The 'R' statistics environment

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Bill Pearson wrp@virginia.edu 4-2818 Pinn 6-057

- A quick introduction to 'R'
  - Variable types:

```
vector=c(0,1,2,3),
mat1 = matrix(vector,nrow=2) (or ncol=2)
dframe1 = data.frame(ved=vector,
            vecx2= vector*2, vecsq=vector**2)
```

  - Input:

```
read.table("filename",header=TRUE,sep="\t")
```

  - Output:

```
plot(), hist(), boxplot()
```

  - Running 'R' ('R'-studio)

To learn more:

1. An introduction to 'R':
   cran.r-project.org/doc/manuals/R-intro.pdf
2. A "short" introduction:
   cran.r-project.org/doc/contrib/Torfs+Brauer-Short-R-Intro.pdf
3. Introducing 'R':
   http://data.princeton.edu/R/introducingR.pdf
4. A different introductory lecture on 'R' (that I borrow from):
   http://www.stat.cmu.edu/~cshalizi/statcomp/13/lectures/01--02/lecture-01--02.pdf

Why 'R' ?

- Open source, statistical programming environment based on 'S' (Bell Labs statistical programming environment)
  - plotting functions, statistical distributions, summary statistics, linear models, etc., etc.
- Universally used for functional bioinformatics (Bioconductor)
- The standard platform for new statistical development (false discovery rate fdr/qvalue)
- Tools for program documentation/reproducibility (`knitr`)
- 'R' analyses on the WWW (`shiny`)

Introduction to 'R' – functional programming

Python is an object oriented "procedural" language. You specify in some detail how to read data into variables, which are then iterated on, or transformed in some way, or used to automate a task.

'R' is a functional language. In some sense, everything in 'R' happens to a vector.

Thus, in Python, to make square all the values in a vector (array), you might write:

```
>>> array = [ 1, 2, 3, 4, 5]
array = [ 1, 2, 3, 4, 5]
>>> [ x * x for x in array ]
[1, 4, 9, 16, 25]
>>> [ 2 * x for x in array ]
[2, 4, 6, 8, 10]
```

in 'R':

```
> vector <- 1:4
> vector
[1] 1 2 3 4
> vector**2
[1]  1  4  9 16
> 2*vector
[1]  2  4  6 8
```

while there are 'for()' loops and 'if/then/else' conditionals in 'R', you will almost never need them to use 'R'. You will need to define functions, and use "apply()" to apply a function to the values in a vector

Introduction to 'R' – data types

- data types:
  - `numbers: 1, 1.0, 12.345`

    numbers are always double precision floating point unless forced to integer with `as.integer()`
  - `boolean: TRUE, FALSE`

    boolean values can be used to retrieve entries in vectors

```
> v1<-1:10
> v1
 [1]  1  2  3  4  5  6  7  8  9 10
> v1<4
 [1]  TRUE TRUE TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
> v1[v1 < 4]
[1] 1 2 3
```

  - `characters: "Jane", "pre-cancerous"`
  - `NaN, NA` - special no-data types

Introduction to 'R' – variable types

- Variable types:
  - `vectors[]` : arrays of the same type (number, string)

```
v1 <- c(1,2,3,4)
v12 <- c(v1,v1) -> 1 2 3 4 1 2 3 4 # c() "flattens"
v2 <- 1:9
v3 <- seq(1,5,0.1)
```

  - `matrices[2,3]` : arrays of arrays (of arrays), multi-dimensional

```
mat1 <- matrix(1:9, nrow=3)
mat1
     [,1] [,2] [,3]
[1,]    1    4    7
[2,]    2    5    8
[3,]    3    6    9
```

```
mat2 <- matrix(1:9,nrow=3,byrow=TRUE)
mat2
     [,1] [,2] [,3]
[1,]    1    2    3
[2,]    4    5    6
[3,]    7    8    9
```

  - `lists[]` : array that can have different types, including vectors and lists, has named entries (like dictionary)
  - `data.frame[]` : like a matrix with named columns (like dictionary), can contain different types

Introduction to 'R' – vector subsets

Selecting and sub-selecting data: vectors

- sub-part of vectors can be selected with vectors of indices

```
> v1 <- c(1.1, 2.2, 4.3, 3.4, 5.5)
> v1[2 , 3]
Error in v1[2, 3] : incorrect number of dimensions
> v1[c(2,3)]      # indices must be in vector
[1] 2.2 4.3
> v1[c(2,4,3)]     # indices can re-order
[1] 2.2 3.4 4.3
> v1[-c(2,3)]     # negative index deletes selection (cannot combine)
[1] 1.1 3.4 5.5
> v1[order(v1)]    # the order() function returns the indexes to sort
[1] 1.1 2.2 3.4 4.3 5.5
```

- sub-parts of vectors can be selected using booleans (TRUE, FALSE)

```
> v1 <- 1:10
> v1
 [1]  1  2  3  4  5  6  7  8  9 10
> v1 <= 5
[1] TRUE TRUE TRUE TRUE TRUE FALSE FALSE FALSE FALSE FALSE
> v1[v1<=5]
[1] 1 2 3 4 5
> v1 %%2 == 0
[1] FALSE TRUE FALSE TRUE FALSE TRUE FALSE TRUE FALSE TRUE
> v1[v1%%2==0]
[1]  2  4  6  8 10
```

- in all of these examples, sub-setting a vector returned a vector.

Introduction to 'R' – matrix subsets

- Selecting and sub-selecting data: matrices

```
> mat1 <- matrix(1:12, nrow=3)
> mat1
     [,1] [,2] [,3] [,4]
[1,]    1    4    7   10
[2,]    2    5    8   11
[3,]    3    6    9   12
> mat1[2,]     # select all columns from one row
[1]  2  5  8 11
> mat1[,4]     # select all rows from one column
[1] 10 11 12
> mat1[,4]**2  # compute on resulting vector
[1] 100 121 144
> mat1[1:2, 3:4] # for matrices, vectors select entries
     [,1] [,2]
[1,]    7   10
[2,]    8   11
> mat1[c(3, 1),c(3,1,2,4)]
     [,1] [,2] [,3] [,4]
[1,]    9    3    6   12
[2,]    7    1    4   10
```

Introduction to 'R' – variable types

- Selecting and sub-selecting data: matrices

```
> mat1 <- matrix(1:12, nrow=3)
> mat1
     [,1] [,2] [,3] [,4]
[1,]    1    4    7   10
[2,]    2    5    8   11
[3,]    3    6    9   12
> mat1[mat1[2,]>=5,]
Error in mat1[mat1[2,]>=5,](subscript)logical subscript
too long
mat1[2,]>=5
[1] FALSE  TRUE  TRUE  TRUE
> mat1[,mat1[2,]>=5] # rows,columns where row=2 entry > 5
     [,1] [,2] [,3]
[1,]    4    7   10
[2,]    5    8   11
[3,]    6    9   12
> mat1[,mat1[,2]<5] # wrong (too short) but no error.
     [,1] [,2]      # Vector extended from c(T,F,F) to
[1,]    1   10      # c(T,F,F,T) by concatenation
[2,]    2   11
[3,]    3   12
> mat1[mat1[,2]<5,]
[1]  1  4  7 10
```


Introduction to 'R' – data.frames

- data.frames are tables (arrays) with different types, typically with labeled columns

```
> head(GSE_FPKM)
        Gene MCF.7_Rep1 MCF.7_Rep2 MCF.7_Rep3 GM12892_Rep1 GM12892_Rep2 GM12892_Rep3
1 1/2-SBSRNA4 0.54253200   0.318766  0.2925300     0.268225   0.50125500    0.4364100
2        A1BG 0.75134200   1.080660  1.3224700     2.389740   0.42191900    0.5300680
3    A1BG-AS1 0.90314900   0.549146  1.5402100     0.701192   0.12630800    0.6629410
4        A1CF 0.00176153   0.000000  0.0000000     0.000000   0.00385721    0.0000000
5       A2LD1 1.37068000   1.040530  1.1445600     2.341310   2.41900000    1.8365700
6         A2M 0.00716990   1.435170  0.0510643     0.137600   0.03139180    0.0299176
```

- typically, columns of the data are extracted by name (`GSE_FPKM$MCF.7_Rep1`) as vectors, but they can also be extracted by index (`GSE_FPKM[2]`)
- data.frames can be reordered, selected and sub-setted just like matrices

```
> head(GSE_FPKM[order(GSE_FPKM$MCF.7_Rep1,decreasing=TRUE),])
       Gene MCF.7_Rep1 MCF.7_Rep2 MCF.7_Rep3 GM12892_Rep1 GM12892_Rep2 GM12892_Rep3
17769  RPL41    9479.40    5999.73    8669.86      8774.13      5197.96      4536.55
17833  RPS29    6909.02    3113.50    3847.84     10579.00      7282.94      5614.69
17829  RPS27    5281.44    2321.00    2883.32     10689.70      9748.79      7855.76
17765  RPL39    5217.51    2396.75    2294.83      6122.56      5146.11      4554.45
```

Introduction to 'R' – variables

- to see what is in a variable, use: str()

```
> str(v1)
 num [1:5] 1.1 2.2 4.3 3.4 5.5
> str(mat1)
 int [1:3, 1:4] 1 2 3 4 5 6 7 8 9 10 ...
> str(GSE_FPKM)
'data.frame':        23197 obs. of  11 variables:
 $ Gene        : Factor w/ 21648 levels "1/2-SBSRNA4",..: 1 2 3 4 5 6 7 8
9 10 ...
 $ MCF.7_Rep1  : num  0.54253 0.75134 0.90315 0.00176 1.37068 ...
 $ MCF.7_Rep2  : num  0.319 1.081 0.549 0 1.041 ...
 $ MCF.7_Rep3  : num  0.293 1.322 1.54 0 1.145 ...
 $ GM12892_Rep1: num  0.268 2.39 0.701 0 2.341 ...
 $ GM12892_Rep2: num  0.50126 0.42192 0.12631 0.00386 2.419 ...
 $ GM12892_Rep3: num  0.436 0.53 0.663 0 1.837 ...
 $ H1.hESC_Rep1: num  0.6699 2.43029 0.42874 0.00798 0.40421 ...
 $ H1.hESC_Rep2: num  0.60306 2.65009 0.37343 0.00259 0.68117 ...
 $ H1.hESC_Rep3: num  0.54942 2.23051 0.44545 0.00536 0.50608 ...
 $ H1.hESC_Rep4: num  0.4247 1.199 0.5754 0.0125 0.6244 ...
```


Introduction to 'R' – variables

- to see what is in a variable, use: summary()

```
> summary(v1)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
    1.1     2.2     3.4     3.3     4.3     5.5
> summary(mat1)
       V1             V2             V3             V4
 Min.   :1.0   Min.   :4.0   Min.   :7.0   Min.   :10.0
 1st Qu.:1.5   1st Qu.:4.5   1st Qu.:7.5   1st Qu.:10.5
 Median :2.0   Median :5.0   Median :8.0   Median :11.0
 Mean   :2.0   Mean   :5.0   Mean   :8.0   Mean   :11.0
 3rd Qu.:2.5   3rd Qu.:5.5   3rd Qu.:8.5   3rd Qu.:11.5
 Max.   :3.0   Max.   :6.0   Max.   :9.0   Max.   :12.0
> summary(GSE_FPKM)
       Gene          MCF.7_Rep1         MCF.7_Rep2          MCF.7_Rep3
 DUX2    :   17   Min.   :   0.000   Min.   :    0.000   Min.   :   0.000
 DUX4    :   13   1st Qu.:   0.009   1st Qu.:    0.000   1st Qu.:   0.005
 DUX4L2  :   12   Median :   1.103   Median :    0.882   Median :   0.875
 REXO1L2P:   10   Mean   :  22.062   Mean   :   23.801   Mean   :  22.559
 STK19   :   10   3rd Qu.:   9.433   3rd Qu.:    9.195   3rd Qu.:   8.305
 TNXB    :   10   Max.   :9479.400   Max.   :14997.700   Max.   :8669.860
 (Other) :23125
```

Reading in datasets (data.frame()s)

- for tab delimited files with headers:

```
Gene      MCF-7_Rep1        MCF-7_Rep2        MCF-7_Rep3
1/2-SB    0.542532 0.318766 0.29253
A1BG      0.751342 1.08066           1.32247
A1BG-     0.903149 0.549146 1.54021  0.701192
```

- you can read directly into a data.frame[] with read.table():

```
> GSE_FPKM <- read.table('GSE49712_ENCODE_FPKM.txt', header=TRUE, sep="\t")
> head(GSE_FPKM)
         Gene MCF.7_Rep1 MCF.7_Rep2 MCF.7_Rep3 GM12892_Rep1 GM12892_Rep2 GM12892_Rep3
1 1/2-SBSRNA4 0.54253200   0.318766  0.2925300     0.268225   0.50125500    0.4364100
2        A1BG 0.75134200   1.080660  1.3224700     2.389740   0.42191900    0.5300680
3    A1BG-AS1 0.90314900   0.549146  1.5402100     0.701192   0.12630800    0.6629410
4        A1CF 0.00176153   0.000000  0.0000000     0.000000   0.00385721    0.0000000
5       A2LD1 1.37068000   1.040530  1.1445600     2.341310   2.41900000    1.8365700
6         A2M 0.00716990   1.435170  0.0510643     0.137600   0.03139180    0.0299176
```

- If every column is not labeled, you may get an error:

```
Error in read.table("GSE49712_ENCODE_FPKM.txt", header = TRUE, sep = "\t") :
  duplicate 'row.names' are not allowed
```

- If you do not have a header, you can provide names:

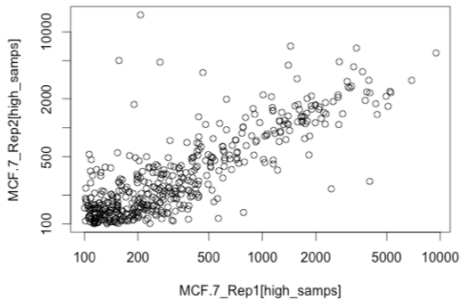
```
> fpe = read.table("noheader.dat",
+  col.names=c("setting","effort","change")) # + for continuation
```


Plotting data

One of the great strengths of 'R' is its ability to plot data in many different ways (this is also why you will be running it on your laptop, rather than on interactive.hpc from the command line)

- x-y plots : plot(x-vector, y-vector)

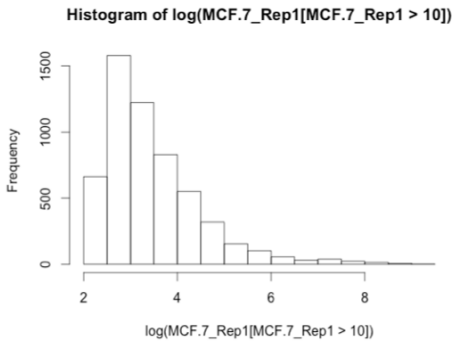
```
> high_samps <- GSE_FPKM$MCF.7_Rep1 > 100
> plot(MCF.7_Rep1[high_samps], MCF.7_Rep2[high_samps],log="xy")
```



Plotting data

- histograms: hist(vector)

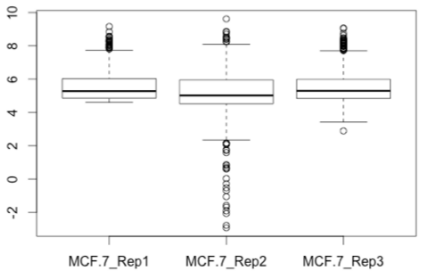
```
> hist(log(MCF.7_Rep1[MCF.7_Rep1 > 10]))
```



Plotting data

- boxplots boxplot(vector1, vector2, vector3)

```
> boxplot(log(GSE_FPKM[GSE_FPKM[2:4]>100,2:4]))
```



'R' functions

Functions may have arguments specified or unspecified when the function is defined

- There may be an arbitrary number of unspecified arguments
- Unspecified arguments denoted by ...
- Specified arguments may be supplied in the same order in which they occurred in the function definition
- Specified arguments may be supplied as name=value in which case their order is not important

```
> help(t.test) # if you know the name of the R built in function, you can use help()
> x = rnorm(10) # 10 numbers randomly drawn from a normal distribution; x ~ N(0, 1)
> y = rnorm(10) # 10 numbers randomly drawn from a normal distribution; y ~ N(0, 1)
> t.test(x, y, "greater")  # arguments in same order in which they are defined in function
> t.test(x=x, alternative="greater", y=y) # argument names specified but in wrong order
 Welch Two Sample t-test  data:  x and y
t = 1.1862, df = 16.896, p-value = 0.1260  alternative hypothesis: true difference in means
is greater than 0
95 percent confidence interval:
  -0.2838161        Inf
sample estimates:
mean of x   mean of y
0.02149336 -0.58618035
```


'R' functions

The R Base Package (so many functions; indexed by alphabet!)

`stat.ethz.ch/R-manual/R-patched/library/base/html/00Index.html`

Basic functions that come with your installation of R

- `mean(); sum(); median(); quantile(); max(); min(); range();`
- `abs(); sign(); log(); log10(), sqrt(); exp(); sin(); cos(); tan(); sinh(); tanh()`
- `sort(); order(); rev();`
- `duplicated(); unique();`
- `seq(); rep();`
- `round(); trunc(), floor(); ceiling()`
- `cat(); paste(); substring(); grep()`
- `merge(); cbind(); rbind()`

Contributed Packages: Currently, the CRAN package repository has more than 1700 packages:

`cran.r-project.org/web/packages/`

Specialized packages implementing the latest methods developed in computational statistics.

Use help() for assistance on usage!

'R' functions – apply()

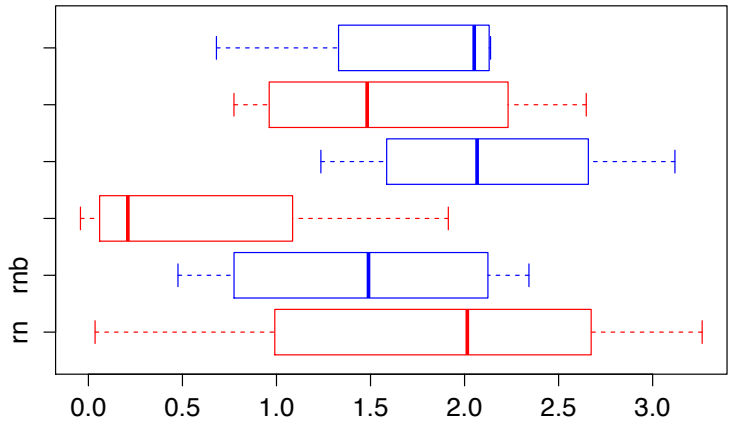
The apply() function allows you to apply functions, like mean() or var(), which apply to a vector, to a row (or row subset) of a matrix or data.frame.

```
> GSE_FPKM[11:15,2:4]
   MCF.7_Rep1 MCF.7_Rep2 MCF.7_Rep3
11   0.000000     0.0000  0.0000000
12   0.014162     0.0000  0.0000000
13  29.783700    23.1135 38.1064000
14  20.810500    21.7803 32.8547000
15   0.104898     0.0000  0.0610452

> var(GSE_FPKM[13,2:4])    # does NOT work – should report one variance per row
           MCF.7_Rep1 MCF.7_Rep2 MCF.7_Rep3
MCF.7_Rep1         NA         NA         NA
MCF.7_Rep2         NA         NA         NA
MCF.7_Rep3         NA         NA         NA

> apply(GSE_FPKM[13,2:4],1,var)   # does work – variance of row 13 is 56.42433
      13
56.42433
> apply(GSE_FPKM[11:15,2:4],1,var)  # five rows, five variances
          11           12           13           14           15
0.000000e+00 6.685408e-05 5.642433e+01 4.477427e+01 2.775529e-03
```


'R' examples – expression analysis 2

```
rn.0<-rnorm(4, mean=1.0, sd=1.0)
rn.1<-rnorm(4, mean=1.0, sd=1.0)
rn.2<-rnorm(4, mean=1.0, sd=1.0)
rnb.0<-rnorm(4, mean=2.0, sd=1.0)
rnb.1<-rnorm(4, mean=2.0, sd=1.0)
rnb.2<-rnorm(4, mean=2.0, sd=1.0)
boxplot(rn.0, rnb.0, rn.1, rnb.1, rn.2, rnb.2,
  horizontal=TRUE,
  border=c("red","blue","red","blue","red","blue"),
  names=c("rn","rnb","","","",""))

t.test(rn.0, rnb.0)
t.test(rn.1, rnb.1)
t.test(rn.2, rnb.2)
t.test(c(rn.0,rn.1,rn.2),c(rnb.0,rnb.1,rnb.2))
```

'R' examples – expression boxplot()



'R' examples – t.test()

```
> t.test(rn.0, rnb.0)
        Welch Two Sample t-test
data:  rn.0 and rnb.0
t = 0.48598, df = 5.0367, p-value = 0.6474
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.637706  2.403365
sample estimates:
mean of x mean of y
 1.832011  1.449182

> t.test(rn.1, rnb.1)

        Welch Two Sample t-test
data:  rn.1 and rnb.1
t = -2.5994, df = 5.8732, p-value = 0.0415
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3.01521343 -0.08321807
sample estimates:
mean of x mean of y
0.5727129 2.1219286
```

'R' examples – p.adjust()

```
nreps <-  4   # number of replicates
ngenes <- 20000
ngenes0 <- 15000
ngenes1 <-  3000
ngenes2 <-  1500
ngenes3 <-  500

data0 <- matrix(rnorm(ngenes*nreps, mean=1, sd=0.3), nrow=ngenes)
data1 <- matrix(rnorm(ngenes*nreps, mean=1, sd=0.3), nrow=ngenes)

diff0 <- matrix(rnorm(ngenes0*nreps, mean=1.0, sd=0.3), nrow=ngenes0)
diff1 <- matrix(rnorm(ngenes1*nreps, mean=1.5, sd=0.4), nrow=ngenes1)
diff2 <- matrix(rnorm(ngenes2*nreps, mean= 10, sd=3.0), nrow=ngenes2)
diff3 <- matrix(rnorm(ngenes3*nreps, mean=100, sd=10.0), nrow=ngenes3)

no_change <- cbind(data0, data1)  # 8 colums, 1:4 data0, 5:8 data1

mix_change <- cbind(data0, rbind(diff0,diff1,diff2,diff3))  # put the data together

nc_pvals <- matrix(apply(no_change, 1, function(x) {
  t.test(x[1:4], x[5:8])$p.value
}), nrow=200)

mix_pvals <- matrix(apply(mix_change, 1, function(x) {
  t.test(x[1:4], x[5:8])$p.value
}), nrow=200)

mix_bon <- matrix(p.adjust(mix_pvals, "bonferroni"), nrow=200)
mix_qvals <- matrix(p.adjust(mix_pvals, "fdr"), nrow=200)

image(nc_pvals < 0.05, axes=F, main="No change, p < 0.05")

image(mix_pvals < 0.05, axes=F, main="Mixed change, p < 0.05")
image(mix_bon < 0.05, axes=F, main="Mixed change, p < 0.05/20K (Bonferroni)")
image(mix_qvals < 0.05, axes=F, main="Mixed change, q < 0.05")

sum(nc_pvals < 0.05)  #  817 in last simulation
sum(mix_pvals < 0.05) # 3617 in last simulation
sum(mix_qvals < 0.05) # 1035 in last simulation
```


'R' examples – p.adjust()

p.adjust {stats}R Documentation

Adjust P-values for Multiple Comparisons

Description

Given a set of p-values, returns p-values adjusted using one of several methods.

Usage

```
p.adjust(p, method = p.adjust.methods, n = length(p))


p.adjust.methods
# c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY",
#   "fdr", "none")
```

Arguments

p	numeric vector of p-values (possibly with NAs). Any other R is coerced by as.numeric
method	correction method. Can be abbreviated.
n	number of comparisons, must be at least length(p); only set this (to non-default) when you know what you are doing!

Details

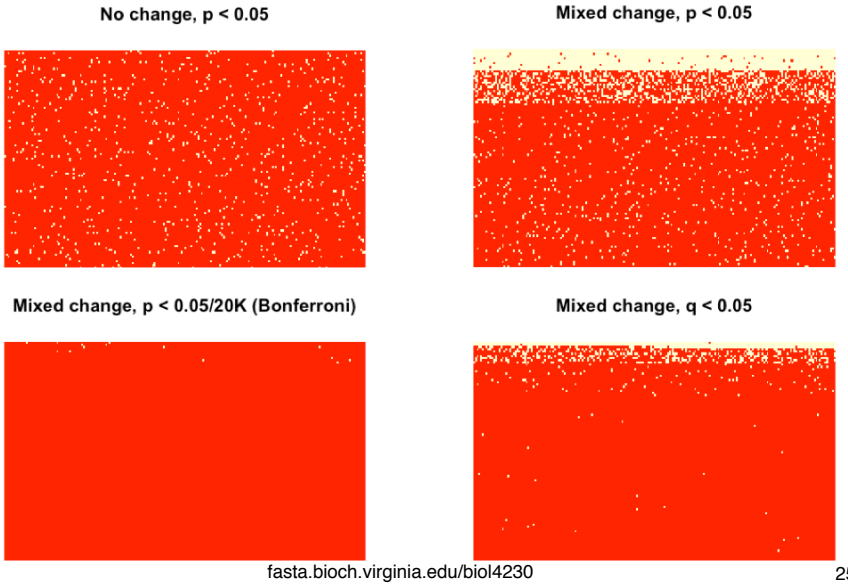
The adjustment methods include the Bonferroni correction ("bonferroni") in which the p-values are multiplied by the number of comparisons. Less conservative corrections are also included by Holm (1979) ("holm"), Hochberg (1988) ("hochberg"), Hommel (1988) ("hommel"), Benjamini & Hochberg (1995) ("BH" or its alias "fdr"), and Benjamini & Yekutieli (2001) ("BY"), respectively. A pass-through option ("none") is also included. The set of methods are contained in the p.adjust.methods vector for the benefit of methods that need to have the method as an option and pass it on to p.adjust.

The first four methods are designed to give strong control of the family-wise error rate. There seems no reason to use the unmodified Bonferroni correction because it is dominated by Holm's method, which is also valid under arbitrary assumptions.

Hochberg's and Hommel's methods are valid when the hypothesis tests are independent or when they are non-negatively associated (Sarkar, 1998; Sarkar and Chang, 1997). Hommel's method is more powerful than Hochberg's, but the difference is usually small and the Hochberg p-values are faster to compute.

The "BH" (aka "fdr") and "BY" method of Benjamini, Hochberg, and Yekutieli control the false discovery rate, the expected proportion of false discoveries amongst the rejected hypotheses. The false discovery rate is a less stringent condition than the family-wise error rate, so these methods are more powerful than the others.

'R' examples – p.adjust()



Introduction to 'R'

- 'R' works on vectors, matrices, and data.frames()
- subsets of vectors/matrices/data.frames can be specified:
  - vectors of indices (c(4,3,1,2), order(v1))
  - boolean vectors ($rep1>10 & rep2 > 10)
  - [,1:3] : all rows, columns 1:3
  - [1:4,] : all columns, rows 1:4
- columns of data.frames() can be named or indexed
- read.table()
- plot, hist, boxplot