Introduction to R:

Using R for statistics and data analysis

BaRC Hot Topics – October 2011

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http://iona.wi.mit.edu/bio/education/R2011/

Why use R?

- To perform inferential statistics (e.g., use a statistical test to calculate a p-value)
- To do real statistics (unlike in Excel)
- To create custom figures
- To automate analysis routines (and make them more reproducible)
- To reduce copying and pasting
  - But Unix commands may be easier – ask us
- To use up-to-date analysis algorithms
- Real statisticians use it
- It’s free

Why not use R?

- A spreadsheet application already works fine
- You’re already using another statistics package
  - Ex: Prism, MatLab
- It’s hard to use at first
  - You have to know what commands to use
- Real statisticians use it
- You don’t know how to get started
  - Irrelevant if you’re here today

Getting started

- Log into tak

```
ssh –l USERNAME tak
```

- Start R

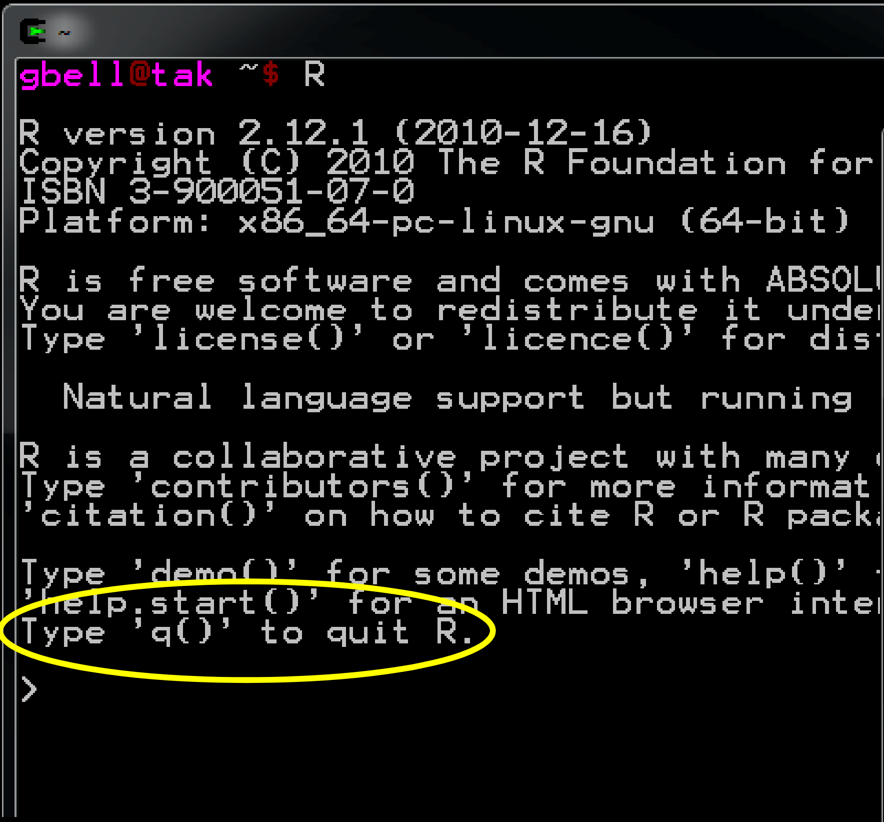
```
R
```

or

- Go to R (http://www.r-project.org/)
- Download "base" from CRAN and install it on your computer
- Open the program

Start of an R session

On tak



```
gbell@tak ~$ R

R version 2.12.1 (2010-12-16)
Copyright (C) 2010 The R Foundation for
ISBN 3-900051-07-0
Platform: x86_64-pc-linux-gnu (64-bit)

R is free software and comes with ABSOLU
You are welcome to redistribute it under
Type 'license()' or 'licence()' for dis

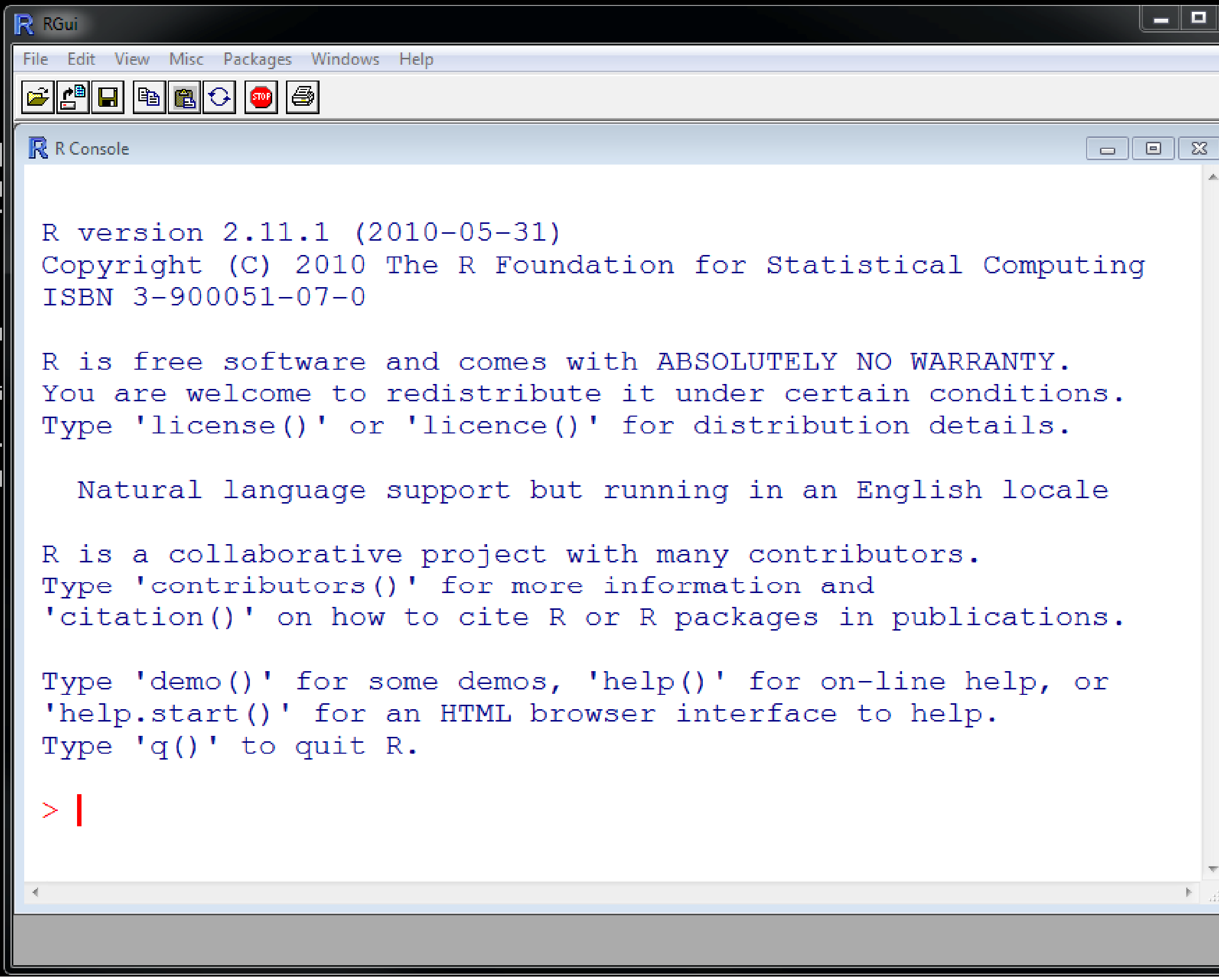
  Natural language support but running 

R is a collaborative project with many 
Type 'contributors()' for more informat
'citation()' on how to cite R or R packa

Type 'demo()' for some demos, 'help()' 
'help.start()' for an HTML browser inte
Type 'q()' to quit R.

>
```

On your own computer



```
R version 2.11.1 (2010-05-31)
Copyright (C) 2010 The R Foundation for Statistical Computing
ISBN 3-900051-07-0

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.

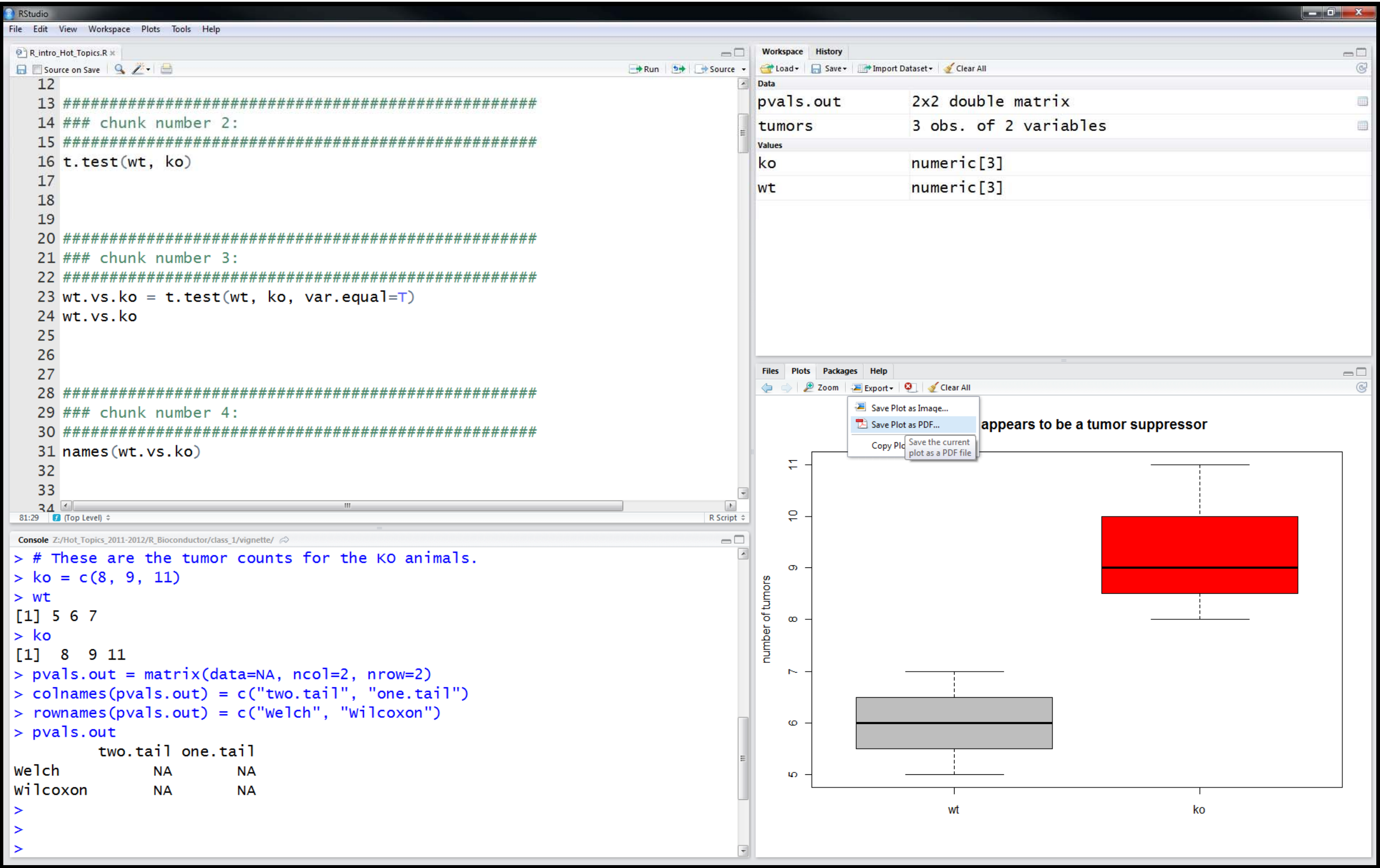
  Natural language support but running in an English locale

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.

> |
```

RStudio interface



```
####################################################
### chunk number 2:
####################################################
t.test(wt, ko)



####################################################
### chunk number 3:
####################################################
wt.vs.ko = t.test(wt, ko, var.equal=T)
wt.vs.ko



####################################################
### chunk number 4:
####################################################
names(wt.vs.ko)
```

Data

pvals.out	2x2 double matrix
tumors	3 obs. of 2 variables

Values

ko	numeric[3]
wt	numeric[3]

```
> # These are the tumor counts for the KO animals.
> ko = c(8, 9, 11)
> wt
[1] 5 6 7
> ko
[1]  8  9 11
> pvals.out = matrix(data=NA, ncol=2, nrow=2)
> colnames(pvals.out) = c("two.tail", "one.tail")
> rownames(pvals.out) = c("Welch", "Wilcoxon")
> pvals.out
         two.tail one.tail
Welch          NA       NA
Wilcoxon       NA       NA
>
>
>
```

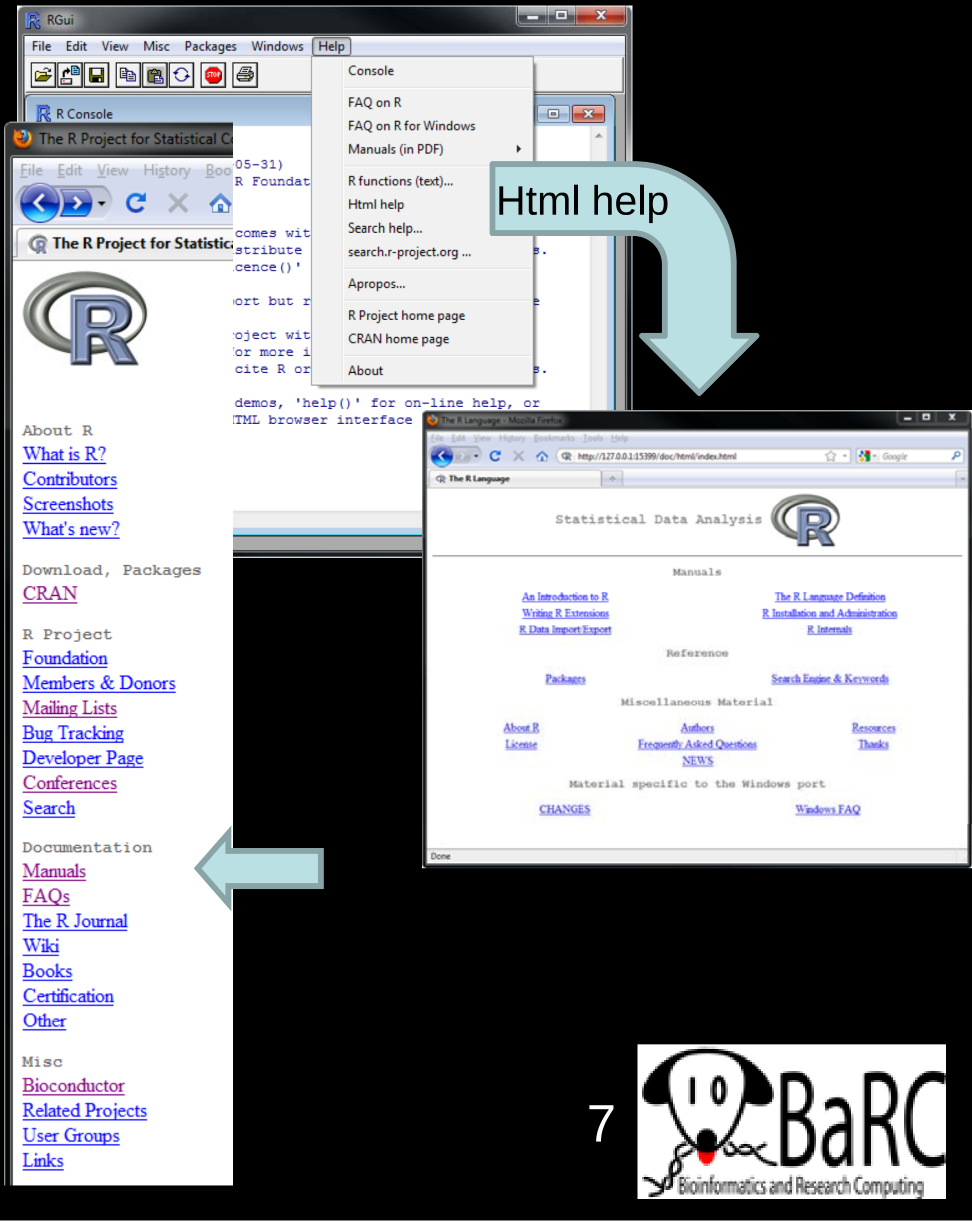
Requires R; free download from http://rstudio.org/

Getting help

- Use the Help menu
- Check out "Manuals"
  - http://www.r-project.org/
  - contributed documentation
- Use R's help

  `?median` [show info]

  `??median` [search docs]
- Search the web
  - "r-project median"
- Our favorite book:
  - Introductory Statistics with R (Peter Dalgard)



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or more i
cite R or
demos, 'help()' for on-line help, or
TML browser interface
```

Handling data

- Data can be numerical or text
- Data can be organized into
  - Vectors (lists of values)
  - Matrices (2-dimensional tables of data)
  - Data frames (a combination of different types of data)
- Data can be entered
  - By typing (using the “c” command to combine things)
  - From files
- Names of data should start with letters
  - Uppercase + lowercase helps (myWTmice)
  - Can include dots (my.WT.mice)

Good practices

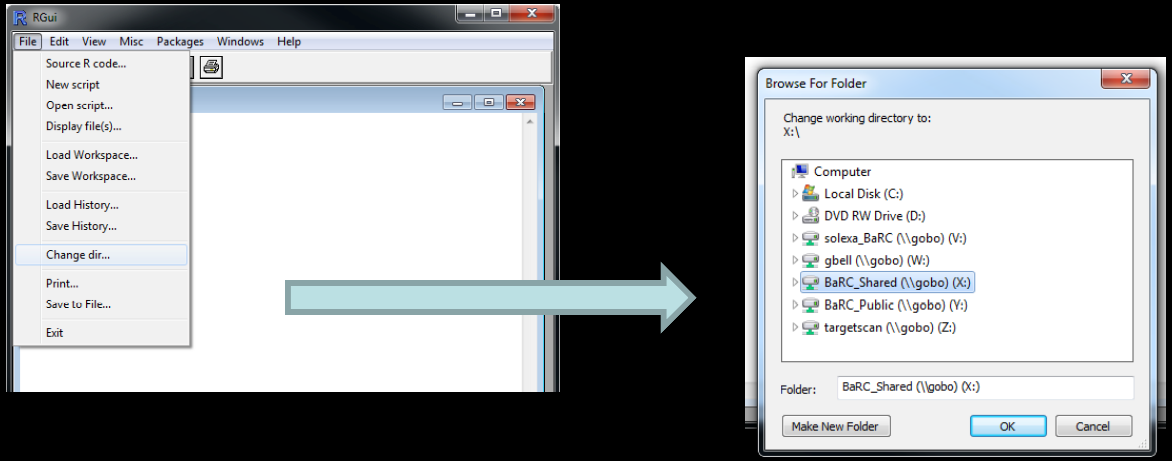
- Save all useful commands and rationale
  - Add comments (starting with “#”)
  - Use history() to get previous commands
- Two approaches
  - Write commands in R and then paste into a text file, or
    - By convention, we end files of R commands with “.R”
    - Use a specific name for file (ex: compare_WT_KO_weights.R)
  - Write commands in a text editor and paste into R session.
- Use the up-arrow to get to previous command
  - Minimize typing, as this increases potential errors.
- To clear your R window, use Ctrl-L

Example commands

```
# Number of tumors (from litter 2 on 11 July 2010)
wt = c(5, 6, 7)
ko = c(8, 9, 11)
# Try default t-test settings (Welch's 2-sample t-test)
t.test(wt, ko)
# Do standard 2-sample t-test
t.test(wt, ko, var.equal=T)
# Save the results as a variable
wt.vs.ko = t.test(wt, ko, var.equal=T)
# What are the different parts of this data frame?
names(wt.vs.ko)
# Just print the p-value
wt.vs.ko$p.value
# What commands did we use?
history(max.show=Inf)
```

Reading files - intro

- Take R to your preferred directory ()



Change working directory to:
X:\

Folder: BaRC_Shared (\\gobo) (X:)

- Check where you are (e.g., get your working directory) and see what files are there

```
> getwd()
[1] "X:/bell/Hot_Topics/Intro_to_R“
> dir()
[1] "compare_WT_KO_weights.R"
```

Running a series of commands

- Copy and paste commands into R session, or
- Execute a script in R, or

```
source("compare_WT_KO_weights.R")
```

[but not so useful in this case, since we aren’t creating any files]

- [tak only]
  - Change to working directory with Unix command

    ```
    cd /nfs/BaRC/Hot_Topics/Intro_to_R
    ```

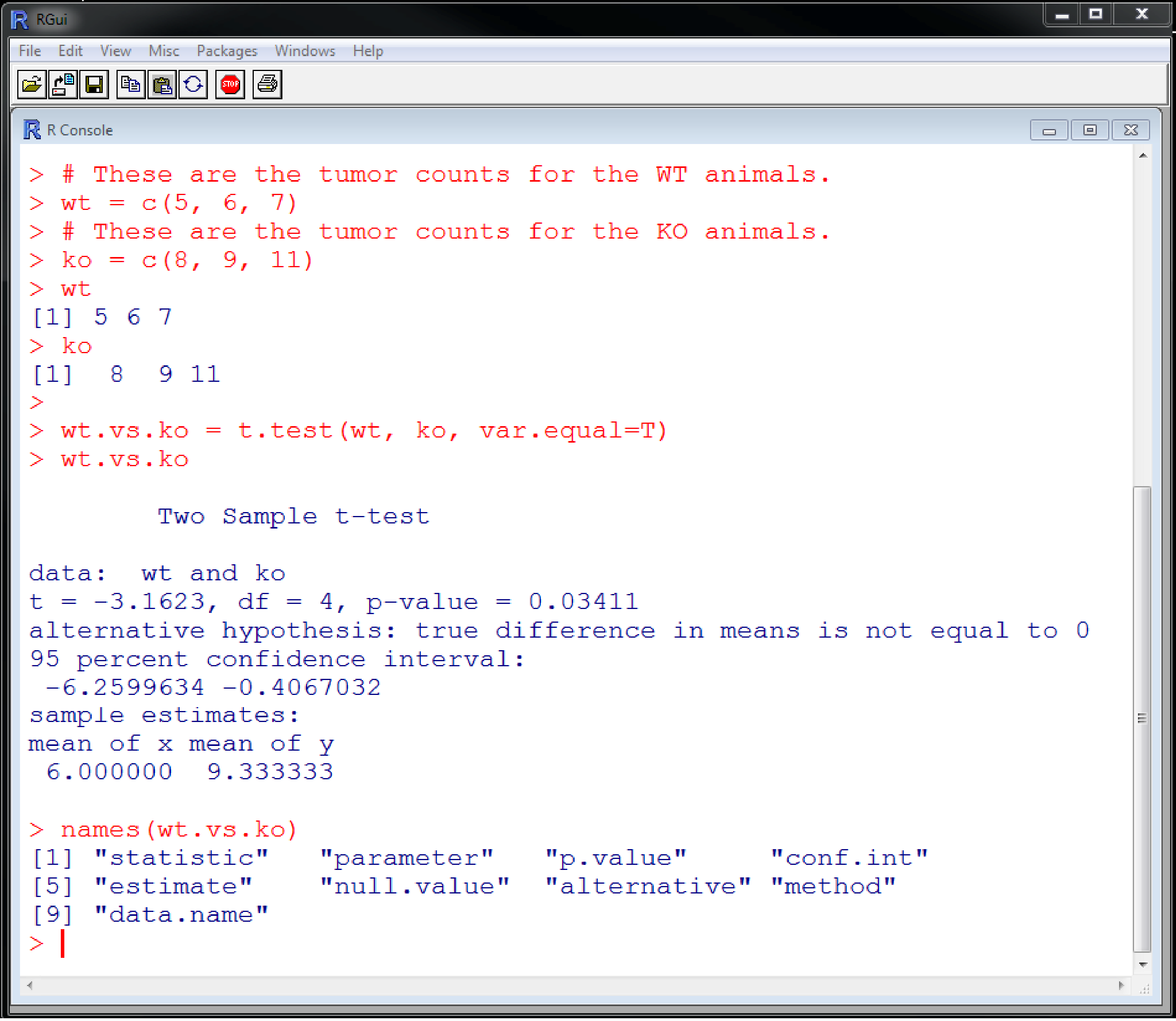
  - Run R, with script as input (print to screen), or

    ```
    R --vanilla < compare WT KO weights.R
    ```

  - Run R, with script as input (save output)

    ```
    R --vanilla < compare_WT_KO_weights.R > R_out.txt
    ```

Command output



```
> # These are the tumor counts for the WT animals.
> wt = c(5, 6, 7)
> # These are the tumor counts for the KO animals.
> ko = c(8, 9, 11)
> wt
[1] 5 6 7
> ko
[1]  8  9 11
>
> wt.vs.ko = t.test(wt, ko, var.equal=T)
> wt.vs.ko

        Two Sample t-test

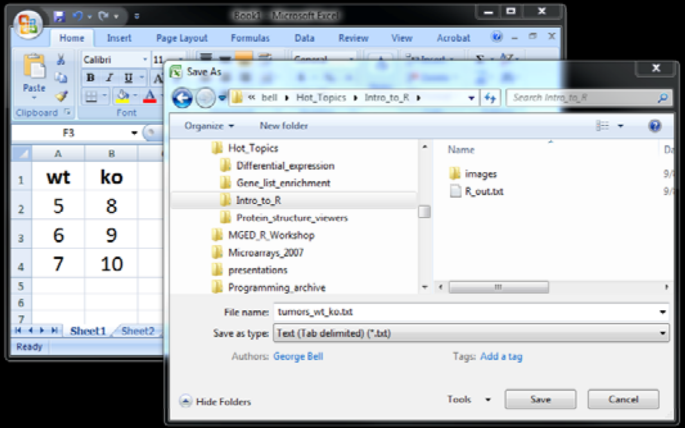
data:  wt and ko
t = -3.1623, df = 4, p-value = 0.03411
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -6.2599634 -0.4067032
sample estimates:
mean of x mean of y
 6.000000  9.333333

> names(wt.vs.ko)
[1] "statistic"   "parameter"   "p.value"     "conf.int"
[5] "estimate"    "null.value"  "alternative" "method"
[9] "data.name"
>
```

Partial output from R on tak, if saved as a file (R_out.txt from previous slide), also looks something like this (but without the colors).

Reading data files

- Usually it’s easiest to read data from a file
  - Organize in Excel with one-word column names
  - Save as tab-delimited text



	A	B
1	wt	ko
2	5	8
3	6	9
4	7	10

File name: tumors_wt_ko.txt

Save as type: Text (Tab delimited) (*.txt)

Authors: George Bell

- Check that file is there

```
list.files()
```

- Read file

```
tumors = read.delim("tumors_wt_ko.txt", header=T)
```

- Check that it’s OK

```
> tumors
  wt ko
1  5  8
2  6  9
3  7 11
```

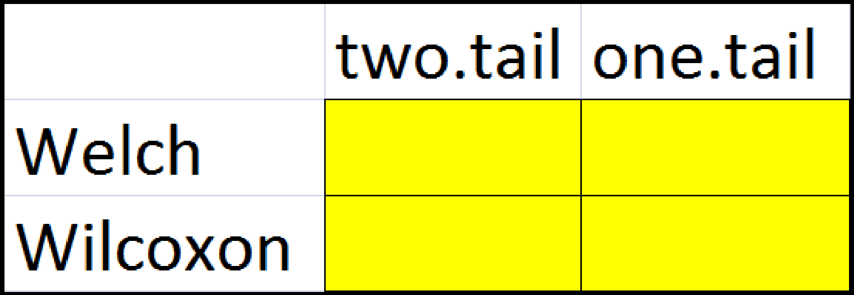
Accessing data

```
> tumors
  wt ko
1  5  8
2  6  9
3  7 11
```

```
> tumors$wt       # Use the column name
[1] 5 6 7
> tumors[1:3,1]   # [rows, columns]
[1] 5 6 7
> tumors[,1]      # missing row or column => all
[1] 5 6 7
> tumors[1:2,1:2] # select a submatrix
  wt ko
1  5  8
2  6  9
> t.test(tumors$wt, tumors$ko)  # t-test as before
```

Creating an output table

- Most analyses involve several outputs
- You may want to create a matrix to hold it all
- Create an empty matrix
  - name rows and columns



	two.tail	one.tail
Welch		
Wilcoxon		

```
pvals.out = matrix(data=NA, ncol=2, nrow=2)
colnames(pvals.out) = c(“two.tail", “one.tail")
rownames(pvals.out) = c("Welch", "Wilcoxon")
pvals.out
```

```
         two.tail one.tail
Welch          NA       NA
Wilcoxon       NA       NA
```

Filling the output table (matrix)

- Do the stats

```
# Welch’s test (t-test with pooled variance)
pvals.out[1,1] = t.test(tumors$wt, tumors$ko)$p.value
pvals.out[1,2] = t.test(tumors$wt, tumors$ko,
alt="less")$p.value
# Wilcoxon rank sum test (non-parametric alternative to
t-test)
pvals.out[2,1] = wilcox.test(tumors$wt,
tumors$ko)$p.value
pvals.out[2,2] = wilcox.test(tumors$wt, tumors$ko,
alt="less")$p.value
pvals.out
```

```
           two.tail   one.tail
Welch    0.04191452 0.02095726
Wilcoxon 0.10000000 0.05000000
```

Printing the output table

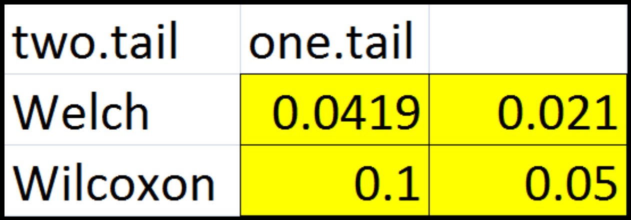
- We may want to round the p-values

```
pvals.out.rounded = round(pvals.out, 4)
```

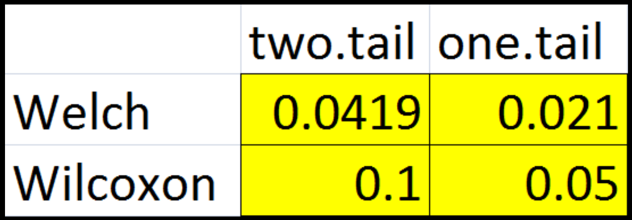
- Print the matrix (table)

```
 write.table(pvals.out.rounded,
   file="Tumor_pvals.txt", quote=F, sep="\t")
```

- Warning: output column names are shifted by 1 when read in Excel



two.tail	one.tail	
Welch	0.0419	0.021
Wilcoxon	0.1	0.05



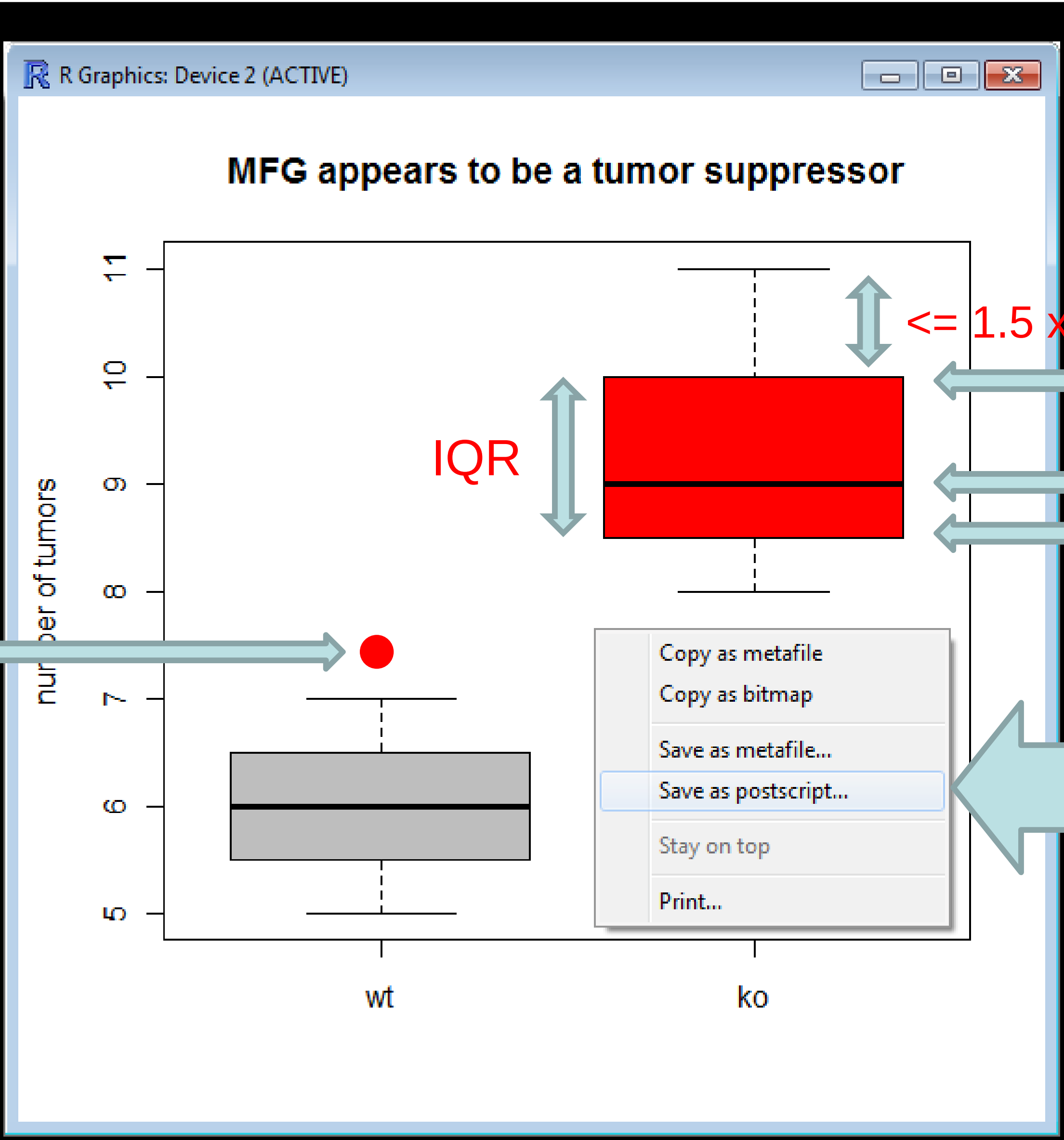
	two.tail	one.tail
Welch	0.0419	0.021
Wilcoxon	0.1	0.05

Introduction to figures

- R is very powerful and very flexible with its figure generation
- Any aspect of a figure should be modifiable
- Some figures aren’t available in spreadsheets
- Boxplot example

```
boxplot(tumors)       # Simplest case
# Add some more details
boxplot(tumors, col=c("gray", "red"), main="MFG
  appears to be a tumor suppressor", ylab="number
  of tumors")
```

Boxplot description



75th percentile

median

25th percentile

Any points beyond the whiskers are defined as “outliers”

Right-click to save figure

Figure formats and sizes

- By default, figures on tak are saved as “Rplots.pdf”
- Helpful figure names can be included in code

- To select name and size (in inches) of pdf file

```
pdf(“tumor_boxplot.pdf”, w=11, h=8.5)
boxplot(tumors) # can have >1 page
dev.off()       # tell R that we’re done
```

- To create another format (with size in pixels)

```
png(“tumor_boxplot.png”, w=1800, h=1200)
boxplot(tumors)
dev.off()
```

Bioconductor and other packages

- Many statisticians have extended R by creating packages (libraries) containing a set of commands to do something special
  - Ex: affy, limma, edgeR, made4
- For a huge list of Bioconductor packages, see http://www.bioconductor.org/packages/release/Software.html
- All require the package to be installed AND explicitly called, for example,

```
library(limma)
```

- Install what you need on your computer or, for tak, ask the IT group to install packages via

  http://tak.wi.mit.edu/trac/newticket

Other useful commands

```
library()    mean()           round(x, n)
dir()        median()         min()
length()     sd()             max()
dim()        rbind()          paste()
nrow()       cbind()          x[x>0]
ncol()       sort()           x[c(1,3,5)]
unique()     rev()            seq(from, to, by)
t()          log(x, base)     commandArgs()
```

More resources from BaRC

- “Statistics for Biologists” course:
  - http://iona.wi.mit.edu/bio/education/stats2007/
- “Microarray Analysis” course
  - http://jura.wi.mit.edu/bio/education/bioinfo2007/arrays/
- R scripts for Bioinformatics
  - http://iona.wi.mit.edu/bio/bioinfo/Rscripts/
- List of R modules installed on tak
  - http://tak/trac/wiki/R
- We’re glad to share commands and/or scripts to get you started

Upcoming Hot Topics

- Introduction to R Graphics (tomorrow)
- Introduction to Bioconductor - microarray and RNA-Seq analysis (Thursday)

- Unix, Perl, and Perl modules (short course)
- Quality control for high-throughput data
- RNA-Seq analysis
- Gene list enrichment analysis
- Galaxy
- Sequence alignment: pairwise and multiple

- See http://iona.wi.mit.edu/bio/hot_topics/
- Other ideas? Let us know.