Introduction to Solving Biological Problems Using R - Day 2

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Day 2 Schedule

1. Further customisation of plots
2. Statistics
3. Data Manipulation Techniques
4. Programming in R
5. Further report writing

1. Further customisation of plots

Recap

- We have seen how to use `plot()`, `boxplot()`, `hist()` etc to make simple plots
- These come with arguments that can be used to change the appearance of the plot
    - `col`, `pch`
    - `main`, `xlab`, `ylab`
    - etc….

- We will now look at ways to modify the plot appearance after it has been created
- Also, how to export the graphs

The painter's model

- R employs a painter's model to construct it's plots
- Elements of the graph are added to the canvas one layer at a time, and the picture built up in levels.
- Lower levels are obscured by higher levels,
  - allowing for blending, masking and overlaying of objects.
- Caution: You can't undo the changes you make to the plot

http://www.inquisitr.com/309687/jesus-painting-restoration-goes-wrong-well-intentioned-old-lady-destroys-100-year-old-fresco/ (http://www.inquisitr.com/309687/jesus-painting-restoration-goes-wrong-well-intentioned-old-lady-destroys-100-year-old-fresco/)

Example data

- We will re-use the patients data from yesterday:

```
age    <- c(50, 21, 35, 45, 28, 31, 42, 33, 57, 62)
weight <- c(70.8, 67.9, 75.3, 61.9, 72.4, 69.9, 63.5,
            71.5, 73.2, 64.8)
firstName  <- c("Adam", "Eve", "John", "Mary", "Peter",
                "Paul", "Joanna", "Matthew", "David", "Sally")
secondName <- c("Jones", "Parker", "Evans", "Davis",
                "Baker","Daniels", "Edwards", "Smith",
                "Roberts", "Wilson")

consent <- c(TRUE, TRUE, FALSE, TRUE, FALSE, FALSE,
             FALSE, TRUE, FALSE, TRUE)

sex <- c("Male", "Female", "Male", "Female", "Male",
         "Male", "Female", "Male", "Male", "Female")
```

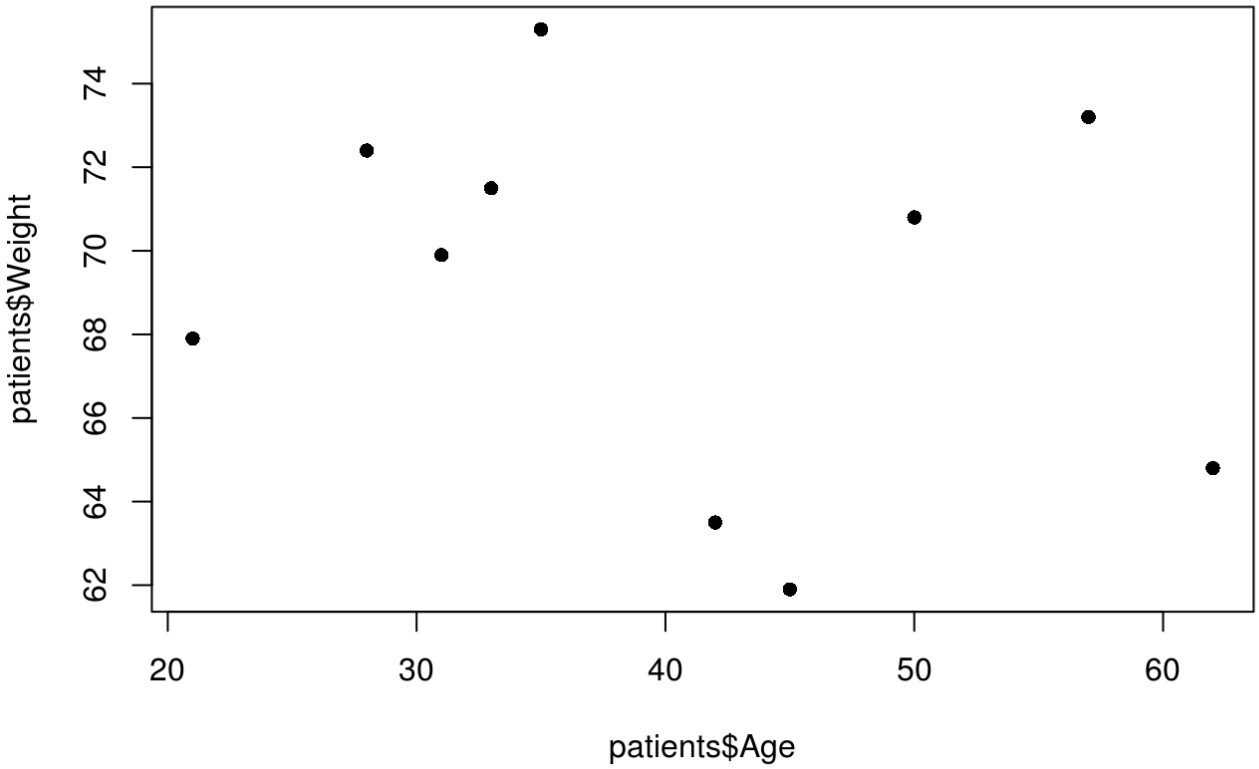
Example data

```
patients <- data.frame(First_Name = firstName,
                       Second_Name = secondName,
                       Full_Name = paste(firstName, secondName),
                       Sex = factor(sex),
                       Age = age,
                       Weight = weight,
                       Consent = consent,
                       stringsAsFactors = FALSE)
```

Initial plot

- Recall our patients dataset from yesterday
  - we might want to display other characteristics on the plot, e.g. gender of individual:

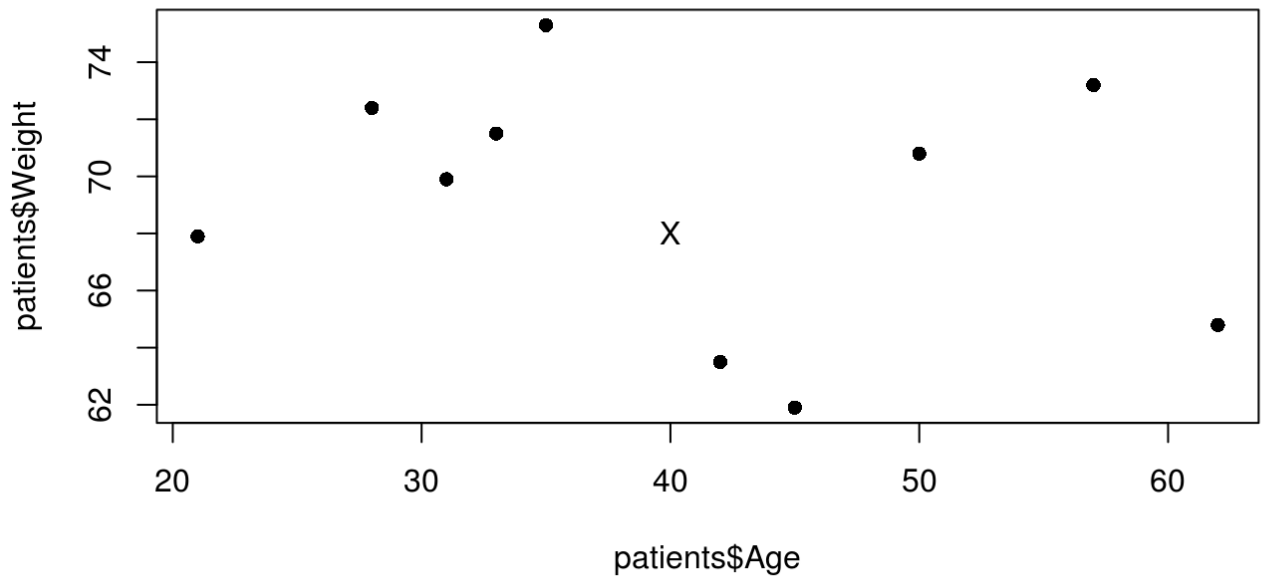
```
plot(patients$Age, patients$Weight, pch=16)
```



The points function

- `points()` can be used to set of points to an *existing* plot
- It requires a vector of x and y coordinates
  - These do not have to be the same length as the number of points in the initial plot:
    - Hence we can use `points()` to highlight observations
    - …or add a set of new observations

```
plot(patients$Age, patients$Weight, pch=16)
points(40,68, pch="X")
```

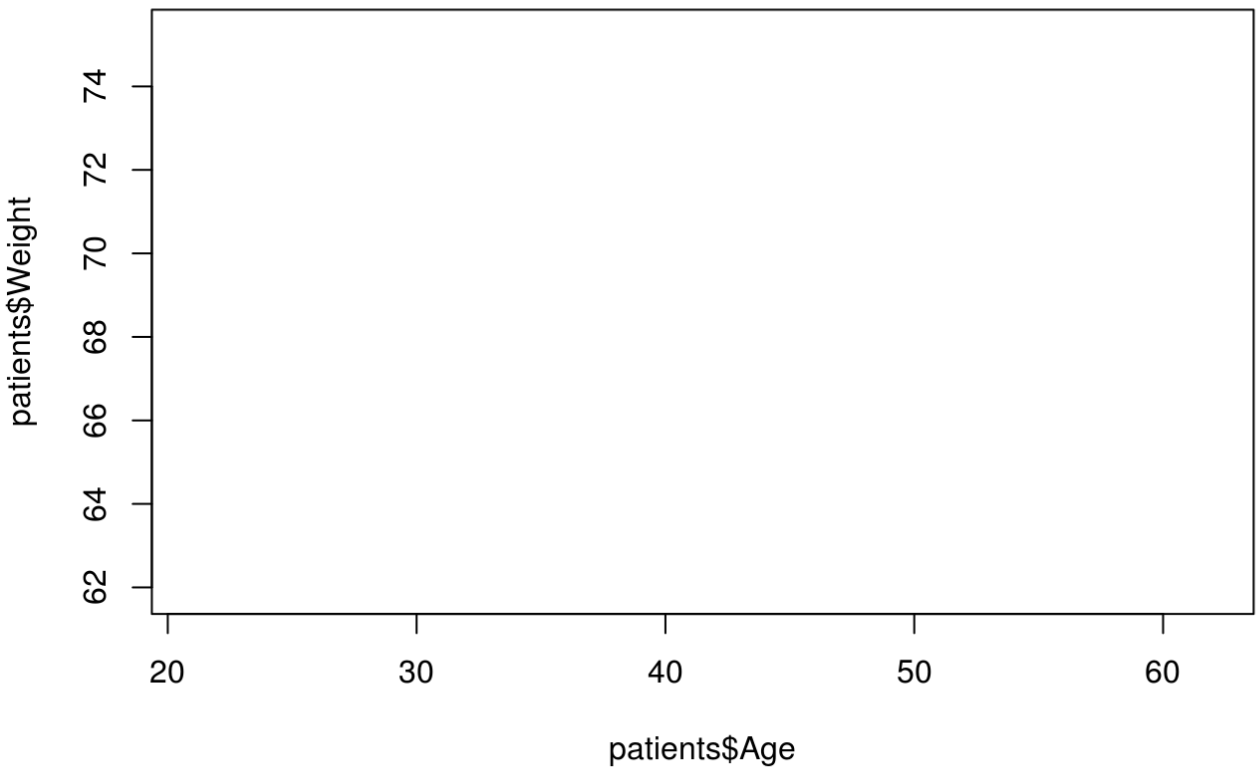


- Note that axis limits of the existing plot are not altered

Creating a blank plot

- Often it is useful to create a blank ‘canvas’ with the correct labels and limits

```
plot(patients$Age, patients$Weight, type="n")
```



Adding points to differentiate gender

- Selecting males using the `==` comparison we saw yesterday
  - Gives a `TRUE` or `FALSE` value
  - Can be used to index the data frame

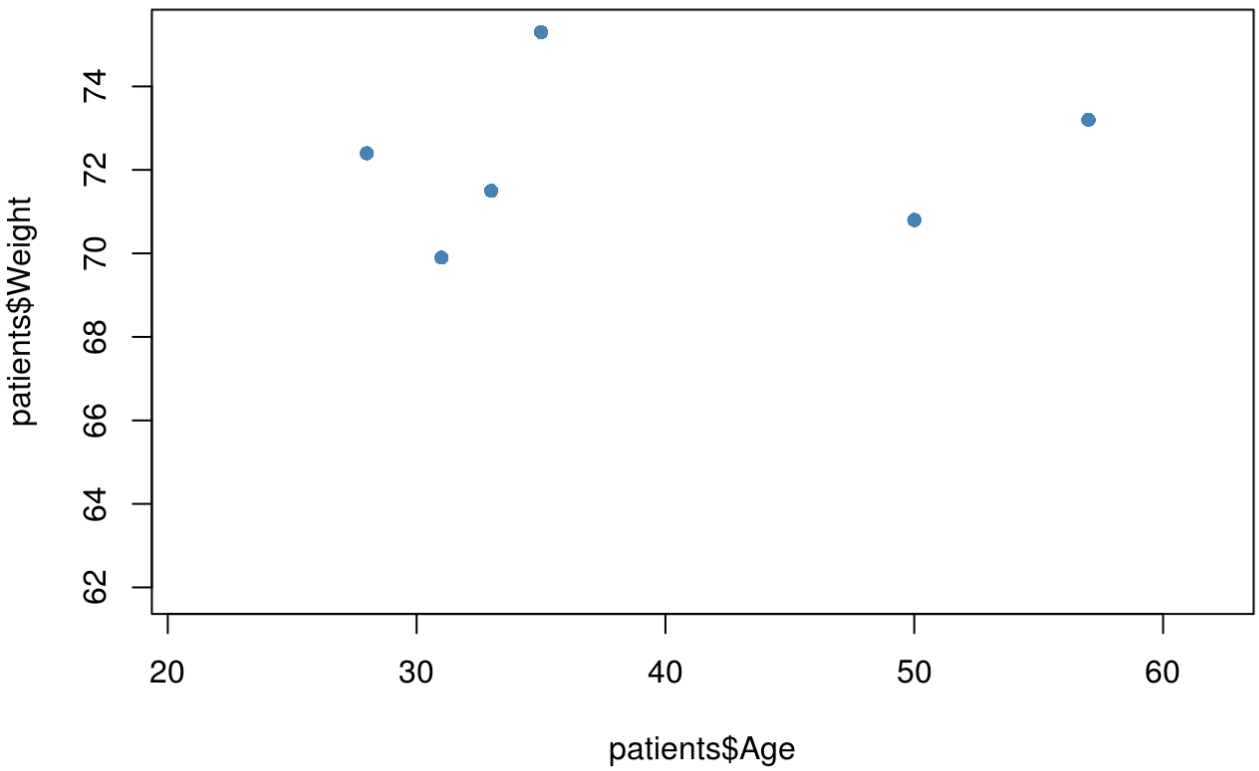
- Which means we can get the relevant Age and Weight values

```
males <- patients$Sex == "Male"
```

```
males
patients[males,]
patients[males, "Age"]
patients[males, "Weight"]
```

Adding points to differentiate gender

```
plot(patients$Age, patients$Weight, type="n")
points(patients$Age[males], patients$Weight[males],
       pch=16, col="steelblue")
```



Adding points to differentiate gender

```
females <- patients$Sex == "Female"
females
```

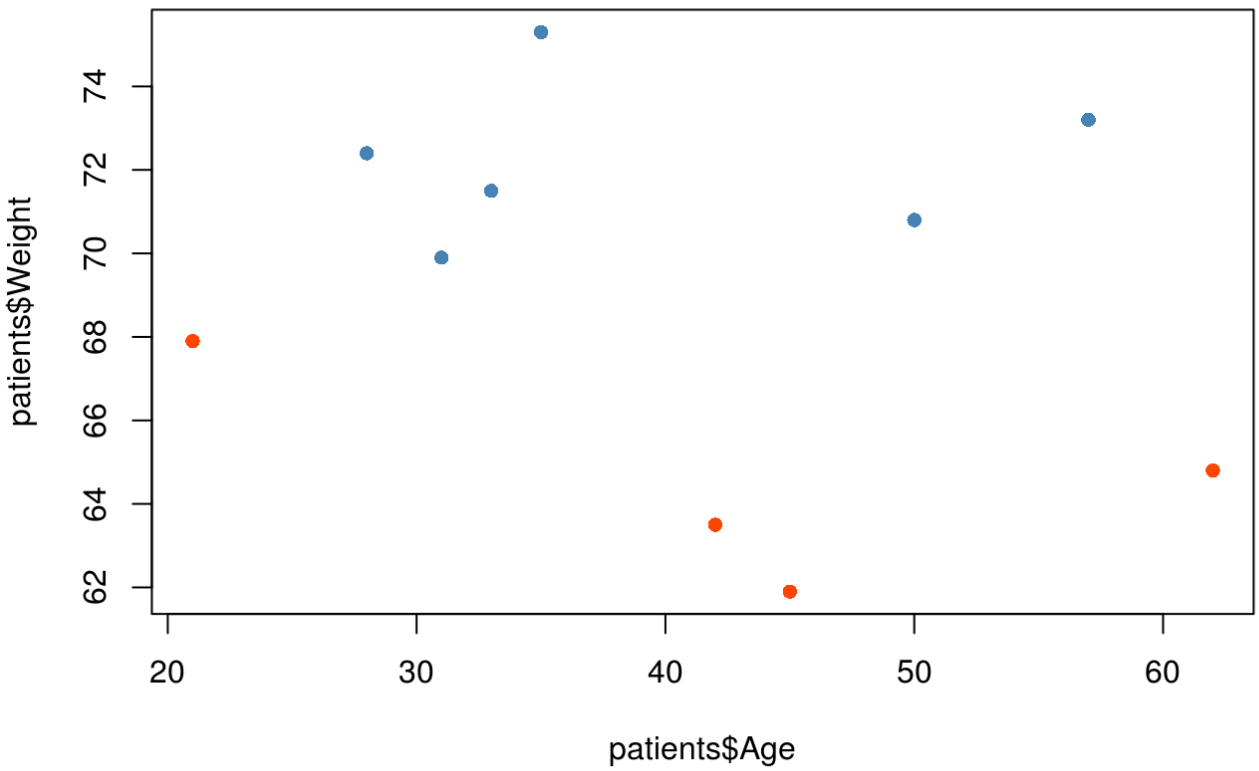
```
 [1] FALSE  TRUE FALSE  TRUE FALSE FALSE  TRUE FALSE FALSE  TRUE
```

```
patients[females,]
```

```
   First_Name Second_Name      Full_Name    Sex Age Weight Consent
2         Eve      Parker     Eve Parker Female  21   67.9    TRUE
4        Mary       Davis     Mary Davis Female  45   61.9    TRUE
7      Joanna     Edwards Joanna Edwards Female  42   63.5   FALSE
10      Sally      Wilson   Sally Wilson Female  62   64.8    TRUE
```

Adding points to differentiate gender

```
plot(patients$Age, patients$Weight, type="n")
points(patients$Age[males], patients$Weight[males],
       pch=16, col="steelblue")
points(patients$Age[females], patients$Weight[females],
       pch=16, col="orangered1")
```

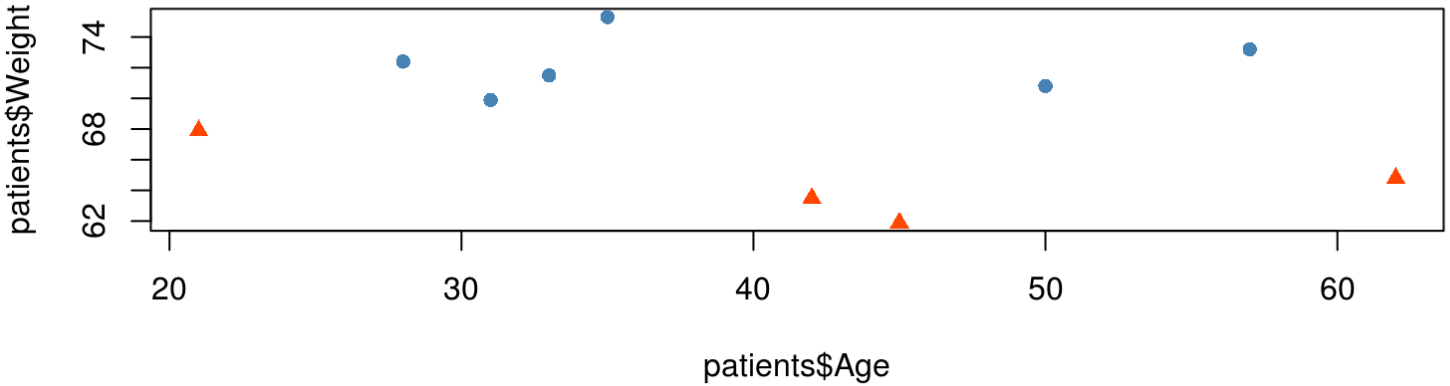


Adding points

- Each set of points can have a different colour and shape
- Axis labels and title and limits are defined by the plot
- You can add points ad-nauseum. Try not to make the plot cluttered!

- Once you've added points to a plot, they cannot be removed
- A call to `plot` will start a new graphics window
  - or typing `dev.off()`

```
plot(patients$Age, patients$Weight, type="n")
points(patients$Age[males], patients$Weight[males],
       pch=16, col="steelblue")
points(patients$Age[females], patients$Weight[females],
       pch=17, col="orangered1")
```



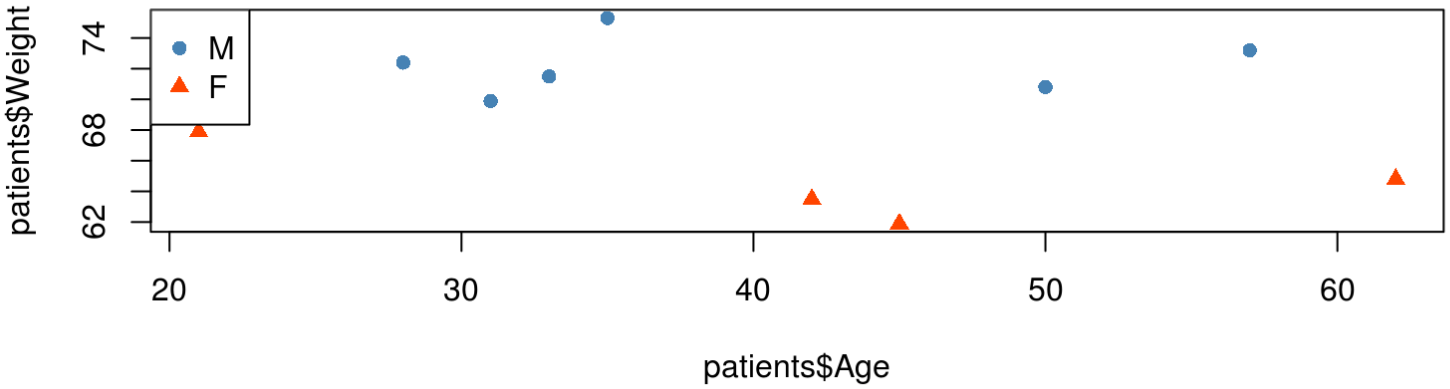
- TIP: try building the same plot, but using a vector of colors fo the attribute `col`, instead of subselecting the observations

Adding a legend

- Should also add a legend to help interpret the plot

- use the `legend` function
- can give x and y coordinates where legend will appear
- also recognises shortcuts such as ***topleft*** and ***bottomright***…

```
plot(patients$Age, patients$Weight, type="n")
points(patients$Age[males], patients$Weight[males],
       pch=16, col="steelblue")
points(patients$Age[females], patients$Weight[females],
       pch=17, col="orangered1")
legend("topleft", legend=c("M","F"),
       col=c("steelblue","orangered1"), pch=c(16,17))
```

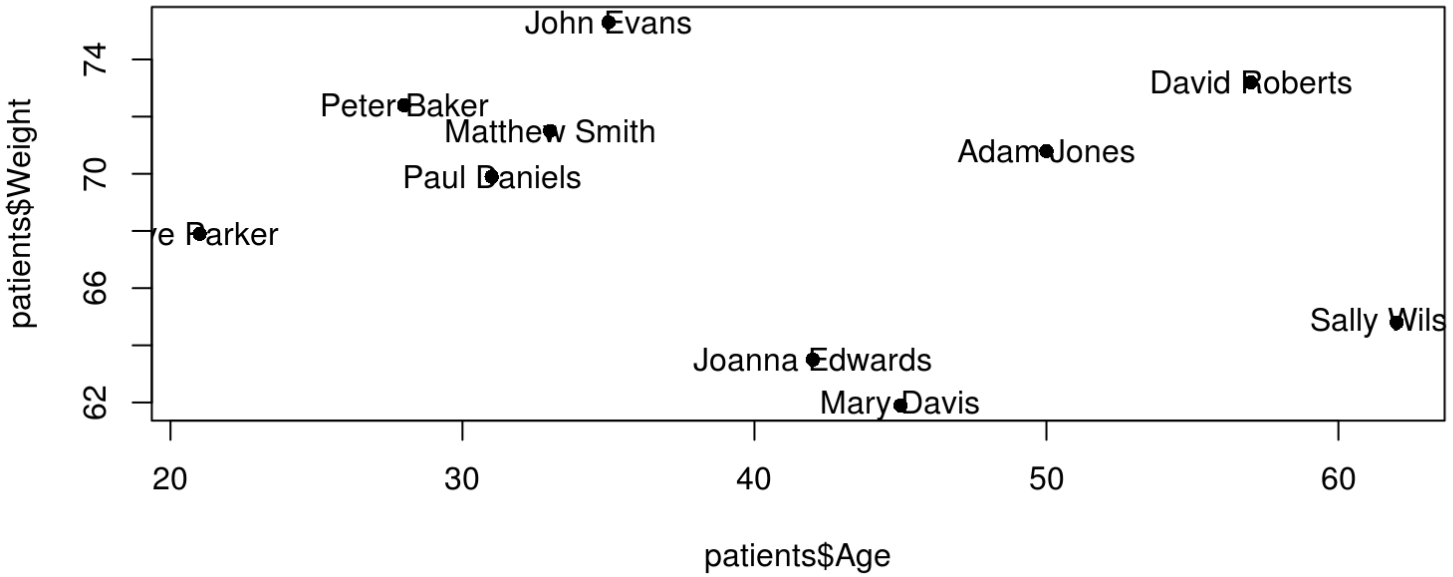


Adding text

- Text can also be added to a plot in a similar manner

- The `labels` argument specifies the text we want to add

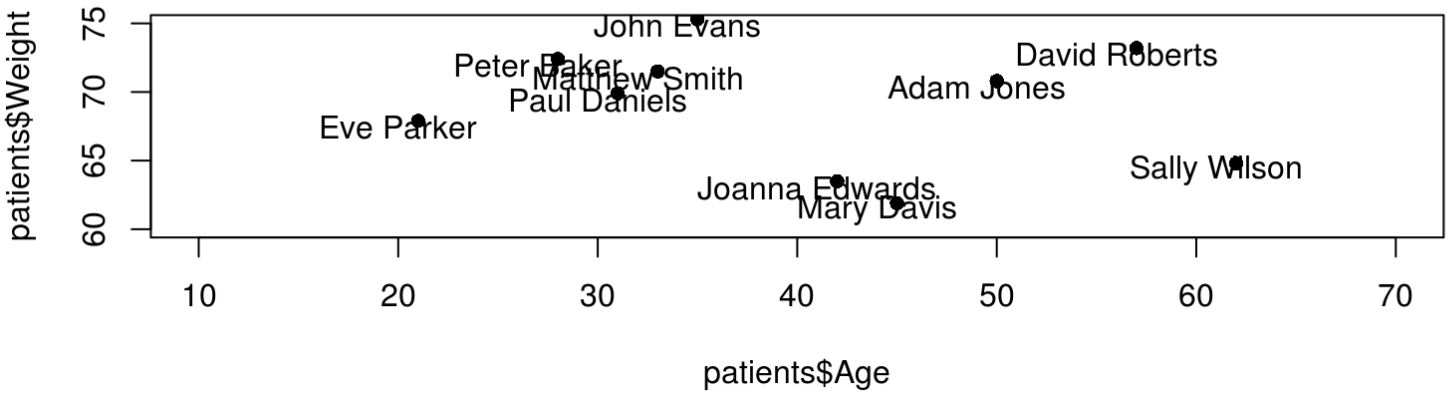
```
plot(patients$Age, patients$Weight, pch=16)
text(patients$Age, patients$Weight, labels=patients$Full_Name)
```



Adding text

- Can alter the positions so they don't interfere with the points of the graph

```
plot(patients$Age, patients$Weight, pch=16,
     xlim=c(10,70), ylim=c(60,75))
text(patients$Age-1, patients$Weight-0.5,
     labels=patients$Full_Name)
```

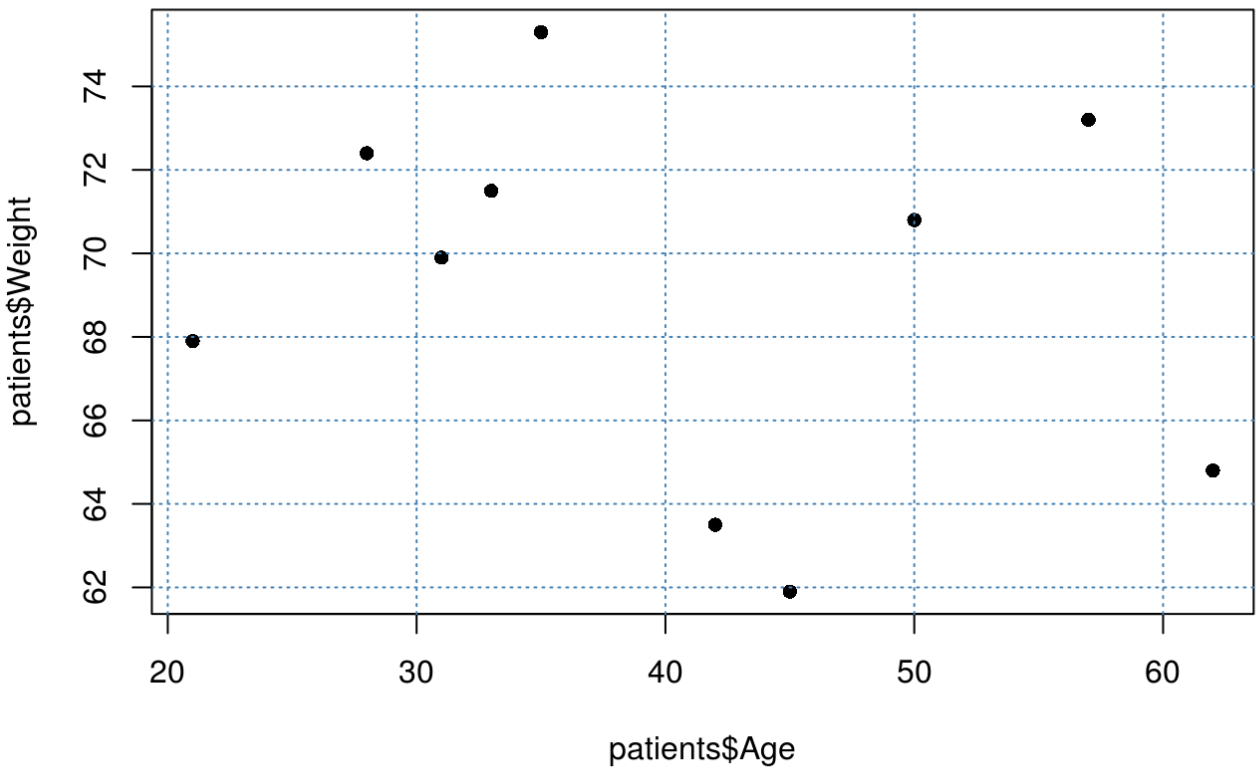


- Alternatively, you can use the argument `adj`

Adding lines

- To aid our interpretation, it is often helpful to add guidelines
  - `grid()` is one easy way of doing this:

```
plot(patients$Age, patients$Weight, pch=16)
grid(col="steelblue")
```

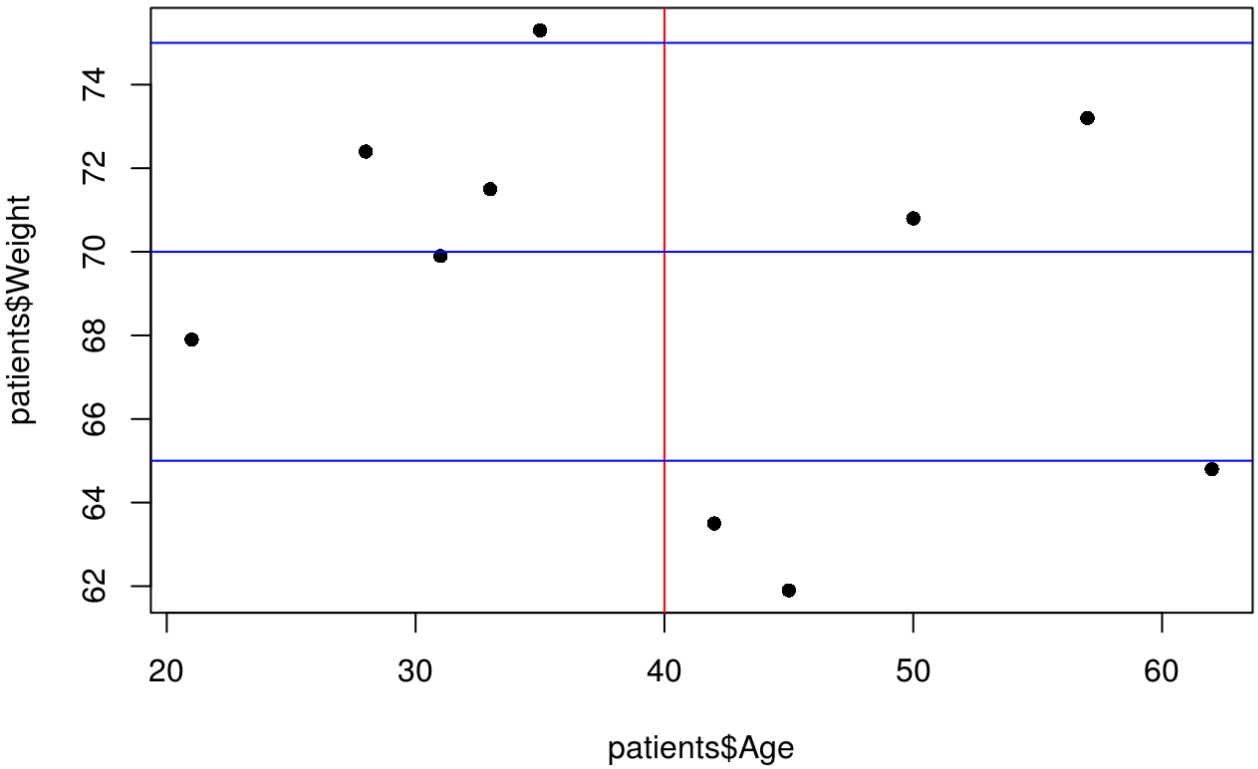


Adding lines

- Can also add lines that intersect the axes:
  - `v` = for vertical lines
  - `h` = for horizontal

- can specify multiple lines in a vector

```
plot(patients$Age, patients$Weight, pch=16)
abline(v=40, col="red")
abline(h=c(65,70,75), col="blue")
```

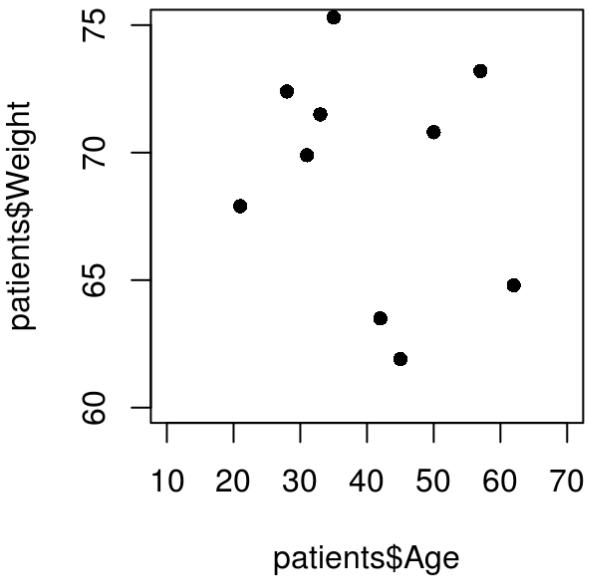


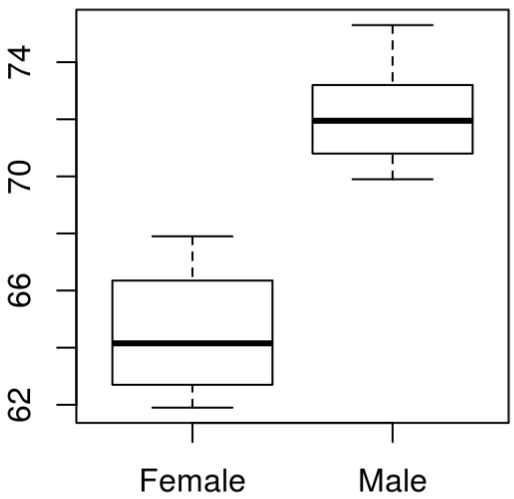
Plot layouts

- The `par` function can be used specify the appearance of a plot
- The settings persist until the plot is closed with **`dev.off()`**
- `?par` and scroll to ***graphical parameters***
- One example is `mfrow` :
  - “multiple figures per row”
  - needs to be a vector of rows and columns:
    - e.g. a plot with one row and two columns `par(mfrow=c(1,2))`
    - don’t need the same kind of plot in each cell

Plot layouts

```
par(mfrow=c(1,2))
plot(patients$Age, patients$Weight, pch=16,
     xlim=c(10,70), ylim=c(60,75))
boxplot(patients$Weight ~ patients$Sex)
```





- See also `mar` for setting the margins:
  - `par(mar=c(...))`

Exporting graphs from RStudio

- Easiest option to to use the *Export* button from the *Plots* panel
- Otherwise, use the `pdf()` function:
  - You will see that the plot does not appear in RStudio

```
pdf("ExampleGraph.pdf")
plot(rnorm(1:10))
```

- You need to use the `dev.off()` to stop printing graphs to the pdf and 'close' the file
  - It allows you to create a pdf document with multiple pages

```
dev.off()
```

- pdf is a good choice for publication as they can be imported into Photoshop, Inkscape, etc.
  - Sometimes it is easier to edit in these tools than R!
  - If it is taking too long to customise a plot in R, consider if you should be using one of these tools instead

Exporting graphs from RStudio

- To save any graph you have created to a pdf, repeat the code you used to create the plot with `pdf()` before and `dev.off()` afterwards
  - you can have as many lines of code in-between as you like

```
pdf("mygraph.pdf")
plot(patients$Age, patients$Weight, pch=16)
abline(v=40, col="red")
abline(h=c(65,70,75), col="blue")
dev.off()
```

```
png
  2
```

Exporting graphs from RStudio

- We can specify the dimensions of the plot, and other properties of the file (`?pdf`)

```
pdf("ExampleGraph.pdf", width=10, height=10)
plot(rnorm(1:10))
dev.off()
```

```
png
  2
```

- Other formats can be created:
  - e.g. ***png***, or others `?jpeg`
  - more appropriate for email, presentations, web page

```
png("ExampleGraph.png")
plot(rnorm(1:10))
dev.off()
```

```
png
  2
```

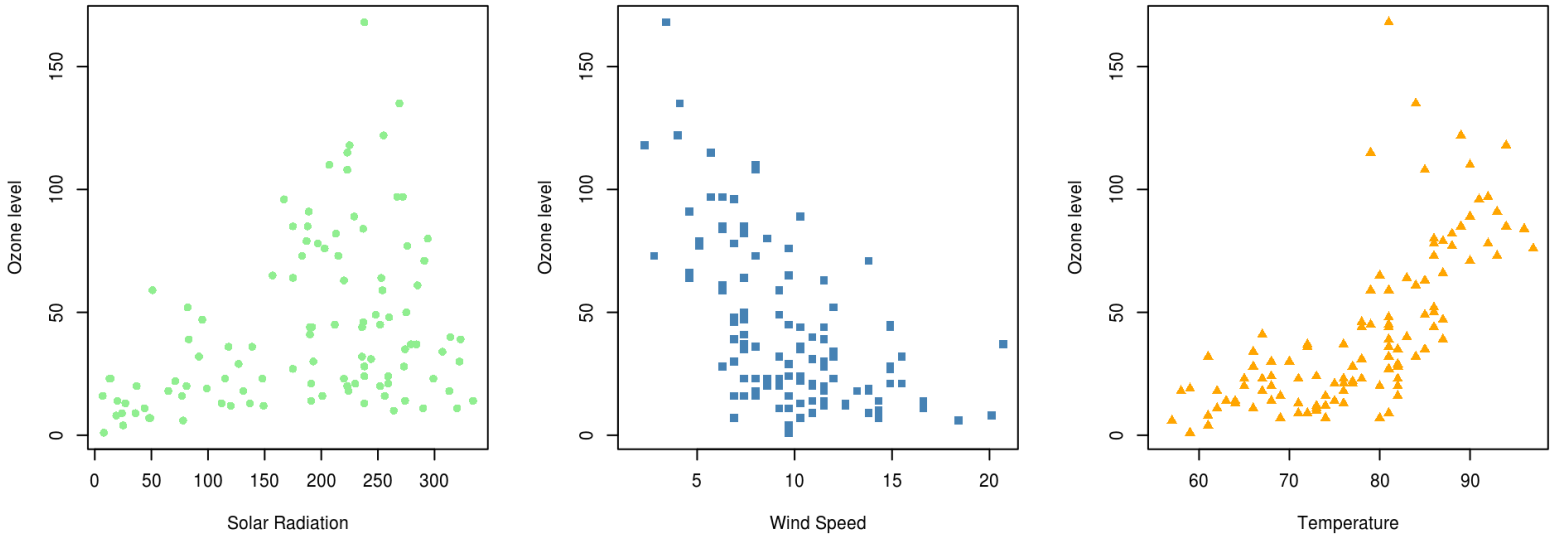
Exercise: exercise5.Rmd

- Return to the weather data from yesterday:

```
weather <- read.csv("ozone.csv")
```

- Using the `par` function, create a layout with three columns
- Plot Ozone versus Solar Radiation, Wind Speed and Temperature on separate graphs

    - use different colours and plotting characters on each plot
- Save the plot to a pdf
- HINT: Create the graph first in RStudio, then when you're happy with it, use the `pdf` function to save to a file



Solution

```
pdf("ozoneCorrelations.pdf")
par(mfrow=c(1,3))
plot(weather$Solar.R, weather$Ozone, pch=16,
     col="lightgreen", ylab="Ozone level",
     xlab="Solar Radiation")
plot(weather$Wind, weather$Ozone, pch=15,
     col="steelblue", ylab="Ozone level",
     xlab="Wind Speed")
plot(weather$Temp,weather$Ozone, pch=17,
     col="orange", ylab="Ozone level",
     xlab="Temperature")
dev.off()
```

Solution

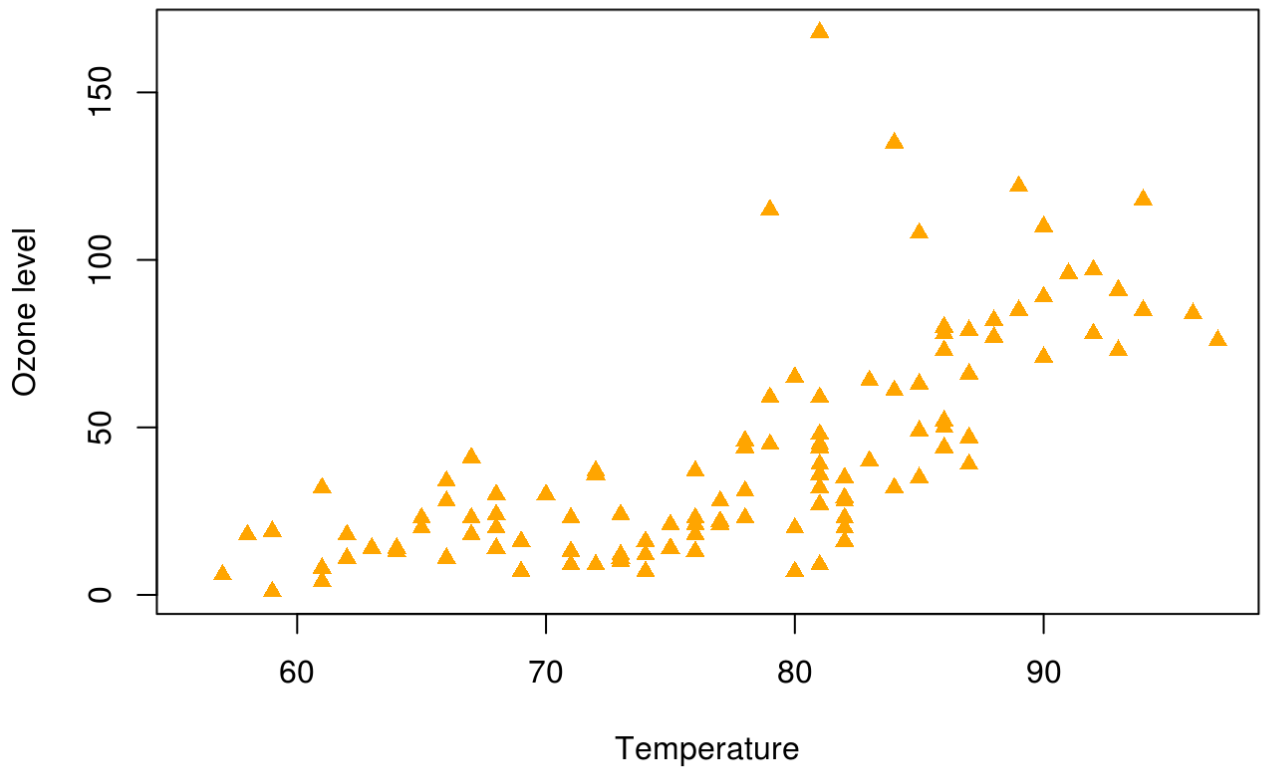
If the graph looks a bit stretched…

```
pdf("ozoneCorrelations.pdf", width=10,height = 6)
par(mfrow=c(1,3))
plot(weather$Solar.R, weather$Ozone, pch=16,
     col="lightgreen", ylab="Ozone level",
     xlab="Solar Radiation")
plot(weather$Wind, weather$Ozone, pch=15,
     col="steelblue", ylab="Ozone level",
     xlab="Wind Speed")
plot(weather$Temp,weather$Ozone, pch=17,
     col="orange", ylab="Ozone level",
     xlab="Temperature")
dev.off()
```

Exercise: exercise5.Rmd

- Temperature and Ozone level seem to be correlated
- However, there are some observations that do not seem to fit the trend
    - those with Ozone level > 100
- Modify the plot so that these outlier observations are in a different colour

```
plot(weather$Temp,weather$Ozone, pch=17,
     col="orange", ylab="Ozone level",
     xlab="Temperature")
```

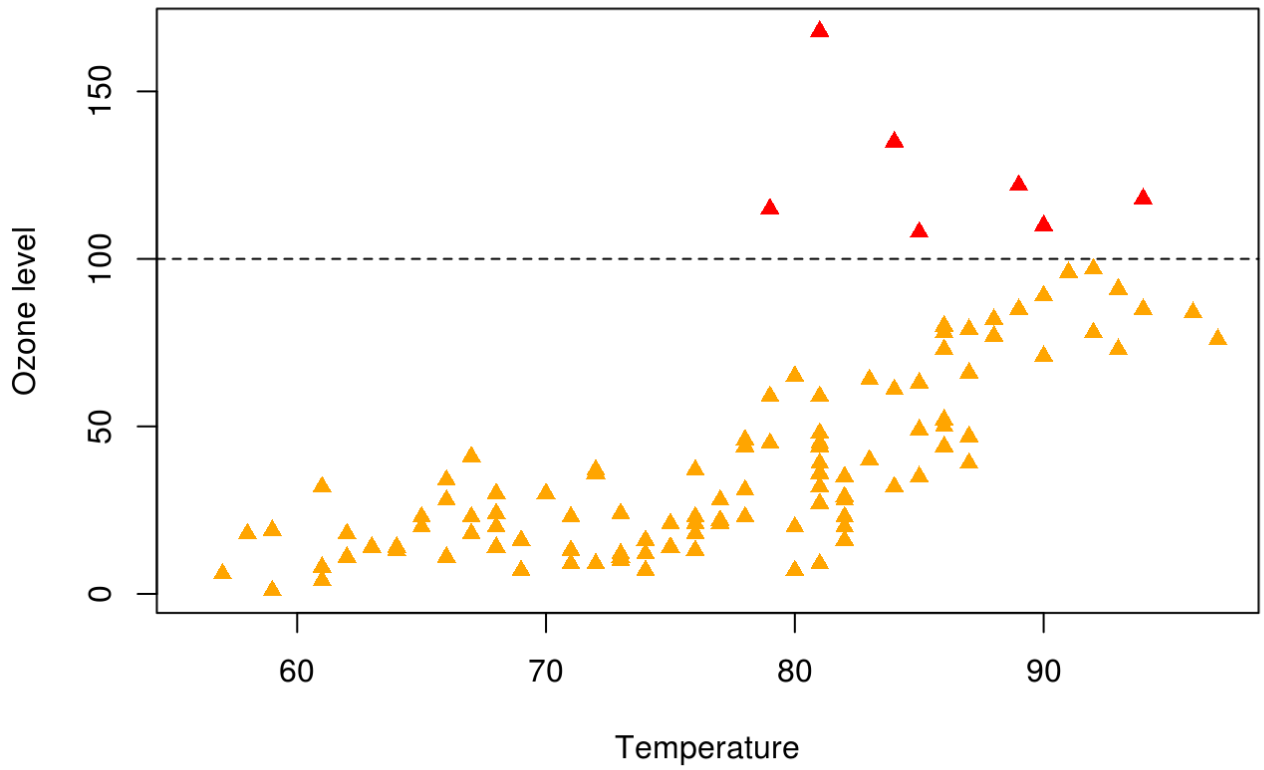


Target graph

HINT: You can break down the problem into the following steps

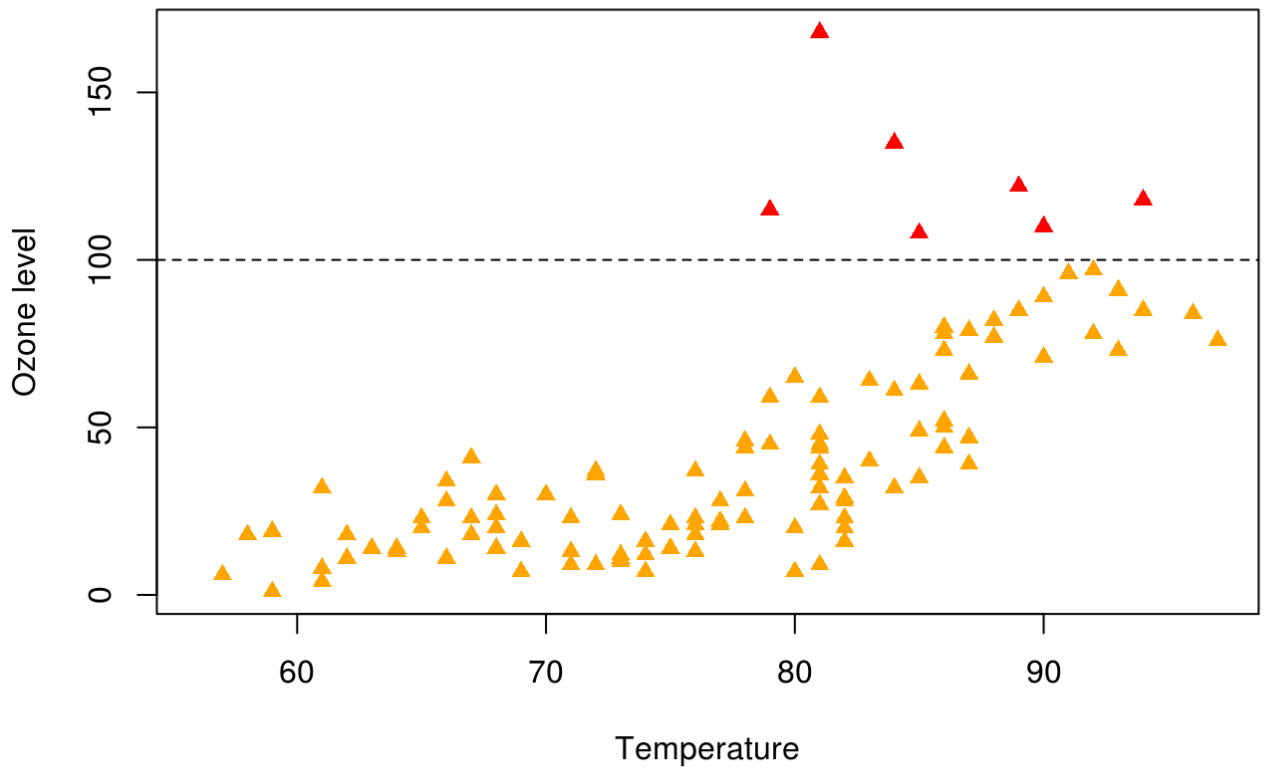
- Create a blank plot

- Identify observations with ozone > 100
  - plot the corresponding Temperature and Ozone values for these in red
- Identify observations with ozone < 100
  - plot the corresponding Temperature and Ozone values for these in orange



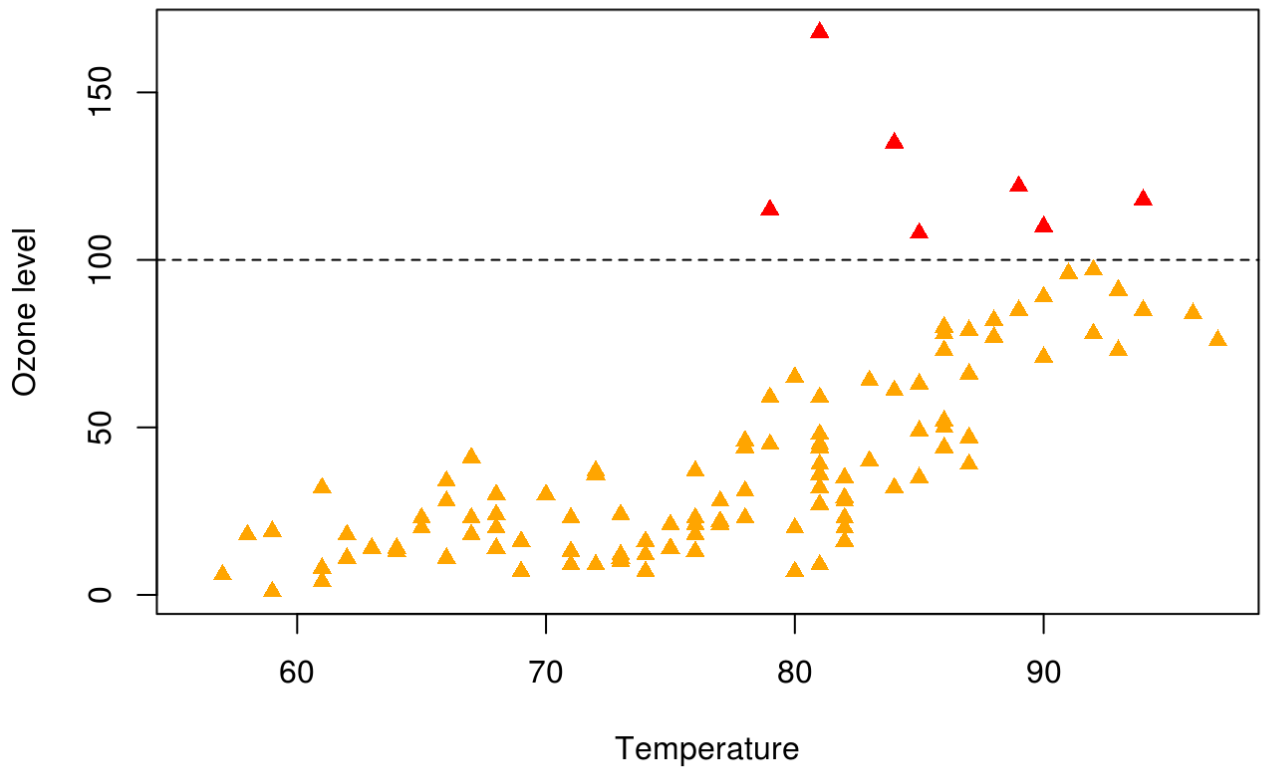
Solution: solution-exercise5.pdf

```
highO <- which(weather$Ozone > 100)
lowO <- which(weather$Ozone < 100)

plot(weather$Temp,weather$Ozone, type="n",
     ylab="Ozone level",
     xlab="Temperature")
points(weather$Temp[highO],weather$Ozone[highO],
       col="red",pch=17)
points(weather$Temp[lowO],weather$Ozone[lowO],
       col="orange",pch=17)
abline(h=100,lty=2)
```



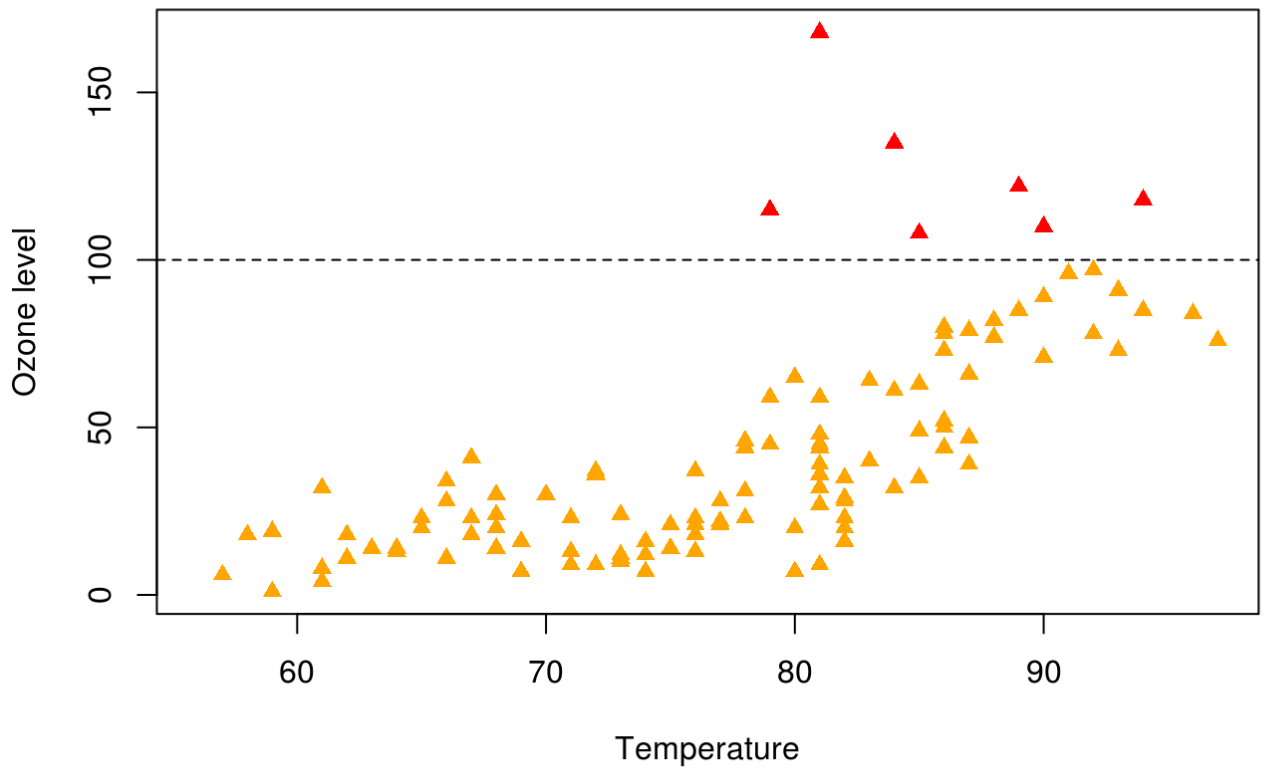
Alternative Solution

```
plot(weather$Temp,weather$Ozone, pch=17,
     col="orange", ylab="Ozone level",
     xlab="Temperature")
highO <- which(weather$Ozone > 100)
abline(h=100,lty=2)
points(weather$Temp[highO],weather$Ozone[highO],pch=17,col="red")
```



Alternative Solution

```
mycol = rep("orange",nrow(weather))
highO <- which(weather$Ozone > 100)
mycol[highO] <- "red"
plot(weather$Temp,weather$Ozone, pch=17,
     col=mycol, ylab="Ozone level",
     xlab="Temperature")
abline(h=100,lty=2)
```



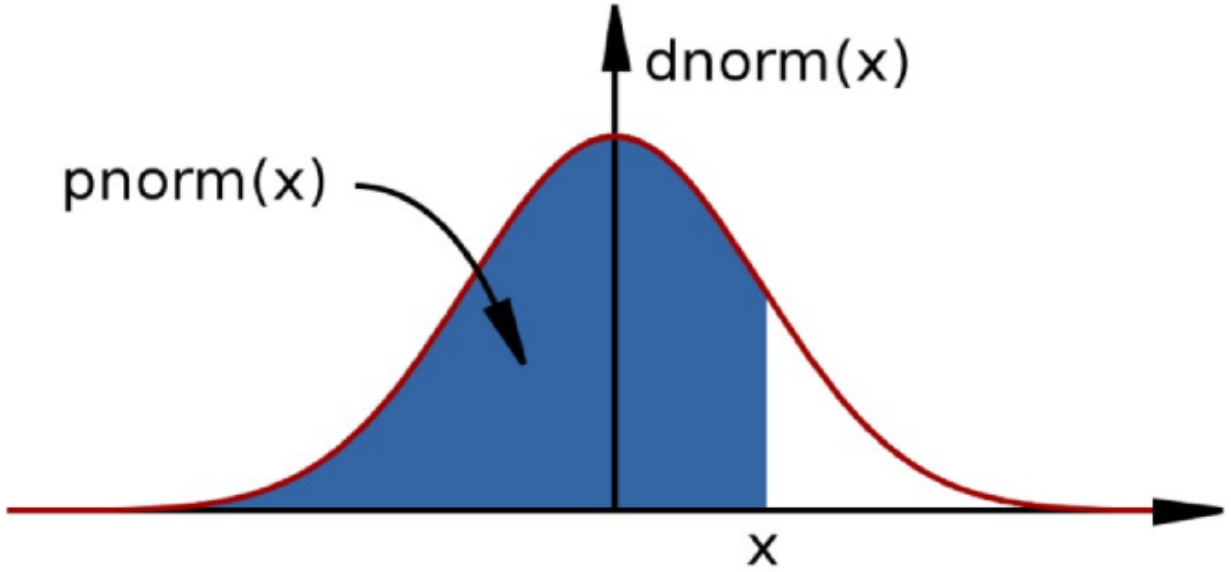
2. Statistics

Built-in support for statistics

- R is a statistical programming language:
  - Classical statistical tests are built-in
  - Statistical modeling functions are built-in
  - Regression analysis is fully supported
  - Additional mathematical packages are available (`MASS` , Waves, sparse matrices, etc)

Distribution functions

- Most commonly used distributions are built-in, functions have stereotypical names, e.g. for normal distribution:
  - **`pnorm`** - cumulative distribution for x
  - **`qnorm`** - inverse of pnorm (from probability gives x)
  - **`dnorm`** - distribution density
  - **`rnorm`** - random number from normal distribution



distributions

- Available for variety of distributions: `punif` (uniform), `pbinom` (binomial), `pnbinom` (negative binomial), `ppois` (poisson), `pgeom` (geometric), `phyper` (hyper-geometric), `pt` (T distribution), pf (F distribution)

Distribution functions

- 10 random values from the Normal distribution with mean 10 and standard deviation 5:

```
rnorm(10, mean=10, sd=5)
```

- The probability of drawing 10 from this distribution:

```
dnorm(10, mean=10, sd=5)
```

```
[1] 0.07978846
```

```
dnorm(100, mean=10, sd=5)
```

```
[1] 3.517499e-72
```

Distribution functions (continued)

- The probability of drawing a value smaller than 10:

```
pnorm(10, mean=10, sd=5)
```

```
[1] 0.5
```

- The inverse of `pnorm()`:

```
qnorm(0.5, mean=10, sd=5)
```

```
[1] 10
```

- How many standard deviations for statistical significance?

```
qnorm(0.95, mean=0, sd=1)
```

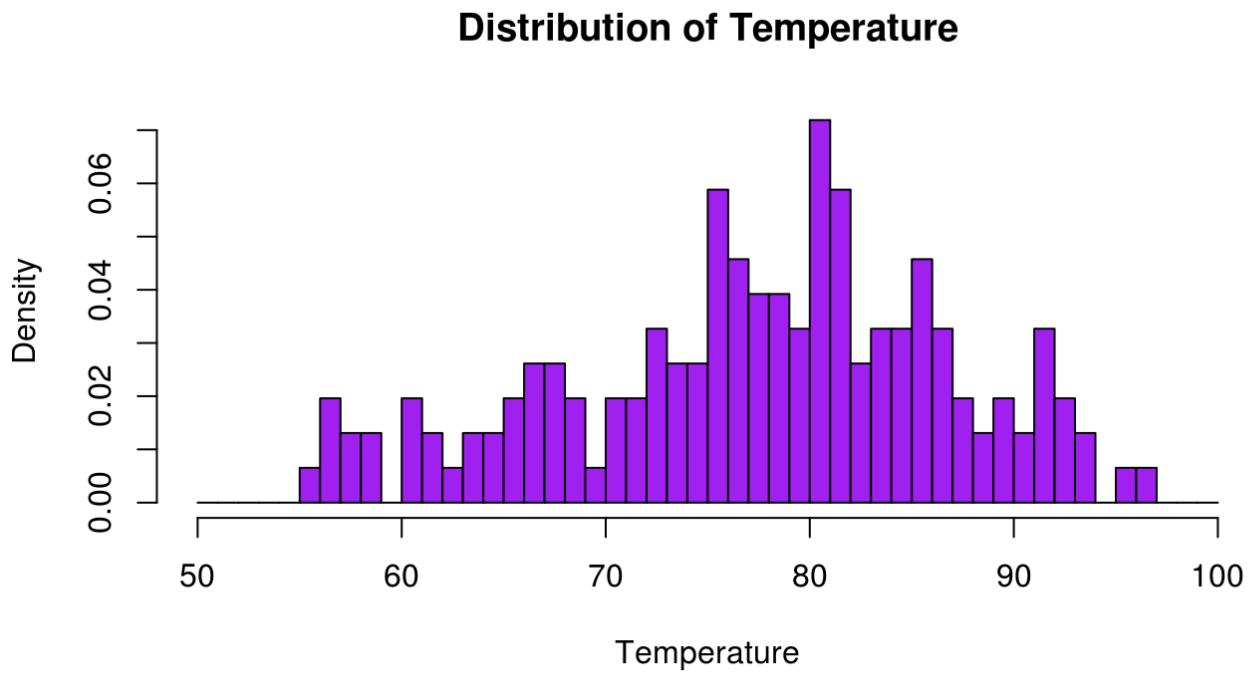
```
[1] 1.644854
```

Example

Recall our histogram of temperature from yesterday:

- The data look to be roughly normally-distributed
- An assumption we rely on for various statistical tests

```
hist(weather$Temp, col="purple", xlab="Temperature",
     main="Distribution of Temperature",
     breaks = 50:100, freq=FALSE)
```



Create a normal distribution curve

- If our data are normally-distributed, we can calculate the probability of drawing particular values.
  - e.g. a temperature of 80

```
tempMean <- mean(weather$Temp)
tempSD <- sd(weather$Temp)
dnorm(80, mean=tempMean, sd=tempSD)
```

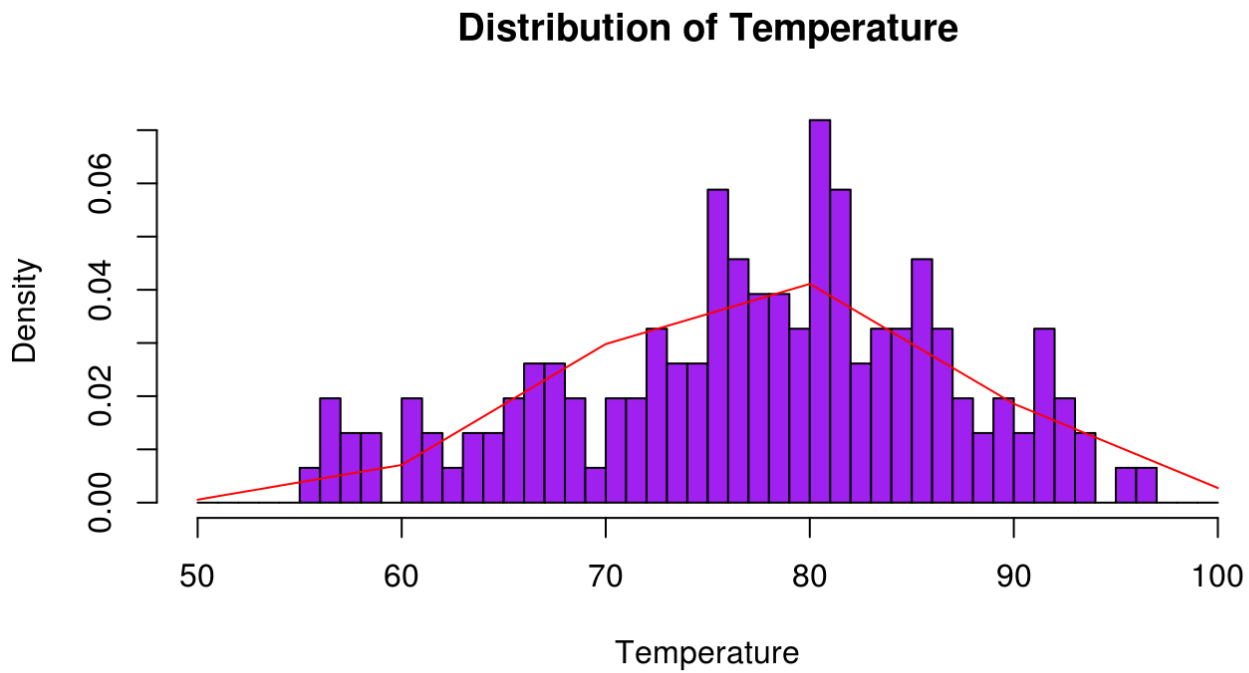
- We can overlay this on the histogram using `points` as we just saw:

```
hist(weather$Temp, col="purple", xlab="Temperature",
     main="Distribution of Temperature",
     breaks = 50:100, freq=FALSE)
points(80, dnorm(80, mean=tempMean, sd=tempSD),
       col="red", pch=16)
```

Create a normal distribution curve

- We can repeat the calculation for a vector of values
  - remember that functions in R are often ***vectorized***
  - use `lines` in this case rather than `points`

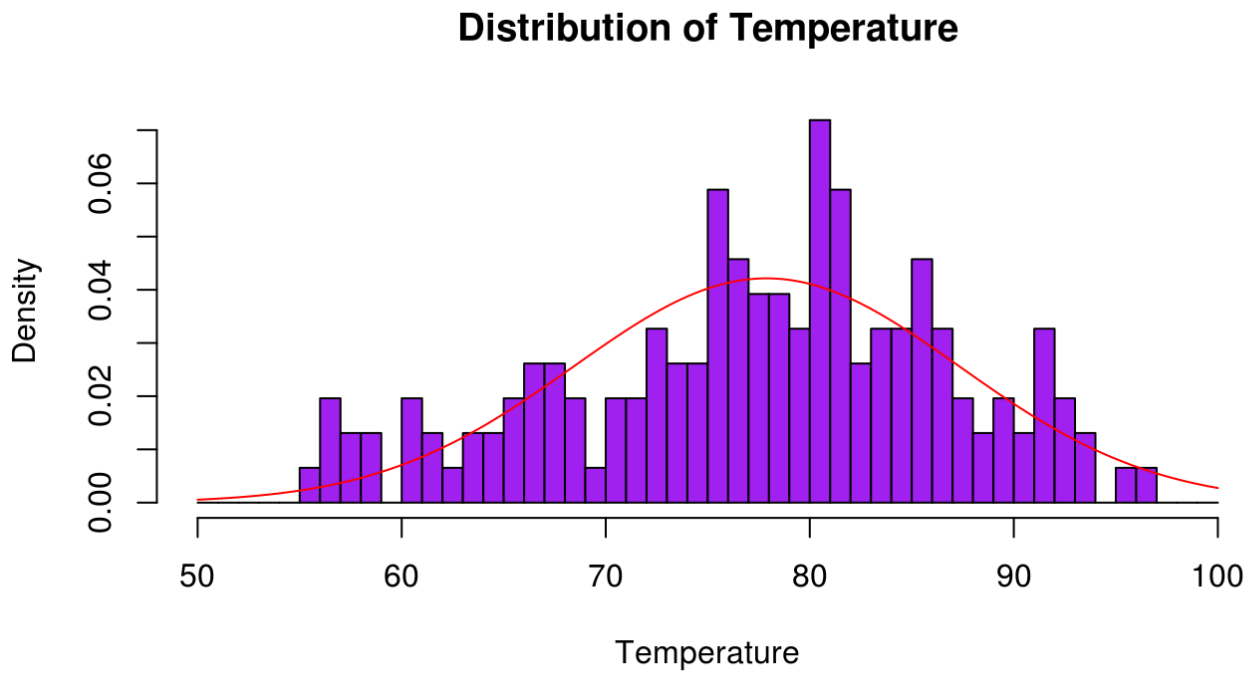
```
xs <- c(50,60,70,80,90,100)
ys <- dnorm(xs, mean=tempMean, sd=tempSD)
lines(xs, ys, col="red")
```



Create a normal distribution curve

- For a smoother curve, use a longer vector:
  - We can generate x values using the `seq()` function

```
xs <- seq(50,100, length.out = 10000)
ys <- dnorm(xs, mean=tempMean, sd=tempSD)
lines(xs, ys, col="red")
```



Simple testing

- If we want to compute the probability of observing a particular temperature, from the same distribution, we can use the standard formula to calculate a t statistic:

$$t = \frac{\bar{x} - \mu_0}{s/\sqrt{(n)}}$$

- Say a temperature of 50; which from the histogram seems to be unlikely

```
t <- (tempMean - 50) / (tempSD/sqrt(length(weather$Temp)))
t
```

```
[1] 36.43696
```

Simple testing

- …or use the **`t.test()`** function to compute the statistic and corresponding p-value

```
t.test(weather$Temp, mu=50)
```

```

    One Sample t-test

data:  weather$Temp
t = 36.437, df = 152, p-value < 2.2e-16
alternative hypothesis: true mean is not equal to 50
95 percent confidence interval:
 76.37051 79.39420
sample estimates:
mean of x
 77.88235
```

Two-sample tests: Basic data analysis

- Comparing 2 variances:
  - Fisher's F test

```
var.test()
```

- Comparing 2 sample means with normal errors:
  - Student's t test

```
t.test()
```

- Comparing 2 means with non-normal errors:
  - Wilcoxon's rank test

```
wilcox.test()
```

Two-sample tests: Basic data analysis

- Comparing 2 proportions:
  - Binomial test

```
prop.test()
```

- Correlating 2 variables:
  - Pearson's / Spearman's rank correlation

```
cor.test()
```

- Testing for independence of 2 variables in a contingency table:
  - Chi-squared / Fisher's exact test

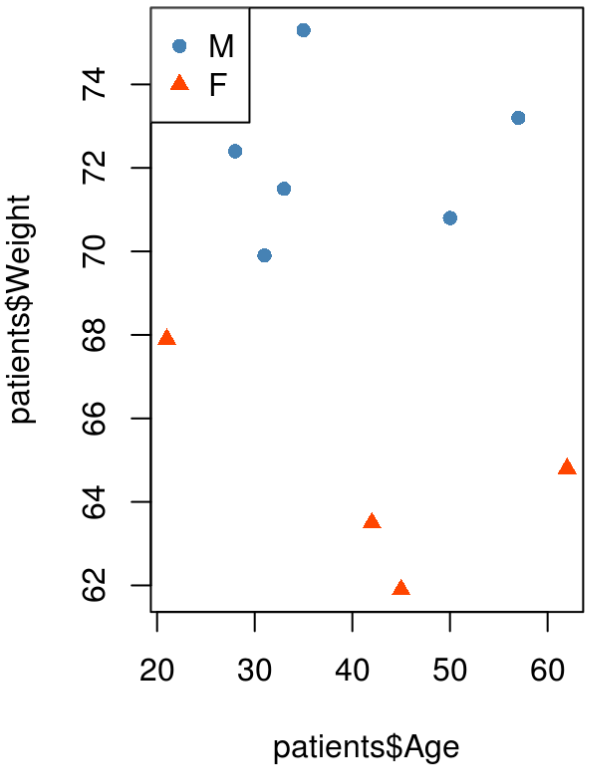
```
chisq.test(); fisher.test()
```

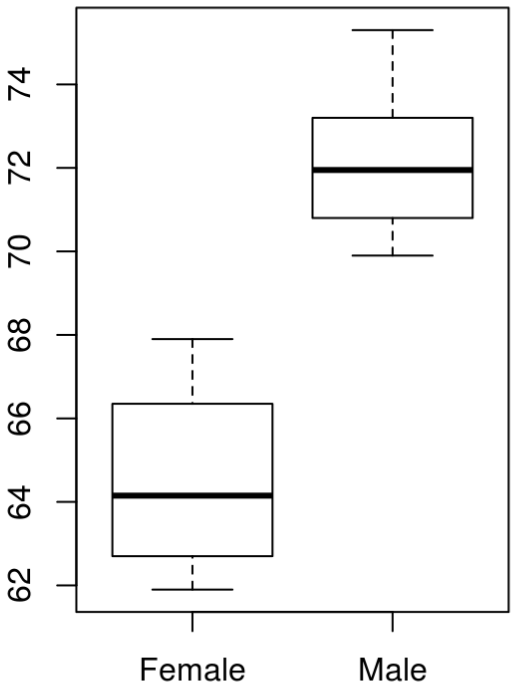
Statistical tests in R

- Bottom-line: Pretty much any statistical test you care to name will probably be in R
  - This is not supposed to be a statistics course (sorry!)
  - None of them are particular harder than others to use
  - The difficulty is deciding which test to use:
    - whether the assumptions of the test are met, etc.
  - Consult your local statistician if not sure
  - An upcoming course that will help
    - Introduction to Statistical Analysis (http://training.csx.cam.ac.uk/bioinformatics/event/1809255)
  - Some good references:
    - Statistical Analysis Using R (Course from the Babaraham Bioinformatics Core) (http://www.bioinformatics.babraham.ac.uk/training.html#rstats)
    - Quick-R guide to stats (http://www.statmethods.net/stats/index.html)
    - Simple R eBook (https://cran.r-project.org/doc/contrib/Verzani-SimpleR.pdf)
    - R wiki (https://en.wikibooks.org/wiki/R_Programming/Descriptive_Statistics)

Example analysis

- We have already seen that men in our `patients` dataset tend to be heavier than women
- We can **test this formally** in R





Test variance assumption

```
var.test(patients$Weight~patients$Sex)
```

```
    F test to compare two variances

data:  patients$Weight by patients$Sex
F = 1.759, num df = 3, denom df = 5, p-value = 0.5417
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
  0.2265757 26.1830147
sample estimates:
ratio of variances
          1.759041
```

Perform the t-test

```
t.test(patients$Weight~patients$Sex, var.equal=TRUE)
```

```
    Two Sample t-test

data:  patients$Weight by patients$Sex
t = -5.4584, df = 8, p-value = 0.0006027
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -10.893759  -4.422908
sample estimates:
mean in group Female   mean in group Male
            64.52500             72.18333
```

- This function can be tuned in various ways (`?t.test`):

- Assumed equal variances, or not (and use Welch's correction)
- Deal with parired samples
- Two-sided, or one-sided p-value

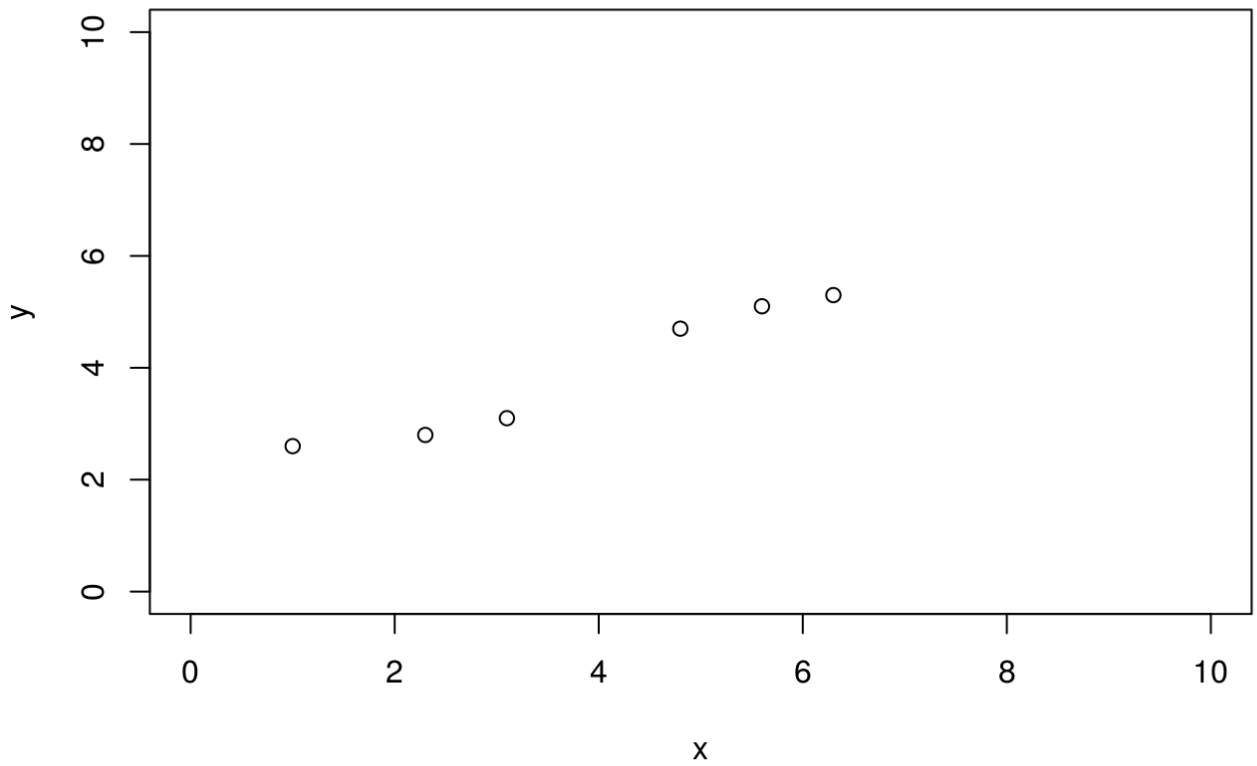
Linear regression: Basic data analysis

- Linear modeling is supported by the function **`lm()`**:
  - `example(lm)`
  - The output assumes you know a fair bit about the subject
- `lm` is really useful for plotting lines of best fit to XY data, in order to determine intercept, gradient and Pearson's correlation coefficient
  - This is very easy in R
- Three steps to plotting with a best fit line:
  1. Plot XY scatter-plot data
  2. Fit a linear model
  3. Add bestfit line data to plot with `abline()` function

Typical linear regression analysis: Basic data analysis

- The ~ (***tilde***) is used to define a ***formula***; i.e. "y is given by x"

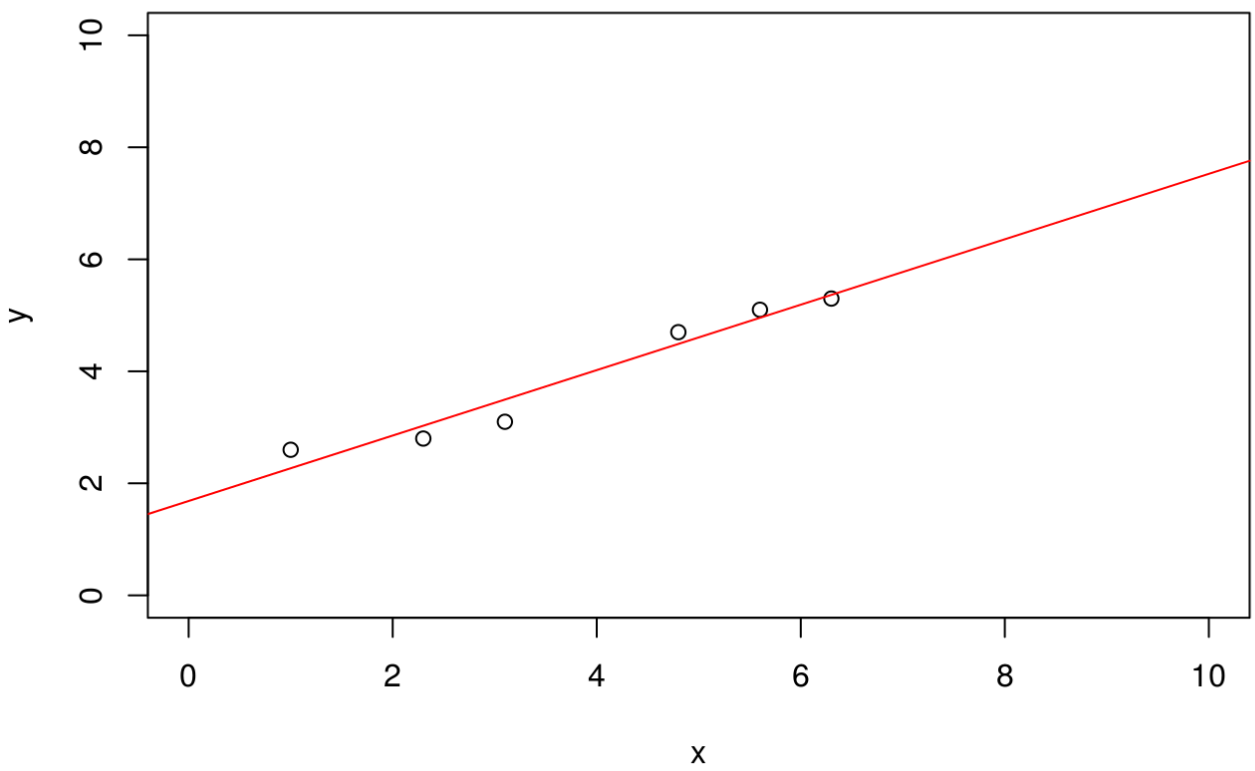
```
x <- c(1, 2.3, 3.1, 4.8, 5.6, 6.3)
y <- c(2.6, 2.8, 3.1, 4.7, 5.1, 5.3)
plot(x,y, xlim=c(0,10), ylim=c(0,10))
```



Typical linear regression analysis: Basic data analysis

The ~ is used to define a formula; i.e. “y is given by x” - Take care about the order of x and y in the plot and lm expressions

```
plot(x,y, xlim=c(0,10), ylim=c(0,10))
myModel <- lm(y~x)
abline(myModel, col="red")
```



In-depth summary

```
summary(myModel)
```

```

Call:
lm(formula = y ~ x)

Residuals:
       1        2        3        4        5        6
 0.33159 -0.22785 -0.39520  0.21169  0.14434 -0.06458

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.68422    0.29056   5.796   0.0044 **
x            0.58418    0.06786   8.608   0.0010 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3114 on 4 degrees of freedom
Multiple R-squared:  0.9488,    Adjusted R-squared:  0.936
F-statistic:  74.1 on 1 and 4 DF,  p-value: 0.001001
```

Typical linear regression analysis: Basic data analysis

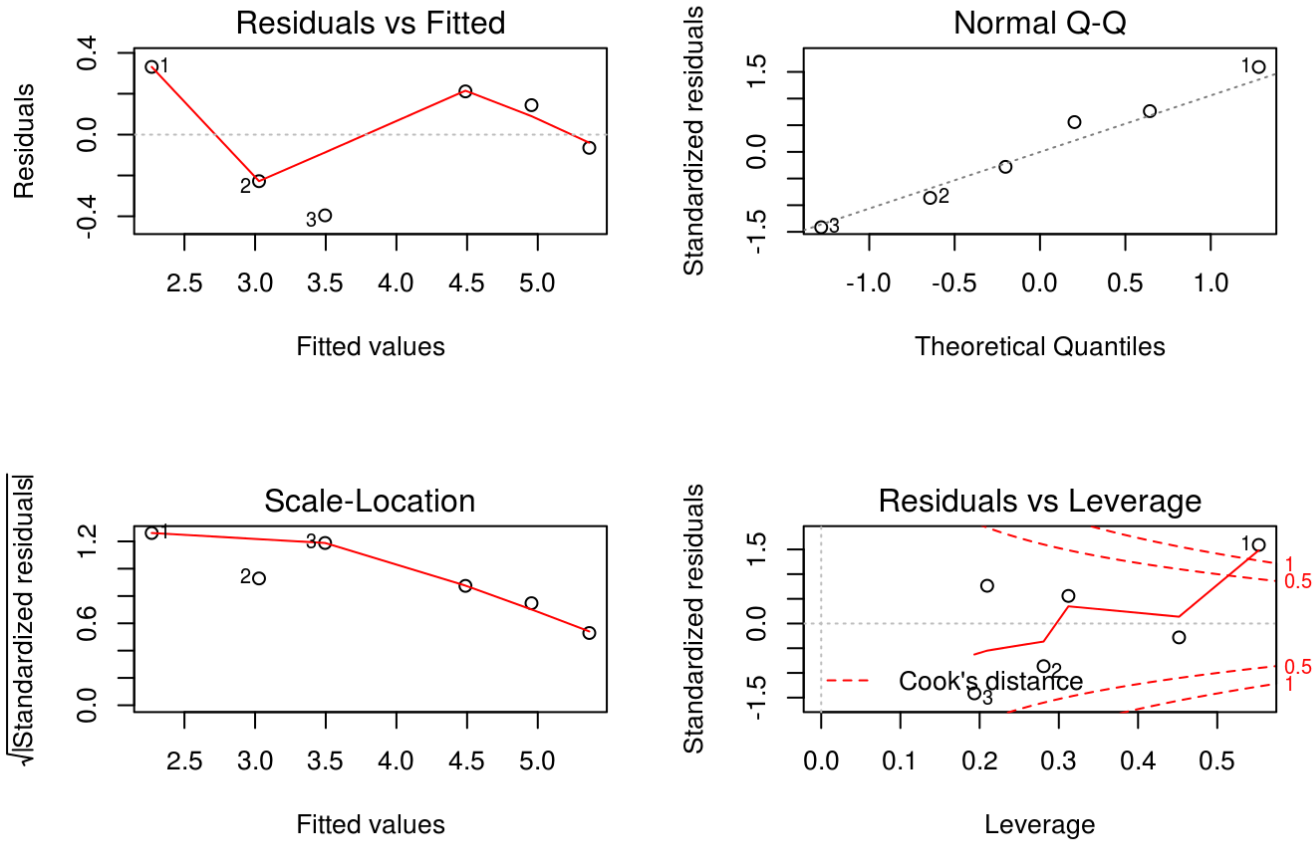
- Get the coefficients of the fit from:

```
coef(myModel)   # Coefficients
resid(myModel)  # Residuals
fitted(myModel) # Fitted values
names(myModel)  # Names of the objects within myModel
```

Diagnostic plots of the fit

- Get QC of fit from:

```
par(mfrow=c(2,2))
plot(myModel)
```



Modelling formulae

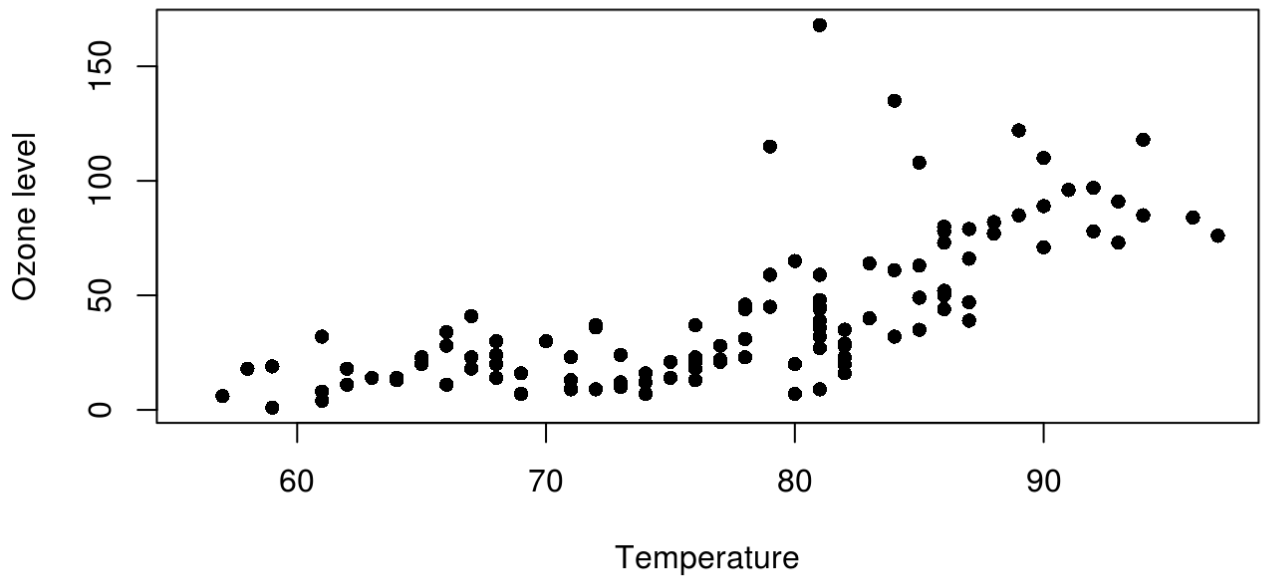
- R has a very powerful formula syntax for describing statistical models
- Suppose we had two explanatory variables `x` and `z`, and one response variable `y`
- We can describe a relationship between, say, `y` and `x` using a tilde `~`, placing the response variable on the left of the tilde and the

explanatory variables on the right:
    - `y~x`
- It is very easy to extend this syntax to do multiple regressions, ANOVAs, to include interactions, and to do many other common modelling tasks. For example

```
y~x       #If x is continuous, this is linear regression
y~x       #If x is categorical, ANOVA
y~x+z     #If x and z are continuous, multiple regression
y~x+z     #If x and z are categorical, two-way ANOVA
y~x+z+x:z # : is the symbol for the interaction term
y~x*z     # * is a shorthand for x+z+x:z
```

Exercise: exercise6.Rmd

- There are suggestions that Ozone level could be influenced by Temperature:



- Perform a linear regression analysis to assess this:
  - Fit the linear model and print a summary of the output
  - Plot the two variables and overlay a best-fit line

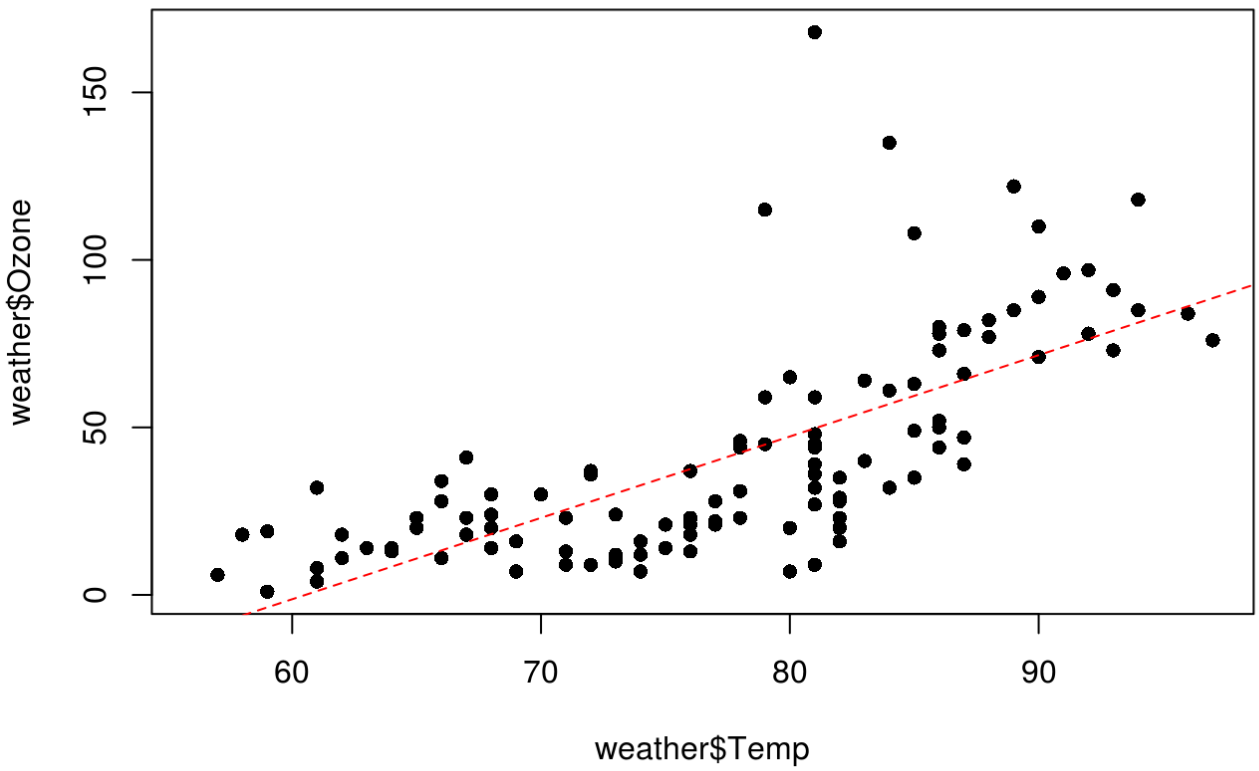
Solution: solution-exercise6.pdf

```
mod1 <- lm(weather$Ozone~weather$Temp)
summary(mod1)
```

```
Call:
lm(formula = weather$Ozone ~ weather$Temp)

Residuals:
    Min      1Q  Median      3Q     Max
-40.729 -17.409  -0.587  11.306 118.271

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  -146.9955    18.2872  -8.038 9.37e-13 ***
weather$Temp    2.4287     0.2331  10.418  < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 23.71 on 114 degrees of freedom
  (37 observations deleted due to missingness)
Multiple R-squared:  0.4877,    Adjusted R-squared:  0.4832
F-statistic: 108.5 on 1 and 114 DF,  p-value: < 2.2e-16
```

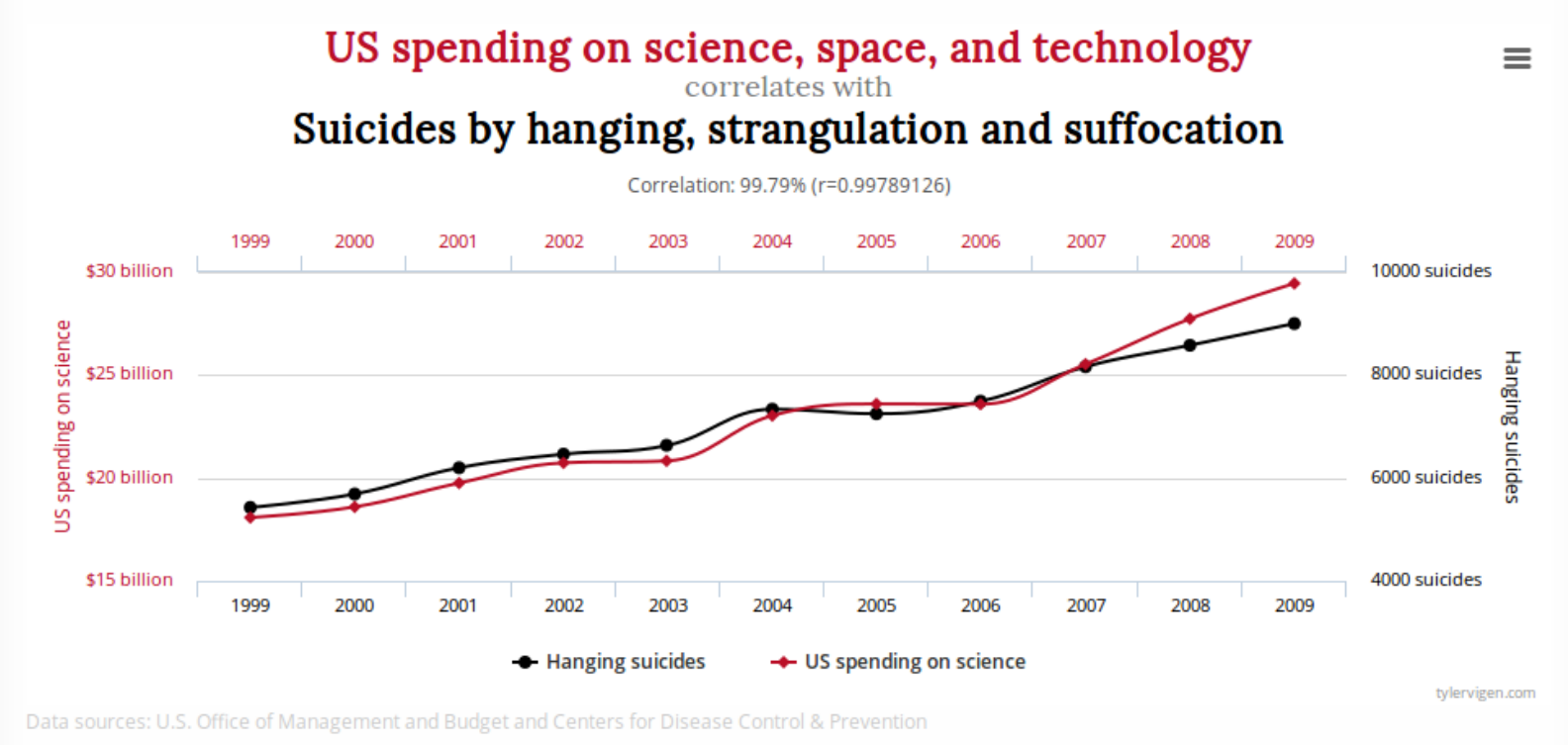
Solution

```
plot(weather$Temp, weather$Ozone, pch=16)
abline(mod1, col="red", lty=2)
```



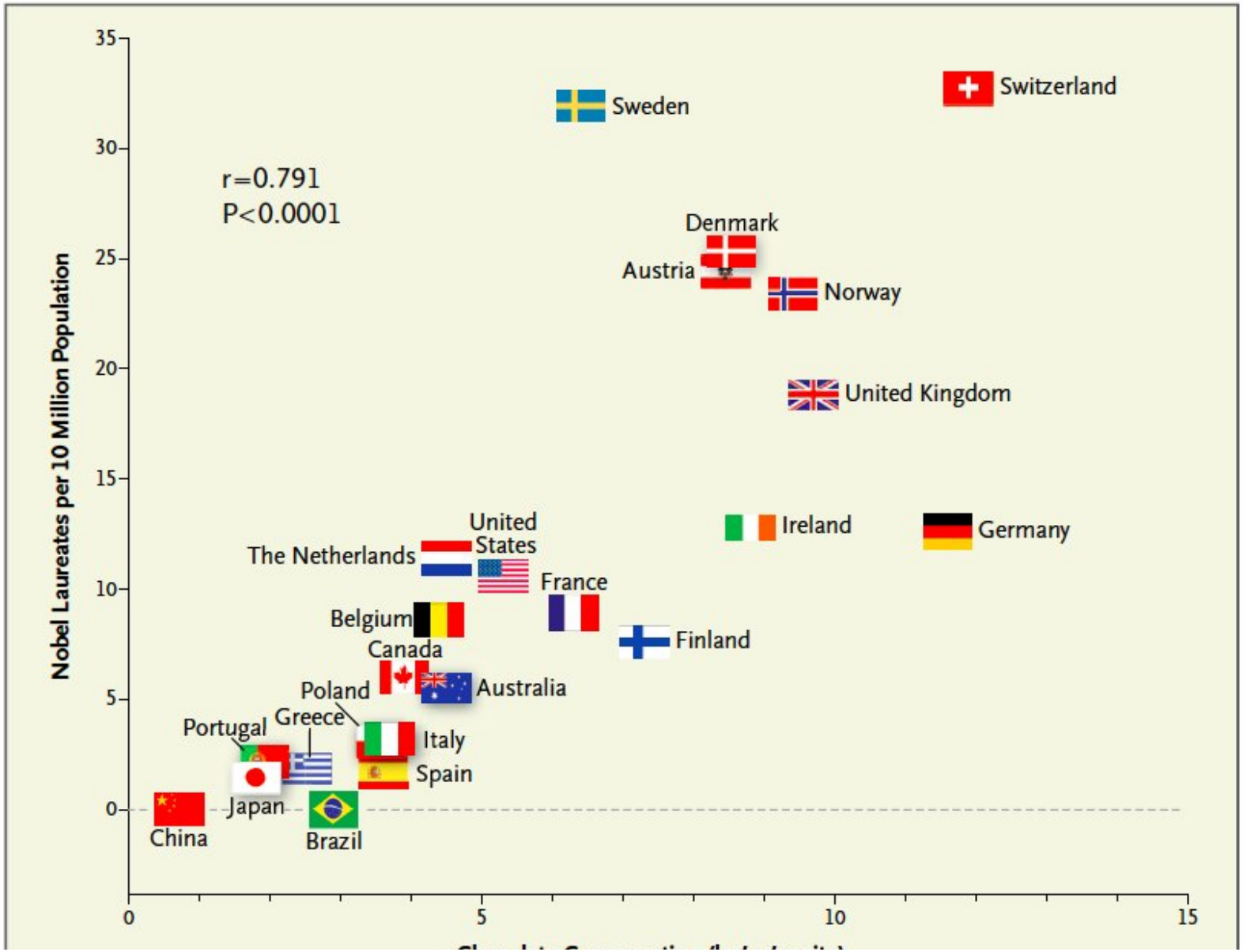
Word of caution

Correlation != Causation



http://tylervigen.com/spurious-correlations (http://tylervigen.com/spurious-correlations)

Word of caution

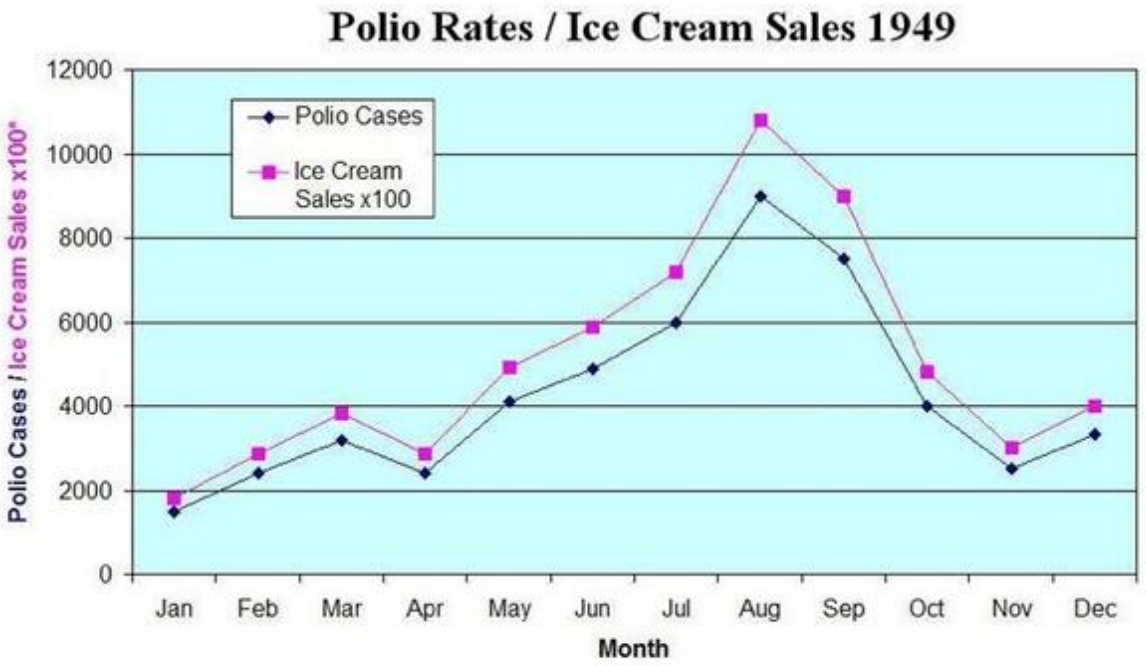

Nobel Laureates per 10 Million Population
r=0.791
P<0.0001
Sweden
Switzerland
Denmark
Austria
Norway
United Kingdom
Ireland
Germany
United States
The Netherlands
France
Belgium
Finland
Canada
Australia
Poland
Greece
Portugal
Italy
Spain
Japan
China
Brazil
0
5
10
15
20
25
30
35

So if I want to win a nobel prize, I should eat even more chocolate?!?!? (http://www.businessinsider.com/chocolate-consumption-vs-nobel-prizes-2014-4?IR=T)

But no-one would ever take such trends seriously….would they?

Wrong!

The Real Cause of Polio!



In the late 1940s, before there was a polio vaccine, public health experts in America noted that polio cases increased in step with the consumption of ice cream and soft drinks, Eliminating such treats was even recommended as part of an anti-polio diet. It turned out that polio outbreaks were most common in the hot months of summer, when people naturally ate more ice cream, showing only an association.

http://www.nytimes.com/2009/08/06/technology/06stats.html

Cutting-down on Ice Cream was recommended as a safeguard against polio!

3. Data Manipulation Techniques

Motivation

- So far we have been lucky that all our data have been in the same file:
  - This is not usually the case
  - Dataset may be spread over several files
    - This takes longer, and is harder, than many people realise
  - We need to combine before doing an analysis

Combining data from multiple sources: Gene Clustering Example

- R has powerful functions to combine heterogeneous data sources into a single data set
- Gene clustering example data:
  - Gene expression values in ***gene.expression.txt***
  - Gene information in ***gene.description.txt***
  - Patient information in ***cancer.patients.txt***
- A breast cancer dataset with numerous patient characteristics:
  - We will concentrate on ***ER status*** (positive / negative)
  - What genes show a statistically-significant different change between ER groups?

Peek at the data

```
evals <- read.delim("gene.expression.txt", stringsAsFactors = FALSE)
evals[1:2,1:5]
dim(evals)
```

```
               NKI_4 NKI_6  NKI_7  NKI_8 NKI_9
Contig56678_RC -0.261 0.346  0.047 -1.140 -0.11
AF026004       -0.064 0.040 -0.165 -0.031  0.33
```

```
[1] 498 337
```

- 498 rows and 337 columns
- One row for each gene:
  - Rows are named according to particular technology used to make measurement
  - The names of each row can be returned by `rownames(evals)` ; giving a vector
- One column for each patient:
  - The names of each column can be returned by `colnames(evals)` ; giving a vector

Peek at the data

```
genes <- read.delim("gene.description.txt",stringsAsFactors = FALSE)
head(genes)
```

```
                       probe HUGO.gene.symbol Chromosome     Start
Contig56678_RC Contig56678_RC            THSD4      chr15  71433788
AF026004             AF026004            CLCN2       chr3 184063973
AB033049             AB033049          ANKRD50       chr4 125585207
AB033050             AB033050            ZMIZ1      chr10  80828792
AB033086             AB033086           NLGN4X       chrX   5808083
NM_003008           NM_003008            SEMG2      chr20  43850010
```

```
dim(genes)
```

```
[1] 498   4
```

- 498 rows and 4 columns
- One for for each gene
- Includes mapping between manufacturer ID and Gene name

Peek at the data

```
subjects <- read.delim("cancer.patients.txt")
head(subjects)
```

```
       samplename age er grade
NKI_4       NKI_4  41  1     3
NKI_6       NKI_6  49  1     2
NKI_7       NKI_7  46  0     1
NKI_8       NKI_8  48  0     3
NKI_9       NKI_9  48  1     3
NKI_11     NKI_11  37  1     3
```

```
dim(subjects)
```

```
[1] 337   4
```

- One for each patient in the study
- Each column is a different characteristic of that patient
    - e.g. whether a patient is ER positive or negative

```
table(subjects$er)
```

```
 0   1
88 249
```

Ordering and sorting

To get a feel for these data, we will look at how we can subset and order

- R allows us to do the kinds of filtering, sorting and ordering operations you might be familiar with in Excel
- For example, if we want to get information about patients that are ER negative
  - these are indicated by an entry of ***0*** in the `er` column

```
subjects$er == 0
```

```
[1] "FALSE" "FALSE" "..."   "FALSE" "FALSE"
```

Ordering and sorting

We can do the comparison within the square brackets

- Remembering to include a `,` to index the columns as well
- Best practice to create a new variable and leave the original data frame untouched

```
erNegPatients <- subjects[subjects$er == 0,]
head(erNegPatients)
```

```
       samplename age er grade
NKI_7       NKI_7  46  0     1
NKI_8       NKI_8  48  0     3
NKI_12     NKI_12  46  0     3
NKI_24     NKI_24  49  0     3
NKI_28     NKI_28  40  0     3
NKI_44     NKI_44  53  0     3
```

Ordering and sorting

Sorting is supported by the **`sort()`** function

- Given a vector, it will return a sorted version of the same length

```
sort(erNegPatients$grade)
```

```
 [1] "1"   "1"   "1"   "1"   "1"   "2"   "2"   "2"   "2"   "2"   "..."
[12] "3"   "3"   "3"   "3"   "3"   "3"   "3"   "3"   "3"
```

- But this is not useful in all cases
  - We have lost the extra information that we have about the patients

Ordering and sorting

- Instead, we can use **`order()`**
- Given a vector, `order()` will give a set of numeric values which will give an ordered version of the vector
  - default is smallest –> largest

```
myvec <- c(9,10,4,3,8,5,6,2,1,7)
myvec
```

```
 [1]  9 10  4  3  8  5  6  2  1  7
```

```
order(myvec)
```

```
 [1]  9  8  4  3  6  7 10  5  1  2
```

- i.e. number in position 9 is the smallest, number in position 8 is the second smallest:

```
myvec[9]
```

```
[1] 1
```

```
myvec[8]
```

```
[1] 2
```

Ordering and sorting

- We can use the result of `order()` to perform a subset of our original vector
- The result is an ordered vector

```
myvec.ord <- myvec[order(myvec)]
myvec.ord
```

```
 [1]  1  2  3  4  5  6  7  8  9 10
```

- Implication: We can use `order` on a particular column of a data frame, and use the result to sort all the rows

Ordering and sorting

- Here we order the `age` column and use the result to re-order the rows in the data frame

```
erNegPatientsByAge <- erNegPatients[order(erNegPatients$age),]
head(erNegPatientsByAge)
```

```
        samplename age er grade
NKI_330    NKI_330  26  0     3
NKI_57      NKI_57  28  0     3
NKI_230    NKI_230  28  0     3
NKI_90      NKI_90  29  0     3
NKI_48      NKI_48  30  0     3
NKI_86      NKI_86  30  0     3
```

Ordering and sorting

- can change the behaviour of `order` to be Largest –> Smallest

```
erNegPatientsByAge <- erNegPatients[order(erNegPatients$age,decreasing = TRUE),]
head(erNegPatientsByAge)
```

```
        samplename age er grade
NKI_96      NKI_96  62  0     3
NKI_93      NKI_93  61  0     3
NKI_119    NKI_119  54  0     3
NKI_44      NKI_44  53  0     3
NKI_75      NKI_75  52  0     3
NKI_76      NKI_76  52  0     2
```

- we can write the result to a fie if we wish

```
write.table(erNegPatientsByAge, file="erNegativeSubjectsByAge.txt", sep="\t")
```

Exercise: exercise7.Rmd

- Imagine we want to know information about chromosome 8 genes that have been measured.

1. Create a new data frame containing information on genes on Chromosome 8
2. Order the rows in this data frame according to start position, and write the results to a file

Solution: solution-exercise7.pdf

```
chr8Genes <- genes[genes$Chromosome=="chr8",]
head(chr8Genes)
```

```
                       probe HUGO.gene.symbol Chromosome     Start
Contig29827_RC Contig29827_RC            FUT10       chr8  33228344
NM_003046           NM_003046           SLC7A2       chr8  17396286
Contig55940_RC Contig55940_RC            CYHR1       chr8 145675315
NM_004133           NM_004133            HNF4G       chr8  76452203
NM_004374           NM_004374            COX6C       chr8 100890223
AF052142             AF052142            NCALD       chr8 102698770
```

```
chr8GenesOrd <- chr8Genes[order(chr8Genes$Start),]
head(chr8GenesOrd)
```

```
                       probe HUGO.gene.symbol Chromosome    Start
NM_004745           NM_004745           DLGAP2       chr8  1449569
NM_018941           NM_018941             CLN8       chr8  1711870
AL117604             AL117604             DLC1       chr8 12940872
NM_003046           NM_003046           SLC7A2       chr8 17396286
Contig58301_RC Contig58301_RC           SLC7A2       chr8 17396286
NM_000662           NM_000662             NAT1       chr8 18067618
```

```
write.table(chr8GenesOrd, "chromosome8.gene.info.txt", sep="\t")
```

Retrieving data for a particular gene

- Gene `ESR1` is known to be hugely-different between ER positive and negative patient
  - let's check that this is evident in our dataset
  - if not, something has gone wrong!
- First step is to locate this gene in our dataset

Character matching in R

- We have already seen various ways of comparing numeric values
  - ==, >, <
  - each of which returns a vector of logical values
  - == will also work with text

```
LETTERS
```

```
 [1] "A" "B" "C" "D" "E" "F" "G" "H" "I" "J" "K" "L" "M" "N" "O" "P" "Q"
[18] "R" "S" "T" "U" "V" "W" "X" "Y" "Z"
```

```
"A" == LETTERS
```

```
 [1]  TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[12] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[23] FALSE FALSE FALSE FALSE
```

Character matching in R

- `match()` and `grep()` are often used to find particular matches
  - CAUTION: by default, match will only return the ***first*** match!

```
match("D", LETTERS)
```

```
[1] 4
```

```
grep("F", rep(LETTERS,2))
```

```
[1]  6 32
```

```
match("F", rep(LETTERS,2))
```

```
[1] 6
```

Retrieving data for a particular gene

- Find the name of the ID that corresponds to gene ***ESR1***
  - mapping between IDs and genes is in the ***genes*** data frame
    - ID in first column, gene name in the second
- Save this ID as a variable

```
ind <- match("ESR1", genes$HUGO.gene.symbol)
genes[ind,]
```

```
             probe HUGO.gene.symbol Chromosome     Start
NM_000125 NM_000125             ESR1       chr6 152128814
```

```
probe <- genes[ind,1]
probe
```

```
[1] "NM_000125"
```

Retrieving data for a particular gene

Now, find which row in our expression matrix is indexed by this ID

- recall that the rownames of the expression matrix are the probe IDs
- save the expression values as a variable

```
match(probe, rownames(evals))
```

```
[1] 384
```

```
evals[match(probe, rownames(evals)), 1:10]
```

```
           NKI_4 NKI_6  NKI_7 NKI_8 NKI_9 NKI_11 NKI_12 NKI_13 NKI_14
NM_000125 -0.007 0.074 -0.767 -0.82 -0.18 -0.296     NA -0.163  0.059
          NKI_17
NM_000125 -0.035
```

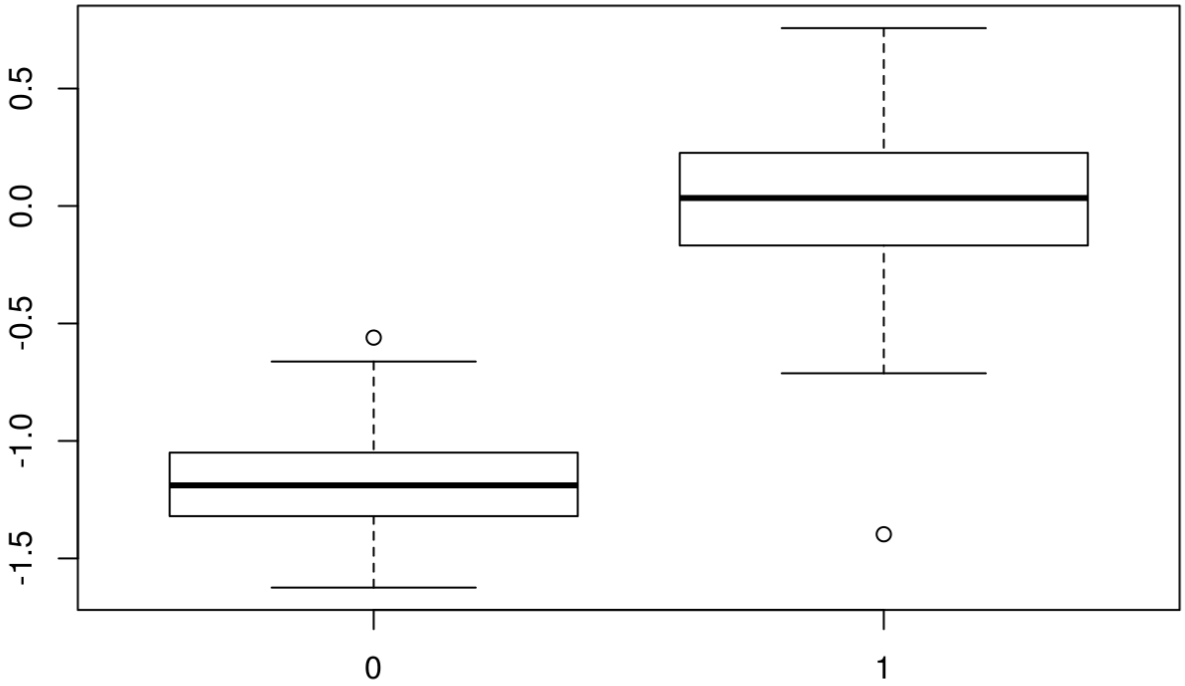
```
genevals <- evals[match(probe,rownames(evals)),]
```

Relating to patient characteristics

We have numeric expression values and want to visualise them against our categorical data

- use a boxplot, for example

```
boxplot(as.numeric(genevals) ~ factor(subjects$er))
```



Relating to patient characteristics

- The p-value is also encouraging

```
t.test(as.numeric(genevals) ~ factor(subjects$er))
```

```
    Welch Two Sample t-test

data:  as.numeric(genevals) by factor(subjects$er)
t = -38.746, df = 205.88, p-value < 2.2e-16
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.246953 -1.126198
sample estimates:
mean in group 0 mean in group 1
    -1.17388506      0.01269076
```

Complete script

esr1Example.Rmd

```
genes    <- read.delim("gene.description.txt")
subjects <- read.delim("cancer.patients.txt")
evals    <- read.delim("gene.expression.txt", stringsAsFactors = FALSE)

ind      <- match("ESR1", genes[,2])
probe    <- genes[ind,1]
genevals <- evals[match(probe,rownames(evals)),]

boxplot(as.numeric(genevals) ~ factor(subjects$er))
t.test(as.numeric(genevals) ~ factor(subjects$er))
```

Exercise: exercise8.Rmd

Repeat the same steps we performed for the gene ESR1, but for GATA3:

- Try and make as few changes as possible from the ESR1 script
- Can you see why making a markdown document is useful for analysis?

4. Programming in R

Motivation

From the previous exercise, you should see how we can easily adapt our markdown scripts:

- e.g. ESR1 versus GATA3
- But what if we want to analyse many genes?
- It would be tedious to create a new markdown document for every gene
- …and prone to error too

Introducing loops

- Many programming languages have ways of doing the same thing many times, perhaps changing some variable each time. This is called **looping**
- Loops are not used in R so often, because we can usually achieve the same thing using vector calculations
- For example, to add two vectors together, we do not need to add each pair of elements one by one, we can just add the vectors

```
x <- 1:10
y <- 11:20
x+y
```

- But there are some situations where R functions can not take vectors as input. For example, `t.test()` will only test one gene at a

time
- What if we wanted to test multiple genes?

Introducing loops

- We could do this:

```
t.test(evals[1,] ~ factor(subjects$er))
t.test(evals[2,] ~ factor(subjects$er))
```

- But this will be boring to type, difficult to change, and prone to error
- As we are doing the same thing multiple times, but with a different index each time, we can use a **loop** instead

Loops: Commands and flow control

- R has two basic types of loop
  - a **`for`** loop: run some code on every value in a vector
  - a **`while`** loop: run some code while some condition is true (*hardly ever used!*)

`for`

```
for(i in 1:10) {
  print(i)
  }
```

`while`

```
i <- 1
while(i <= 10 ) {
  print(i)
  i <- i + 1
  }
```

Loops: Commands and flow control

- Here's how we might use a `for` loop to test the first 10 genes

```
for(i in 1:10) {
  t.test(as.numeric(evals[i,]) ~ factor(subjects$er))
  }
```

- This is exactly the same as:

```
i <- 1
t.test(evals[i,] ~ factor(subjects$er))
i <- 2
t.test(evals[i,] ~ factor(subjects$er))
i <- 3
...
```

Storing results

However, this for loop is doing the calculations but not storing the results

- The output of `t.test()` is an object with data placed in different slots
  - the `names()` of the object tells us what data we can retrieve, and what name to use

- N.B it is a “list” object

```
t <- t.test(as.numeric(evals[1,]) ~ factor(subjects$er))
names(t)
```

```
[1] "statistic"   "parameter"   "p.value"     "conf.int"    "estimate"
[6] "null.value"  "alternative" "method"      "data.name"
```

```
t$statistic
```

```
       t
-20.12546
```

Storing results

- When using a loop, we often create an empty “dummy” variable
- This is used store the results at each stage of the loop

```
stats <- NULL
for(i in 1:10) {
  tmp <- t.test(as.numeric(evals[i,]) ~ factor(subjects$er))
  stats[i] <- tmp$statistic
  }
stats
```

```
 [1] -20.1254643  -1.7973581  -9.2625540  -3.3080720   0.7512869
 [6]  -0.6220547  -0.2596520  -4.1309155  -1.7027881 -16.1224377
```

Practical application

Previously we have identified probes on chromosome 8

- Lets say that we want to do a t-test for each gene on chromosome 8

```
head(chr8GenesOrd)
```

```
                      probe HUGO.gene.symbol Chromosome    Start
NM_004745           NM_004745           DLGAP2       chr8  1449569
NM_018941           NM_018941             CLN8       chr8  1711870
AL117604             AL117604             DLC1       chr8 12940872
NM_003046           NM_003046           SLC7A2       chr8 17396286
Contig58301_RC Contig58301_RC           SLC7A2       chr8 17396286
NM_000662           NM_000662             NAT1       chr8 18067618
```

- The first step is to extract the expression values for chromosome 8 genes from our expression matrix, which has expression values for all genes
- We can use the `match` function to tell us which rows in the matrix correspond to chromosome 8 genes

```
match(chr8GenesOrd$probe, rownames(evals))
```

```
 [1] 215 494 161   8 481 461 140 478   7  87 256 139 449 128 138 176 201
[18]  77
```

```
chr8Expression <- evals[match(chr8GenesOrd$probe, rownames(evals)),]
dim(chr8Expression)
```

```
[1]  18 337
```

Exercise: exercise9.Rmd

- Create a for loop to perform to test if the expression level of each gene on chromosome 8 is significantly different between ER positive and negative samples
- Store the ***p-value*** from each individual test

Solution: solution-exercise9.pdf

```
pvals <- NULL
for(i in 1:18) {
  tmp <- t.test(as.numeric(chr8Expression[i,]) ~ factor(subjects$er))
  pvals[i] <- tmp$p.value
  }
pvals
```

```
 [1] 5.464153e-03 2.408701e-01 5.842811e-05 6.611391e-05 2.590922e-57
 [6] 2.564435e-69 9.382548e-01 7.555477e-01 7.955434e-01 2.088048e-01
[11] 2.695280e-01 5.440249e-01 3.764754e-02 2.297528e-37 2.077849e-04
[16] 2.188104e-03 1.340043e-12 2.169950e-08
```

Conditional branching: Commands and flow control

- Use an `if` statement for any kind of condition testing
- Different outcomes can be selected based on a condition within brackets

```
if (condition) {
  ... do this ...
  } else {
    ... do something else ...
    }
```

- `condition` is any logical value, and can contain multiple conditions.
  - e.g. `(a == 2 & b < 5)`, this is a compound conditional argument
- The condition should return a *single* value of `TRUE` or `FALSE`

Other conditional tests

- There are various tests that can check the type of data stored in a variable
  - these tend to be called **`is...()`**.
    - try *tab-complete* on `is.`

```
is.numeric(10)
```

```
[1] TRUE
```

```
is.numeric("TEN")
```

```
[1] FALSE
```

```
is.character(10)
```

```
[1] FALSE
```

- `is.na()` is useful for seeing if an `NA` value is found
  - cannot use `== NA` !

```
match("foo", genes[,2])
```

```
[1] NA
```

```
is.na(match("foo", genes[,2]))
```

```
[1] TRUE
```

Conditional branching: Commands and flow control

- Using the **`for`** loop we wrote before, we could add some code to plot the expression of each gene
  - a boxplot would be ideal
- However, we might only want plots for genes with a “significant” pvalue
- Here’s how we can use an `if` statement to test for this
  - for each iteration of the the loop:
    1. test if the p-value from the test is below 0.05 or not
    2. if the p-value is less than 0.05 make a boxplot
    3. if not, do nothing

```
pdf("Chromosome8Genes.pdf")
pvals <- NULL
for (i in 1:18) {
  tmp <- t.test(as.numeric(chr8Expression[i,]) ~ factor(subjects$er))
  pvals[i] <- tmp$p.value
  if(tmp$p.value < 0.05){
    boxplot(as.numeric(chr8Expression[i,]) ~ factor(subjects$er),
            main=chr8Genes$HUGO.gene.symbol[i])
    }
  }
pvals
dev.off()
```

Code formatting avoids bugs!

Compare:

```
f<-26
while(f!=0){
print(letters[f])
f<-f-1}
```

to:

```
f <- 26
while(f != 0 ){
  print(letters[f])
  f <- f-1
  }
```

- The code between brackets `{}` *always* is *indented*, this clearly separates what is executed once, and what is run multiple times
- Trailing bracket `}` always alone on the line at the same indentation level as the initial bracket `{`
- Use white spaces to divide the horizontal space between units of your code, e.g. around assignments, comparisons

5. Report Writing

Creating a markdown file from scratch

File → New File → R Markdown

- Choose 'Document' and the default output type (HTML)
- A new tab is created in RStudio
- The header allows you to specify a Page title, author and output type

```
---
title: "Untitled"
author: "Mark Dunning"
date: "18/08/2015"
output: html_document
---
```

Format of the file

- **Lines 8 - 10**: Plain text description
- **Lines 12 - 14**: An R code 'chunk'
- **Lines 18 to 20**: Another code chunk, this time producing a plot

```
7
8  This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents.
   For more details on using R Markdown see <http://rmarkdown.rstudio.com>.
9
10 When you click the **Knit** button a document will be generated that includes both content as well as the output of any
   embedded R code chunks within the document. You can embed an R code chunk like this:
11
12 ```{r}
13 summary(cars)
14 ```
15
16 You can also embed plots, for example:
17
18 ```{r, echo=FALSE}
19 plot(cars)
20 ```
```

md-format

- Pressing the ***Knit HTML*** button will create the report:
  - Note that you need to 'save' the markdown file before you will see the compiled report in your working directory

Text formatting

See ***?*** → ***Markdown Quick Reference*** in RStudio:

- Enclose text in * to format in *italics*
- Enclose text in ** to format in **bold**
- *** for ***bold italics***
- ` to format like `code`
- $ to include equations: $e = mc^2$
- > quoted text:

> To be or not to be

- See *Markdown Quick Reference** for more:
  - Adding images
  - Adding web links
  - Tables

Not quite enough for a reproducible document

- Minimally, you should record what version of R, and the packages you used.
- Use the `sessionInfo()` function
  - e.g. for the version of R I used to make the slides

```
sessionInfo()
```

```
R version 3.3.0 (2016-05-03)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 14.04.2 LTS

locale:
 [1] LC_CTYPE=en_GB.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_GB.UTF-8
 [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_GB.UTF-8
 [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] knitr_1.13

loaded via a namespace (and not attached):
 [1] magrittr_1.5        formatR_1.4         parallel_3.3.0
 [4] tools_3.3.0         htmltools_0.3.5     yaml_2.1.13
 [7] Rcpp_0.12.5         Biobase_2.32.0      stringi_1.0-1
[10] rmarkdown_0.9.6     BiocGenerics_0.18.0 stringr_1.0.0
[13] digest_0.6.9        evaluate_0.9
```

Defining chunks

- It is not great practice to have one long, continuous R script
- Better to break-up into smaller pieces; '*chunks*'
- You can document each chunk separately

- Easier to catch errors
- The characteristics of each chunk can be modified:
  - You might not want to print the R code for each chunk
  - …or the output
  - etc.

Chunk options

Code chunks are encapsulated between backticks. Options for the chunk can be put inside the curly brackets `{...}`

```
'''{r}
my code here...
'''
```

- It's a good idea to name each chunk
  - Easier to track-down errors
- We can display R code, but not run it
  - `eval=FALSE`
- We can run R code, but not display it
  - `echo=FALSE`
  - e.g. setting display options
- Suppress warning messages
  - `warning=FALSE`

Chunk options: eval

- Sometimes we want to format code for display, but not execute; we want to show the code for how we read our data, but want our report to compile quickly

```
'''{r, eval=FALSE}
data <- read.delim("path.to.my.file")
'''
```

Chunk options: echo

- Might want to load some data from disk
  - e.g. the R object from reading the data in the previous slide

```
'''{r echo=FALSE}
load("mydata.rda")
'''
```

- Your P.I. wants to see your results, but doesn't really want to know about the R code that you used

Chunk options: results

- Some code or functions might produce lots of output to the screen that we don't need

```
for(i in 1:100) {
  print(i)
  }
```

Chunk options: message and warning

- Loading an R package will sometimes print messages and / or warnings to the screen
- This is not always helpful in a report:

```
'''{r}
library(DESeq)
'''
```

```
Loading required package: BiocGenerics
```

```
Loading required package: parallel
```

```

Attaching package: 'BiocGenerics'
```

```
The following objects are masked from 'package:parallel':

    clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
    clusterExport, clusterMap, parApply, parCapply, parLapply,
    parLapplyLB, parRapply, parSapply, parSapplyLB
```

```
The following objects are masked from 'package:stats':

    IQR, mad, xtabs
```

```
The following objects are masked from 'package:base':

    anyDuplicated, append, as.data.frame, cbind, colnames,
    do.call, duplicated, eval, evalq, Filter, Find, get, grep,
    grepl, intersect, is.unsorted, lapply, lengths, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, setdiff,
    sort, table, tapply, union, unique, unsplit
```

```
Loading required package: Biobase
```

```
Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
Loading required package: locfit
```

```
locfit 1.5-9.1   2013-03-22
```

```
Loading required package: lattice
```

```
    Welcome to 'DESeq'. For improved performance, usability and
    functionality, please consider migrating to 'DESeq2'.
```

Chunk options: message and warning

- Using `message=FALSE` and `warning=FALSE`

```
'''{r message=FALSE, warning=FALSE}
library(DESeq)
'''
```

- Could also need `suppressPackageStartupMessages`

Chunk options: cache

- The argument `cache=TRUE` will stop certain chunks from being evaluate if their code does not change
- It speeds-up the compilation of the document
  - we don't want to reload our dataset if we've only made a tiny change downstream

```
'''{r echo=FALSE, cache=TRUE}
load("mydata.rda")
'''
```

Running R code from the main text

- We can add R code to our main text, which gets evaluated
  - make sure we always have the latest figures, p-values etc

```
'''
...the sample population consisted of 'r table(gender)[1]' females
and 'r table(gender)[2]' males...
'''
```

…the sample population consisted of 47 females and 50 males…

- Alternatively:

```
'''
...the p-value of the t-test is 'r pval', which indicates that...
'''
```

…the p-value of the t-test is 0.05, which indicates that…

- We call this **“in-line” code**

Running R code from the main text

- Like the rest of our report these R statements will get updated each time we compile the report

```
'''
...the sample population consisted of 'r table(gender)[1]' females
and 'r table(gender)[2]' males...
'''
```

…the sample population consisted of 41 females and 54 males…

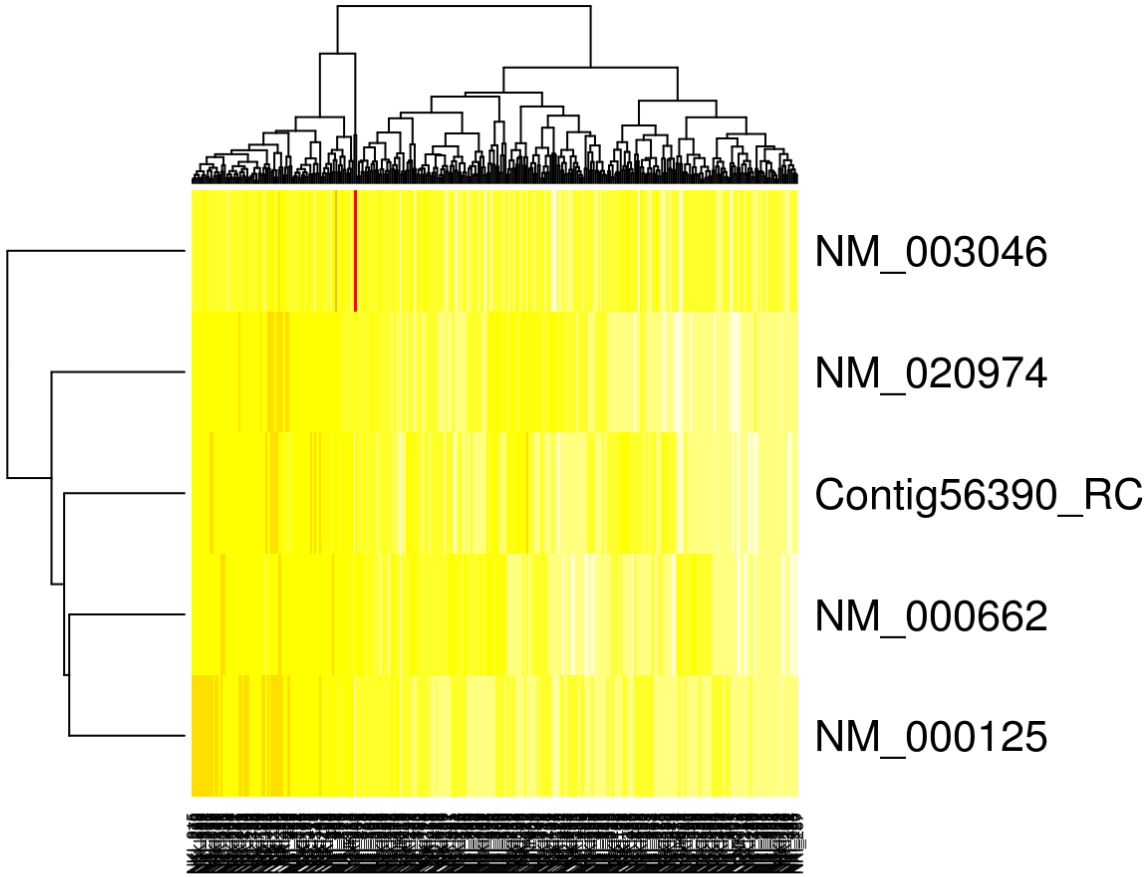
```
'''
...the p-value of the t-test is 'r pval', which indicates that...
'''
```

…the p-value of the t-test is 0.1, which indicates that…

Making a heatmap

- A heatmap is often used to visualise how the expression level of a set of genes vary between conditions
- Making the plot is actually quite straightforward
  - providing you have processed the data appropriately!
  - here, we use `na.omit()` to ensure we have no `NA` values

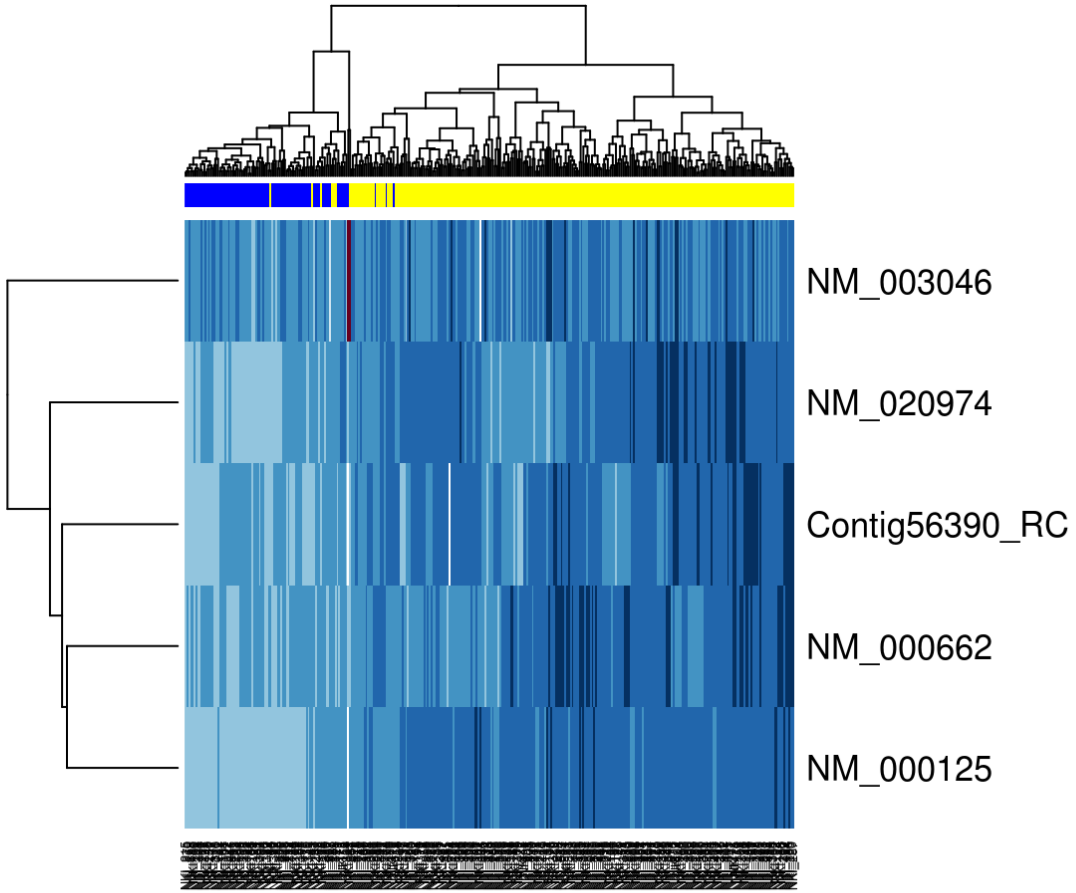
```
genelist <- c("ESR1", "NAT1", "SUSD3","SLC7A2" ,"SCUBE2")
probes   <- na.omit(genes[match(genelist, genes[,2]), 1])
exprows  <- match(probes, rownames(evals))

heatmap(as.matrix(evals[exprows,]))
```



Heatmap adjustments

- We can provide a colour legend for the samples
- Adjust colour of cells

```
library(RColorBrewer)
sampcol <- rep("blue", ncol(evals))
sampcol[subjects$er == 1 ] <- "yellow"
rbPal <- brewer.pal(10, "RdBu")
heatmap(as.matrix(evals[exprows,]), ColSideColors = sampcol, col=rbPal)
```



- see also
  - `heatmap.2` from `library(gplots)` ; `example(heatmap.2)`
  - `heatmap.plus` from `library(heatmap.plus)` ; `example(heatmap.plus)`

Exercise

This analysis is recorded in `exercise10.Rmd`.

- Use in-line R code to report how many patients were involved in the study
- Hide the code chunk used to produce the plot (`echo=FALSE`)
- Cache the code chunk used to read the raw data (`cache=TRUE`)

Solution: solution-exercise10.Rmd

End of Course

Wrap-up

- **Thanks for your attention**
- Practice, practice, practice
  - … & persevere
- Need inspiration? R code is freely-availabe, so read other people's code!
  - Read blogs (http://www.r-bloggers.com/)
  - Follow the forums (http://stackoverflow.com/questions/tagged/r)
  - Download datasets (http://vincentarelbundock.github.io/Rdatasets/datasets.html) to practice with
  - Bookmark some reference (https://en.wikibooks.org/wiki/R_Programming) guides
  - on twitter @rstudio, @Rbloggers, @RLangTip
  - Attend the follow-on course (http://training.csx.cam.ac.uk/bioinformatics/event/1800066) on data manipulation and graphics
- Please fill in the feedback form for us to improve the course