Writing custom scripts & running R batch mode analysis

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The R scripting language
Scripting

- A script is a series of instructions that when executed sequentially automates a task
  - A script is a good solution to a repetitive problem
  - The art of good script writing is
    - understanding exactly what you want to do
    - expressing the steps as concisely as possible
    - making use of error checking
    - including descriptive comments
- R is a powerful scripting language, and embodies aspects found in most standard programming environments
  - procedural statements
  - loops
  - functions
  - conditional branching
- Scripts may be written in any standard text editor, e.g. notepad, gedit, kate
  - RGui (Mac and Windows) has a built-in text editor

An example script
Scripting

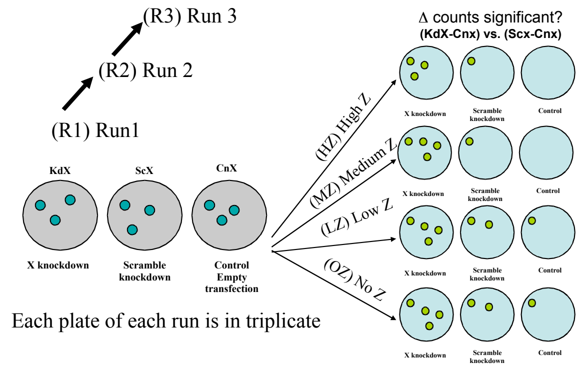
- Colony forming assays provide a measure of cellular proliferation. They are used as read outs for various biological systems
  - A well controlled study may involve multiple samples, treatments and controls (probably replicated).
  - This produces a lot of ‘count’ data, ideally suited to routine script processing

- Encapsulating the analysis into an R script requires a clear understanding of the problem and data structure

CFA experimental design
Scripting

- Expression of gene X may prevent cells from proliferating in high concentrations of compound Z. The theory is tested by knocking down gene X and growing cells in varying concentrations of compound Z.
  - Three repeat runs (same cell line)
    - Gene X knockdown --> KdX
    - Scramble gene X knockdown control --> ScX
    - Control (transfect empty vector) --> CnX
  - 4 concentrations of compound Z
    - High (HZ), Medium (MZ), Low (LZ), None (OZ)
  - The experiment is replicated over 3 successive weeks
    - Run1 (R1), Run2 (R2) and Run3 (R3)
  - 108 counts in total

Colony forming assay experimental design



Preparing the calculation(s)
Scripting

- We need to make barplots of counts for the KDX-CNX and SCX-CNX for each concentration of Z.
- We will group the repeat runs & replicates, and take an average.
- A Wilcoxon Rank Sum test will tell us whether there is a significant level of protection for KDX in concentrations of Z
- We'll add in some data quality checks
  - Boxplots of repeat runs
  - Variance within replicates

We can copy & paste lines of code into a blank text document, try them out and keep the ones that work!

Importing data
Scripting

	R1									R2									R3								
Plate	KDX			SCX			CNX			KDX			SCX			CNX			KDX			SCX			CNX		
Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
OZ																											
LZ																											
MZ																											
HZ																											

Fine for humans, bad for R

Run	Plate	Treatment	Replicate	Count
R1	KDX	OZ	1	100
R1	KDX	OZ	2	104
R1	KDX	OZ	3	91
R1	KDX	LZ	1	85
R1	KDX	LZ	2	71
R1	KDX	LZ	3	69
R1	KDX	MZ	1	32
R1	KDX	MZ	2	12
R1	KDX	MZ	3	45
R1	KDX	HZ	1	5
R1	KDX	HZ	2	11
R1	KDX	HZ	3	3
R1	SCX	OZ	1	136
R1	SCX	OZ	2	99
R1	SCX	OZ	3	154
R1	SCX	LZ	1	79
R1	SCX	LZ	2	45
R1	SCX	LZ	3	35
R1	SCX	MZ	1	3
R1	SCX	MZ	2	7
R1	SCX	MZ	3	9
R1	SCX	HZ	1	1
R1	SCX	HZ	2	0
R1	SCX	HZ	3	3
R1	CNX	OZ	1	110
R1	CNX	OZ	2	136
R1	CNX	OZ	3	79
R1	CNX	LZ	1	12
R1	CNX	LZ	2	31
R1	CNX	LZ	3	22
R1	CNX	MZ	1	0
R1	CNX	MZ	2	1
R1	CNX	MZ	3	0
R1	CNX	HZ	1	0
R1	CNX	HZ	2	0
R1	CNX	HZ	3	1

Factors **Response**

We will create a data frame of factors comprising Run, Plate, Treatment, Replicate
The response variable is counts.

We have 3 spreadsheets of data
Run1counts.csv
Run2counts.csv
Run3counts.csv

We will need to write a procedure that reads in the data
Note that our script will require a consistent data format

Prepare for raw data
Script walkthrough 1

- Open a blank text document, and prepare to write this script
  - The data is contained in three files:
    - 11_CFA_Run1Counts.csv
    - 11_CFA_Run2Counts.csv
    - 11_CFA_Run3Counts.csv
  - Load in the data and concatenate it into a single data frame

```
# load in the data from the three runs into three separate data frames t1,
      t2, t3
t1 = read.csv("11_CFA_Run1Counts.csv")
t2 = read.csv("11_CFA_Run2Counts.csv")
t3 = read.csv("11_CFA_Run3Counts.csv")

# concatenate the three data frames into a single data frame
colony = rbind(t1, t2, t3)

# (or use one of the loops from yesterday...)
```

Example code:
11_CFAcountData.R

Import raw data
Script walkthrough 2

- Data is by default read in as factors, i.e. all input strings are enumerated and stored as numbers
- The three separate data frame have no indication of which number they came from. We will add a column indicating this:

```
# add the missing Run column - factors are stored as numbers !
runNum <- factor( rep( 1:3, each=36 ), labels=c("Run1","Run2","Run3") )
colony <- cbind( "Run" = runNum, colony )

# reorder factor levels in their natural order (instead of alphabetical)
colony$Treatment <- factor(colony$Treatment, c("OZ", "LZ", "MZ", "HZ"))
colony$Plate <- factor(colony$Plate, c("KDX","SCX","CNX"))

# show the full table
colony
```

The tapply function
a brief digression

- Assume we have the following data for heights of 5 males and females:

```
    data <- data.frame(gender=c("Male", "Male", "Female",
            "Female", "Female"), height=c(6, 6.1, 5.8, 6, 5.95))
gender height
1   Male   6.00
2   Male   6.10
3 Female   5.80
4 Female   6.00
5 Female   5.95
```

- By calling mean() on the height column we can get the average of all 5 people, but how do we get average separately for males and females?
- `tapply()` lets us do exactly this
  - It applies a function to grouped data:
- `tapply( data$height, data$gender, mean )`

  data   groups   function

Undertake data analysis
Script walkthrough 3

- We need the means of the triplicate counts for each Run
  - Broken down by plate type (KDX,SCX,CNX) and Z treatment concentration (OZ,LZ,MZ,HZ)

```
### Part 2.  Investigating data ###
tapply(colony$Count, list(colony$Run, colony$Plate,
colony$Treatment), mean)
```

```
, , OZ

           KDX      SCX      CNX
Run1  98.33333 129.6667 108.3333
Run2 180.33333 206.0000 188.6667
Run3 282.33333 288.6667 265.6667

, , LZ

          KDX      SCX      CNX
Run1  75.0000  53.0000 21.66667
Run2 136.3333 103.6667 32.00000
Run3 157.0000 180.6667 46.66667

, , MZ

          KDX       SCX       CNX
Run1 29.66667  6.333333 0.3333333
Run2 47.00000 11.666667 2.0000000
Run3 73.00000 17.333333 3.6666667

, , HZ

           KDX       SCX       CNX
Run1  6.333333 1.3333333 0.3333333
Run2 12.000000 0.3333333 0.0000000
Run3 18.666667 0.3333333 0.0000000
```

We can plot a graph of this. It gives us the variation in counts per run

```
par(oma=c(4,2,2,2))
boxplot(Count~Run*Plate*Treatment, las=2, cex=0.2,
data=colony)
```

Better still, lets plot a grouped bar chart of mean counts per plate type per Z treatment

```
barplot(tapply(colony$Count, list(colony$Plate,
colony$Treatment), mean),beside=T)
```

Summarize & save the analysis
Script walkthrough 4

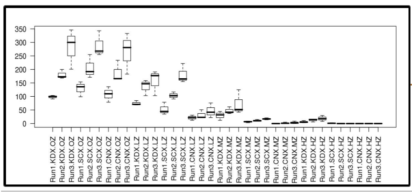
- we need a reshaped, background corrected, table of results on which to perform our tests
- for clarity where possible use dollar ($) notation (work only with data frames)

```
### Part 3.  Summarizing data ###
result <- tapply(colony$Count, list(colony$Treatment, colony$Plate), mean)
result <- data.frame(result) # result of tapply is matrix, convert to dataframe
result

# calculate kdx and scx values after background correction
kdx = result$KDX - result$CNX
scx = result$SCX - result$CNX

result <- cbind(kdx, scx)
# remove the 0Z entry
result <- result[-1,]
barplot(result,beside=T)

wilcox.test(result[,1],result[,2],paired=T)
cor.test(result[,1],result[,2],paired=T)
write.csv(result,"CFAresults.csv")
```

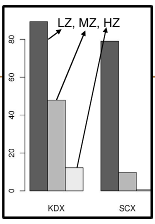
-ve subscripts mean 'delete'

We can plot the results as a barchart, and undetake an appropriate two sample classical test

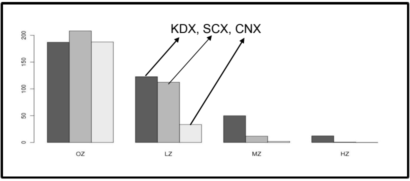
We find that the difference in means is not significant (would expect observation to occur 1:4 times), and that the scramble and knockdown counts have a 90% correlation



Boxplot shows variation in replicates. Run 3 had greatest count variation.



Barchart shows KDX had greater viability in Z compared to SCX. Treatments are Low, Medium and High.



Barchart shows run average counts of various plate types in different Z treatments. KDX is the only plate type that had growth in high Z.

```
> wilcox.test(result[,1],result[,2],paired=TRUE)

    Wilcoxon signed rank test

data:  result[, 1] and result[, 2]
V = 6, p-value = 0.25
alternative hypothesis: true location shift is not equal
to 0
```

```
> cor.test(result[,1],result[,2],paired=TRUE)

    Pearson's product-moment correlation

data:  result[, 1] and result[, 2]
t = 2.5584, df = 1, p-value = 0.2372
alternative hypothesis: true correlation is not equal
to 0
sample estimates:
      cor
0.9313792
```

Would expect to see trend 1 in 4 times. There is a 93% correlation between the knockdown of gene X and scramble control and cell counts response when grown in compound Z.

Script steps review
Script walkthrough 5

- Excel formatted data needs to be exported as comma separated values text (or tab!)
- Get the data into R
  - `read.csv()` … to assign the data to an object
- Produce exploratory plots
  - `boxplot()`
  - `barplot()`
- Undertake statistical tests
  - `cor.test()`
    - Spearman's rank correlation test
  - `wilcox.test()`
    - Wilcoxon test with two sets of paired data … Mann-Whitney U test
- Write out the results
  - `write.csv()`
    - exports data as comma separated list
  - `save.image()`
    - could also save the R environment after analysis (we didn't do this)

Exercise
Colony forming assay script

- Enter the text of the count data script, and save the file.
  - To run the count data script in R, type
  - `source("filename") # the script is available as 11_countData.R`
- Each step of the script is executed, and the results displayed.
- We need to export the graphical output to a file, and the R objects also need to be saved.
  - Modify the script as follows:

Section 3, line directly after ***tapply*** command insert:

```
jpeg(file="fig1.jpg",width=1600,height=800,res=150)
par(oma=c(4,2,2,2))
… <boxplot commands in middle> …
dev.off()
jpeg(file="fig2.jpg",width=675,height=900,res=150)
… <barplot commands in middle> …
dev.off()
```

Section 4, line directly after ***result*** command insert:

```
jpeg(file="fig3.jpg",width=1600,height=800,res=150)
… <barplot commands in middle> …
dev.off()
```

Batch processing R scripts
Scripting

- Scripts can be run without ever launching R, using R CMD batch mode.

quit R and type the following in a linux terminal

```
R CMD BATCH –no-restore 11_CFAcountData.R
```

or if you write all of graphical output to files:

`Rscript 11_CFAcountData.R` (works only with recent R versions)

Advanced example: multiple file handling
Reading files using loops and regular expressions

- Earlier we read in each of the three colony files one-by-one
- Yesterday, we saw how to load files using a loop
- But what if we didn't know how many files we had?
- We can use a regular expression to look up the list of available files

```
# look for patterns like 'Counts.csv'
this.pattern <- "*Counts.csv"

# find all filenames in the current directory containing this pattern
matching.filenames <- dir(pattern=glob2rx(this.pattern))

# see what has been found
matching.filenames

[1] "11_CFA_Run1Counts.csv" "11_CFA_Run2Counts.csv" "11_CFA_Run3Counts.csv"
```

Advanced example: multiple file handling
Reading files using loops and regular expressions

- Using '*' in the pattern is a 'wild card' – means we search for anything that ends in 'Counts.csv'
- glob2rx() is a function which translates this pattern into something the file system can recognise
- Now loop through the matching filenames and open each in turn

```
# start with an empty data frame
colony <- data.frame()

# loop through vector of file names
for(filename in matching.filenames){
      # open each file
      t <- read.csv(matching.filename)
      # append rows
      colony <- rbind(colony, t)
}
```