, y, alpha, fill

n + geom_raster(aes(fill = z), hjust=0.5,

Continuous Bivariate Distribution

i <- ggplot(movies, aes(year, rating))

linetype, size, weight

geom_density2d()

x, y, alpha, colour, linetype, size

geom_bin2d(binwidth = c(5, 0.5))

xmax, xmin, ymax, ymin, alpha, color, fill,

https://www.rstudio.com/wp-content/uploads/2015/03/ggplot2-cheatsheet.pdf

Stats - An alternative way to build a layer

Some plots visualize a transformation of the original data set. Use a stat to choose a common transformation to visualize,

Each stat creates additional variables to map aesthetics to. These variables use a common ...name.. syntax.

stat functions and geom functions both combine a stat with a geom to make a layer, i.e. stat_bin(geom="bar")

does the same as geom_bar(stat="bin")	
stat function layer specific variable created by transformation	
i + stat_density2d(aes(fill =level),	
geom = "polygon", n = 100)	scale_*
geom for layer parameters for stat	scale_*
a + stat_bin(binwidth = 1, origin = 10) 1D distributions	scale_*
x, y count,ncount,density,ndensity	scale_*
a + stat_bindot(binwidth = 1, binaxis = "x")	manu
x, y, count., .ncount	
x, y, .count., .density., .scaled.	
f+stat_bin2d(bins = 30, drop = TRUE) 2D distributions	scale x
x, y, fill .count., .density	break
f + stat_binhex(bins = 30)	values
x, y, htt count,density	scale_x
r + stat_densityzd(contour = TRUE, n = 100)	same
	scale_x
m + stat_contour(aes(z = z)) 3 Variables	scale x
x, y, z, order _level m+ stat_snoke(pac(radius= z_pagio = zi))	scale x
angle, radius, x, xend, y, yend x.,xendy.,yend	acute_A
m + stat_summary_hex(aes(z = z), bins = 30, fun = mean)	
x, y, z, fill value	
m + stat_summary2d(aes(z = z), bins = 30, fun = mean)	
x, y, z, mi [vatue	
g+stat_boxplot(coef = 1.5) Comparisons	n -
x, y _lower.,middle.,upper., _outliers	
g + stat_ydensity(adjust = 1, kernel = "gaussian", scale = "area")	
x, y _density.,scaled, _count,n., _violinwidth,width	
f+stat_ecdf(n = 40) Functions	n -
x, y	1000
<pre>r + stat_quantile(quantiles = c(0.25, 0.5, 0.75), tormula = y - log(x), method = "m")</pre>	
x,y].guantile	
f + stat_smooth(method = "auto", formula = y - x, se = TRUE, n = 80,	
fullrange = FALSE, level = 0.95)	
x, y	P .
ggplot() + stat_function(aes(x = -3:3), General Purnose	TA:
fun = dnorm, n = 101, args = list(sd=0.5))	0,0
x _y	MA −
<pre>r+ stat_identity() emplot() + stat_ad(ac(cample=1:100)_distribution = at</pre>	. 0
dparams = list(df=5))	TX.
sample, x, y .x., .y.	
f+stat_sum()	
x, y, sizesize	

Scales

Scales control how a plot maps data values to the visual values of an aesthetic. To change the mapping, add a custom scale. h <- b + geom_bar(aes(fill = fl))</p> prepackaged scale to use scale_fill_manual(values = c("skyblue", "royalblue", "blue", "navy"), limits = c("d", "e", "p", "r"), breaks =c("d", "e", "p", "r"), name = "fuel", labels = c("D", "E", "P", "R")) title to use in labels to use in breaks to use

General Purpose scales Use with any aesthetic: alpha, color, fill, linetype, shape, size

continuous() - map cont' values to visual values discrete() - map discrete values to visual values identity() - use data values as visual values manual(values = c()) - map discrete values to ally chosen visual values

> X and Y location scales Use with x or y aesthetics (x shown here)

date(labels = date_format("%m/%d"), s = date_breaks("2 weeks")) - treat x as dates. See ?strptime for label formats. datetime() - treat x values as date times. Use arguments as scale_x_date(). log10() - Plot x on log10 scale

_reverse() - Reverse direction of x axis sqrt() - Plot x on square root scale

Color and fill scales

b+geom_bar(a + geom_dotplot(aes(fill = fl) aes(fill = .x.)) scale fill brewer(scale_fill_gradient(palette = "Blues") high = "yellow") For palette choices: scale_fill_gradient2(library(RcolorBrewer) display.brewer.all() low = "red", fright = "blue", mid = "white", midpoint = 25) scale_fill_grey(scale_fill_gradientn(start = 0.2, end = (na.value = "red") colours = terrain.colors(6)) so: rainbow(), heat.colors(), opo.colors(), cm.colors(), RColorBrewer:brewer.pal()

Shape scales

Coordinate Systems

r <- b + geom_bar()

xlim, ylim The default cartesian coordinate system + coord_fixed(ratio = 1/2)

Cartesian coordinates with fixed aspect ratio between x and y units

coord_cartesian(xlim = c(0, 5))

+ coord_flip() xlim, ylim

Polar coordinates + coord_trans(ytrans = "sqrt") xtrans, ytrans, limx, limy

Transformed cartesian coordinates. Set extras and strains to the name of a window function.

z + coord_map(projection = "ortho", orientation=c(41, -74, 0))

projection, orientation, xlim, ylim Map projections from the mapproj package (mercator (default), azequalarea, lagrange, etc.)

Position Adjustments

Position adjustments determine how to arrange geoms that would otherwise occupy the same space.

Arrange elements side by side

< - ggplot(mpg, aes(fl, fill = drv))

s + geom_bar(position = "fill") Stack elements on top of one another, normalize height

of each element to avoid overplotting

Each position adjustment can be recast as a function with manual width and height arguments

s + geom_bar(position = position_dodge(width = 1))

Facets divide a plot into subplots based on the values of one or more discrete variables. t <- ggplot(mpg, aes(cty, hwy)) + geom_point() t + facet_grid(. ~ fl) facet into columns based on fl t + facet_grid(year ~ .) facet into rows based on year t + facet_grid(year ~ fl) facet into both rows and columns t + facet_wrap(~ fl) wrap facets into a rectangular layout Set scales to let axis limits vary across facets t + facet_grid(y ~ x, scales = "free") x and y axis limits adjust to individual facets "free_x" - x axis limits adjust "free_y" - y axis limits adjust Set labeller to adjust facet labels t + facet_grid(. ~ fl, labeller = label_both) fic fid fie fip fir

Faceting

to update legend

t + facet_grid(. ~ fl, labeller = label_bquote(alpha ^ .(x))) $\alpha^{c} = \alpha^{d} = \alpha^{e} = \alpha^{p} = \alpha^{r}$ t + facet_grid(. ~ fl, labeller = label_parsed) c d e p r

Labels

- t + ggtitle("New Plot Title") Add a main title above the plot
- t + xlab("New X label") Change the label on the X axis
- t + ylab("New Y label")
- Change the label on the Y axis
- t + labs(title =" New title", x = "New x", y = "New y") All of the above

Legends

- t + theme(legend.position = "bottom") Place legend at "bottom", "top", "left", or "right"
- t + guides(color = "none")
- Set legend type for each aesthetic: colorbar, legend, or none (no legend)
- t + scale fill discrete(name = "Title". labels = c("A", "B", "C"))
- Set legend title and labels with a scale function.

Zooming Without clipping (preferred) n t+coord_cartesian(

xlim = c(0, 100), ylim = c(10, 20))

With clipping (removes unseen data points)

t + xlim(0, 100) + ylim(10, 20) + scale_x_continuous(limits = c(0, 100)) + scale_y_continuous(limits = c(0, 100))

Learn more at docs.ggplot2.org • ggplot2 0.9.3.1 • Updated: 3/15

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f+stat_unique()

https://www.rstudio.com/wp-content/uploads/2015/03/ggplot2-cheatsheet.pdf

s + geom_bar(position = "stack")

Stack elements on top of one another

f + geom_point(position = "jitter") Add random noise to X and Y position

