# Reproducible Research

*Mark Dunning*

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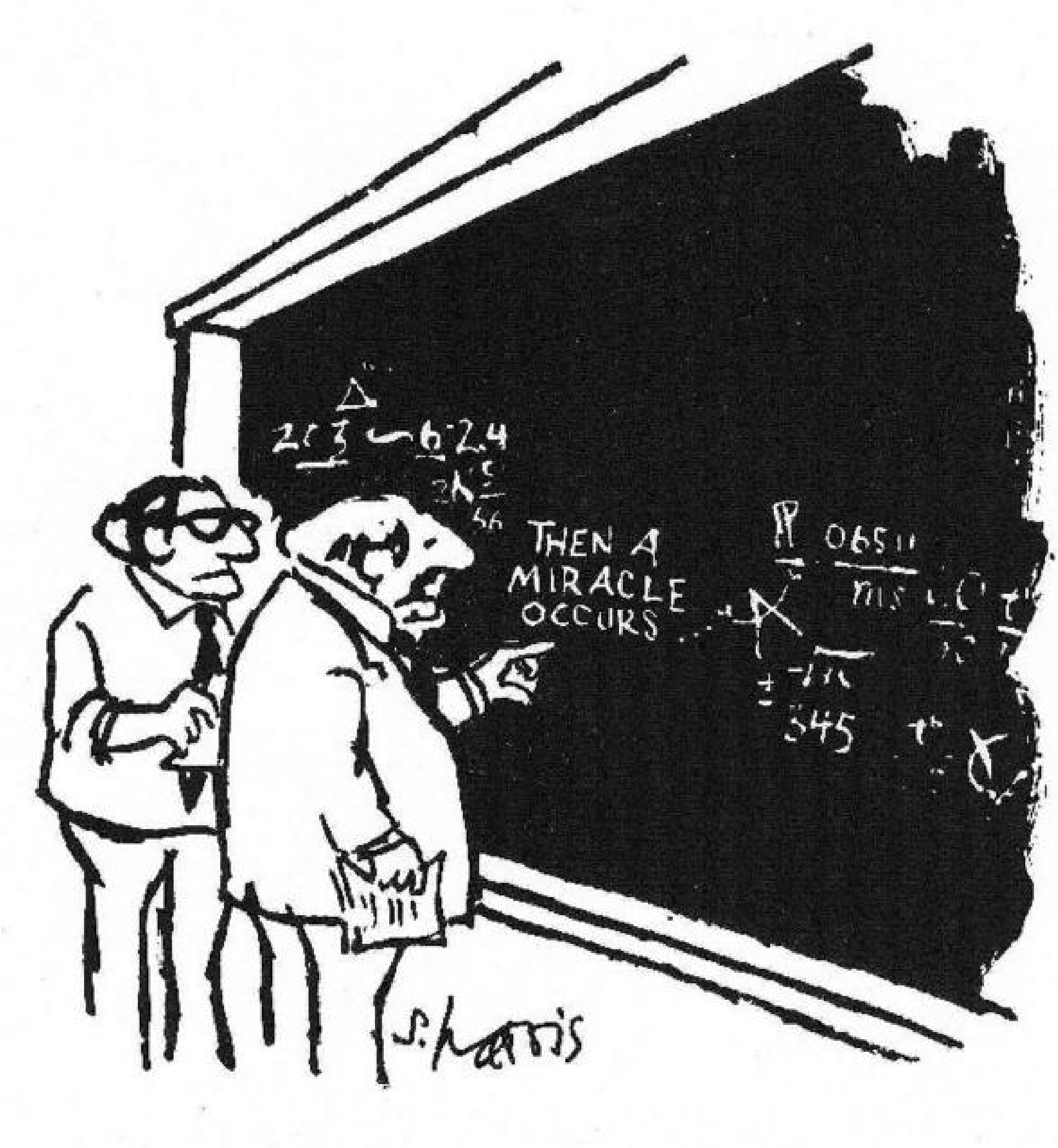
# Principles of Reproducible Research



"I think you should be more explicit here in step two."

Sidney Harris - New York Times

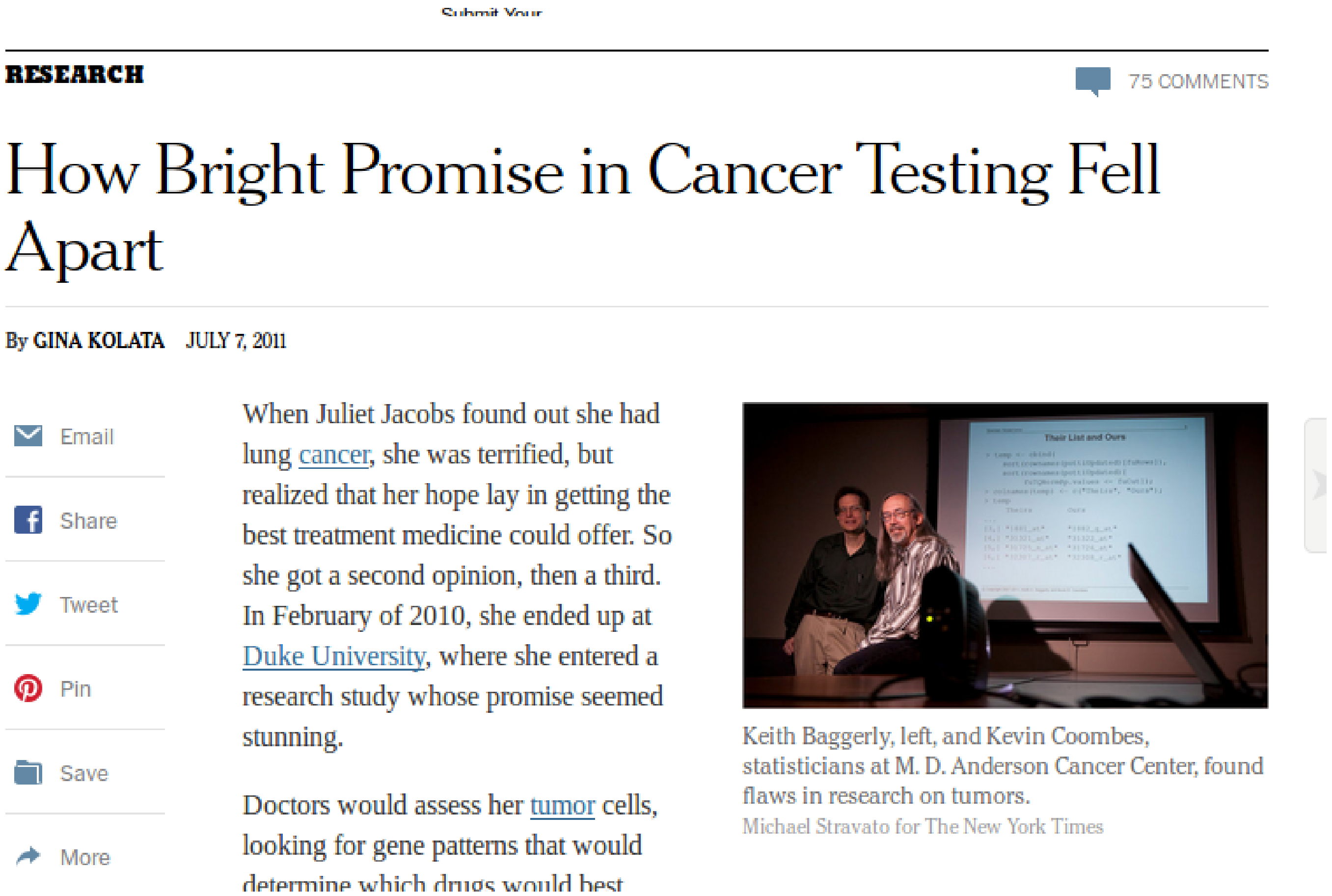
# Why should we do reproducible research?

Five selfish reasons - Florian Markowetz Blog (https://scientificbsides.wordpress.com/2015/07/15/five-selfish-reasons-for-working-reproducibly/)

1. Avoid disaster
2. Easier to write papers
3. Easier to talk to reviewers
4. Continuity of your work in the lab
5. Reputation

# It is a hot topic at the moment

- Statisticians at MD Anderson tried to reproduce results from a Duke paper and unintentionally unravelled a web of incompetence and skullduggery
    - as reported in the ***New York Times***



**RESEARCH**

75 COMMENTS

## How Bright Promise in Cancer Testing Fell Apart

By GINA KOLATA JULY 7, 2011

When Juliet Jacobs found out she had lung cancer, she was terrified, but realized that her hope lay in getting the best treatment medicine could offer. So she got a second opinion, then a third. In February of 2010, she ended up at Duke University, where she entered a research study whose promise seemed stunning.

Doctors would assess her tumor cells, looking for gene patterns that would determine which drugs would best

Keith Baggerly, left, and Kevin Coombes, statisticians at M. D. Anderson Cancer Center, found flaws in research on tumors.
Michael Stravato for The New York Times

# Hear the full account

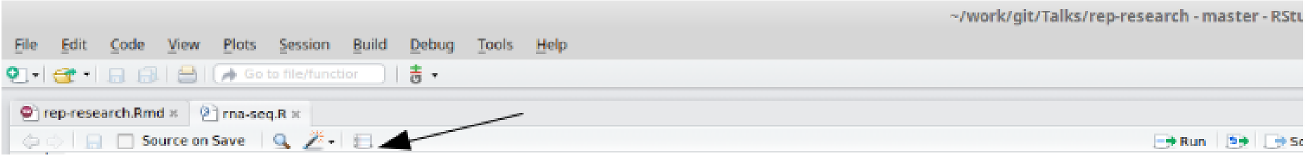
- Very entertaining talk from Keith Baggerly in Cambridge 2010

# What can we do about it?

- Use scripts / R
- Use version control
- Document early
- Document everything
- Write comments and explanations
- Automatically-generate your plots, tables, etc from the data
    - always ensure that you have the latest version

# Simple example in RStudio

- See `rna-seq.R`
    - an analysis of a RNA-seq dataset using edgeR
- Use the Compile Notebook button in RStudio
- Take an R script and turn into HTML, PDF or even Word
- All code will be displayed and the outputs printed
- A compiled report will be generated in your working directory



# What is going on?

- The `knitr` package is being used convert the R script into ‘markdown’ format, which it then compiles into the output of your choosing
- `knitr` is distributed with RStudio
  - `knitr` is the modern-day equivalent of `Sweave`
- markdown is a easy-to-read, easy-to-write text format often used to write HTML, readme files etc
- the following should create the file `rna-seq.Rmd` in your working directory

```
library(knitr)
spin(hair="rna-seq.R",knit=FALSE)
```

# 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 3.2.0 (2015-04-16)
## 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
##
## loaded via a namespace (and not attached):
##  [1] magrittr_1.5    formatR_1.2     tools_3.2.0     htmltools_0.2.6
##  [5] yaml_2.1.13     stringi_0.5-5   rmarkdown_0.6.1 knitr_1.10.5
##  [9] stringr_1.0.0   digest_0.6.8    evaluate_0.7
```

- Lets add this to the R scripts and see what happens

# 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

# Create a markdown file from scratch

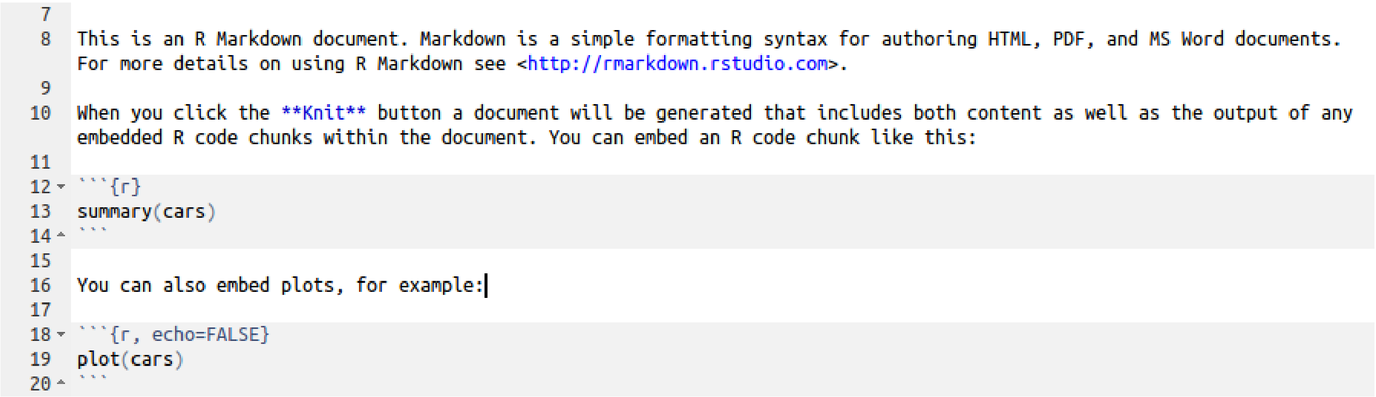
File - > New File - > R Markdown

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

```
---
title: "Untitled"
author: "Mark Dunning"
date: "16/06/2015"
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  ```
```

- 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

# Chunk options

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

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

# 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
    - 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 object is masked from 'package:stats':
## 
##     xtabs
## 
## The following objects are masked from 'package:base':
## 
##     anyDuplicated, append, as.data.frame, as.vector, cbind,
##     colnames, do.call, duplicated, eval, evalq, Filter, Find, get,
##     intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rep.int, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unlist, 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

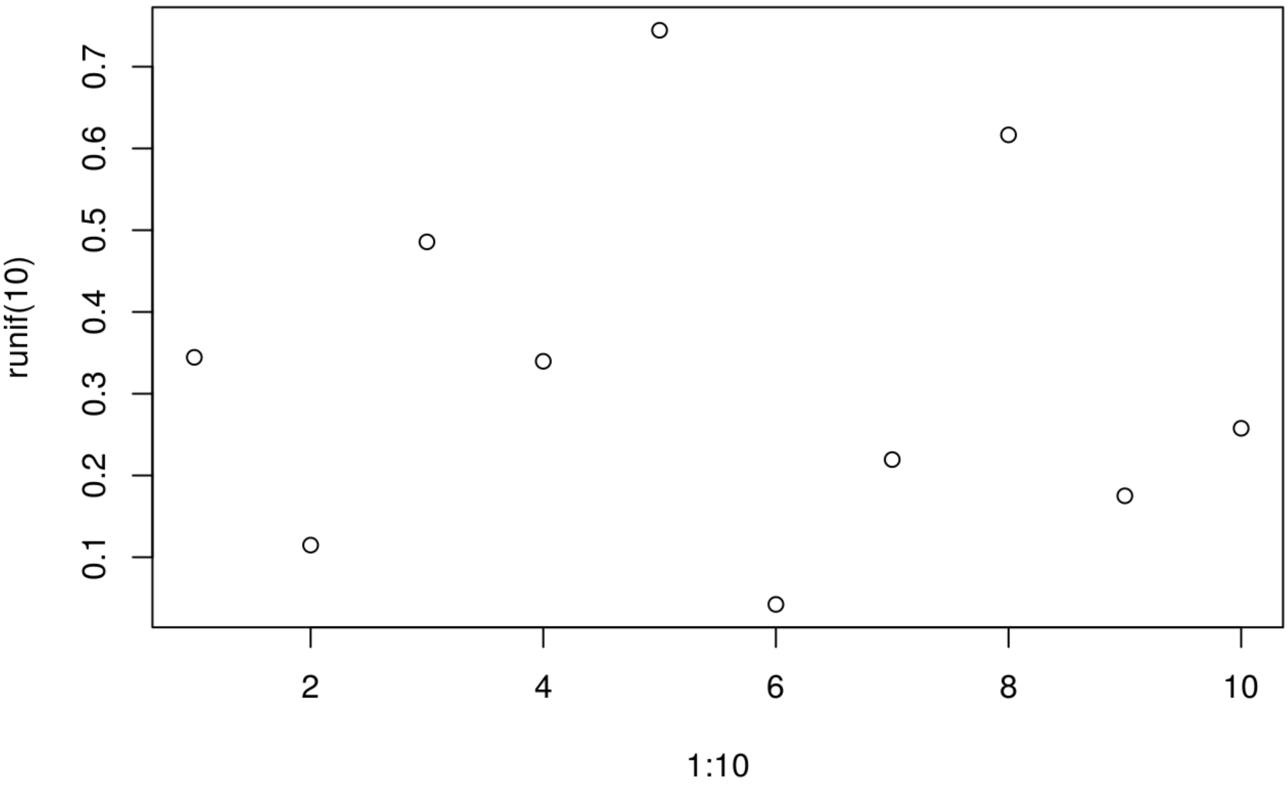
- `cache=TRUE` will stop certain chunks from being evaluate if their code does not change
- speeds-up the compilation of the document
    - we don’t want to reload our dataset if we’ve only made a tiny change downstream

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

# Including plots

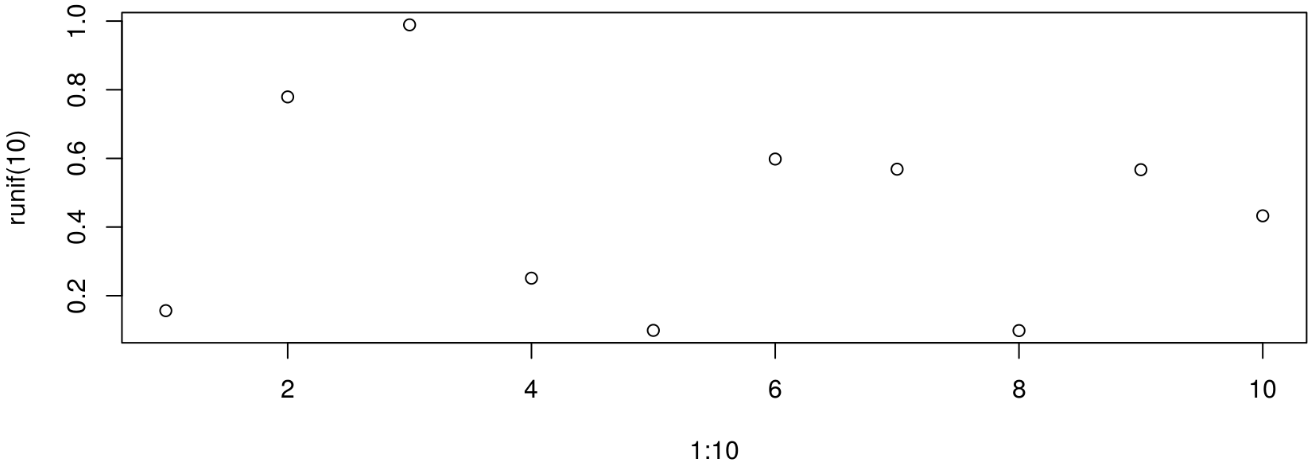
- Use a plotting function ( `plot` , `boxplot` , `hist` etc) will include the plot at the relevant point in the document

```
'''{r}
plot(1:10, jitter(1:10))
'''
```



# Control over plots

```
'''{r fig.height=2,fig.align='right', fig.height=4,fig.width=9}
plot(1:10, jitter(1:10))
'''
```



# 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 ta
ble(gender)[2]' males.....
```

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

```
.....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…..

# 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 ta
ble(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…..

# Conditional output

```
pval <- 0.1
```

```
.....The statistical test was 'r ifelse(pval < 0.05, "", "not")' significant...
.
```

The statistical test was not significant

```
pval <- 0.01
```

```
.....The statistical test was 'r ifelse(pval < 0.05, "", "not")' significant...
.
```

The statistical test was significant

# Printing vectors

```
The months of the year are 'r month.name'
```

The months of the year are January, February, March, April, May, June, July, August, September, October, November, December

# Exercise

- You will find a ‘template’ analysis `pasilla-template.Rmd` in the Day4/rep-research/ folder
- Use this template to report on the analysis of the Pasilla RNA-seq dataset
- Aim for something like `pasilla-analysis.pdf`
- Once you have a report that you are happy with
  - Modify the report so that single-end samples are used in the analysis
  - Modify your p-value cut-off to 0.01
    - In each case, see how the report changes

# References

- Useful reference:
  - Reproducible Research in R and RStudio
    - http://christophergandrud.github.io/RepResR-RStudio/ (http://christophergandrud.github.io/RepResR-RStudio/)
    - Useful exercise is to compile the book from the source code (https://github.com/christophergandrud/Rep-Res-Book)
  - Implementing Reproducible Research (https://osf.io/s9tya/wiki/home/)