Advanced data processing

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Combining data from multiple sources
Gene clustering example

- R has powerful functions to combine heterogeneous data into a single data set
- Gene clustering example data:
  - five sets of differentially expressed genes from various experimental conditions
  - file with names of experimentally verified genes
- Gene clustering exercise:
  1. combine this dataset into a single table and cluster to see which conditions are similar
  2. repeat the clustering but only on a subset of experimentally verified genes

Combining gene tables

- input files have two columns: gene names and fold change
- we want to combine all five tables into a single table, with 0 for missing values

LpR2	3.5795
fs(1)h	3.1376
CG6954	2.7492
Psa	2.7012
zfh2	2.6247
Fur1	2.4413
ct	2.3804
S	2.3674
rux	2.3574
RhoBTB	2.26
CG14889	2.1735
oc	2.1421
pros	2.0882
Kr-h1	-2.0447
CG5149	-2.1521
tna	-2.2102
CG14888	-2.4346
CG31368	-2.4793
Trim9	-2.616
Awd	-3.0595

+

Psa	3.8529
vnd	3.6457
ct	3.201
fs(1)h	3.1489
btd	3.1229
zfh2	2.8421
RhoBTB	2.6022
pros	2.5679
CG1124	2.5475
S	2.5424
oc	2.5111
Fur1	2.43
PHDP	2.304
CG31241	2.2802
rux	2.2232
CG14889	2.1752
CG31163	2.1606
HmgZ	2.0795
svp	-2.0404
TER94	-2.1807
corto	-2.3481
olf413	-2.4404
brat	-2.7256
CG31368	-2.7293
mub	-2.9555
Awd	-3.1413
lola	-3.8882

+

lola	3.0121
CG31368	2.8063
Kr-h1	2.7262
svp	2.7055
mub	2.6475
CG5149	2.5248
run	2.4759
tna	2.4302
CG6954	2.4235
CG11153	2.3045
Awd	2.2295
CG6919	2.1324
CG14888	2.067
Psa	-2.0276
rux	-2.093
fs(1)h	-2.141
CG1124	-2.155
Fur1	-2.1588
S	-2.2539
corto	-2.2618
oc	-2.3017
CG14889	-2.4393
zfh2	-2.5884
HmgZ	-3.6328
btd	-3.7627
brat	-3.7716

+

lola	3.3019
CG6919	2.9965
CG31368	2.817
CG5149	2.7675
Kr-h1	2.7647
TER94	2.6286
tna	2.5748
CG11153	2.4795
run	2.3831
CG14888	2.0938
S	-2.0243
rux	-2.0668
oc	-2.3437
corto	-2.5556
fs(1)h	-2.6211
brat	-2.9904
ct	-3.3404
zfh2	-4.4947
CG6954	-4.7244

+

brat	5.2812
ct	4.828
CG31163	4.3345
LpR2	3.6882
vnd	3.6866
zfh2	3.5314
pros	3.4307
Psa	3.3998
fs(1)h	3.3869
CG31241	2.9973
HmgZ	2.9226
Fur1	2.7469
RhoBTB	2.7189
oc	2.6543
Toll-7	2.6161
rux	2.5975
CG14889	2.3054
S	2.2324
CG1124	2.0216
Kr-h1	-2.1439
tna	-2.1793
CG5149	-2.1892
run	-2.2194
Trim9	-2.251
olf413	-2.3821
btd	-3.0293
CG6919	-3.3719

Gene clustering
Script walkthrough 1

- To make the big table we first need to find out all the genes present in at least one of the files
- Make sure not to use factors in read.delim()

```
# start with en empty collection of genes
genes <- c()
for( fileNum in 1:5 ){
   # load in files 13_DiffGenes1.tsv ...
   t <- read.delim(paste("13_DiffGenes", fileNum, ".tsv", sep=""),
                   as.is=TRUE, header=FALSE)
   # label the input columns to help code readability
   names(t) <- c("gene", "expression")
   genes <- union(genes, t$gene)
}

# for tidiness order our genes by name
genes <- sort(genes)

genes # show all genes
```

when loading in character data use **as.is=T** to prevent it being converted to factors!

union() is a set operation, combines two vectors by eliminating duplicates. There are also intersect() and setdiff()

Example code:
13_geneClustering.R

Gene clustering
Script walkthrough 2

- Using the complete list of genes, we can create the big table and fill in the values:

```
# make the destination table [rows = unique genes, cols = file numbers]
values <- matrix(0, nrow=length(genes), ncol=5)
rownames(values) <- genes # name the rows with the complete gene names

for(fileNum in 1:5){
  # read in the file again
     t <- read.delim(paste("13_DiffGenes", fileNum, ".tsv", sep=""),
            as.is=T, header=F)
     names(t) <- c("gene", "expression")

     # match the names of the genes to the rows in our big table
     index <- match(t$gene, rownames(values))
     # copy the expression levels
     values[index,fileNum] <- t$expression
}
```

match() returns the index of first argument in the second, i.e. index of input file genes in the big table

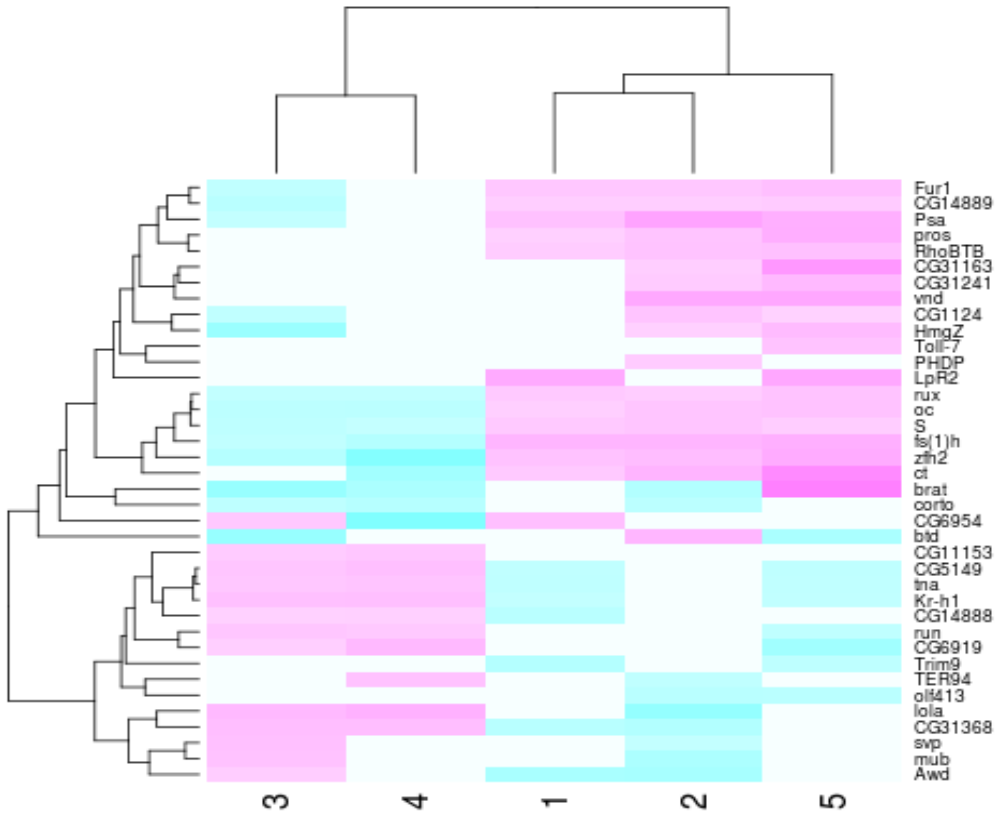
we use index to pick the rows in such way that they match the gene order in the input file

Gene clustering
Script walkthrough 3

- Now we can do hierarchical clustering:

```
heatmap(values, scale="none", col = cm.colors(256))
```

Values from the matrix are colour-coded. Rows and columns are re-arranged according to similarity



Gene clustering

Script walkthrough 4

- In a second part of our analysis, we want to produce the same heatmap but only based on a list of experimentally verified genes

- The problem is data is not formatted in the most convenient way:

genes	citation
oc,run,RhoBTB,CG5149,CG11153,S,Fur1	Segal et al, Development 2001
tna,Kr-h1,rux	Krejci et al, Development 2002

Gene clustering

Script walkthrough 5

- We load in this table, and only extract the gene names, then we use them to select a subset of values matrix

```
# load in the tab-delimited file with genes and citations
t.exp <- read.delim("13_ExperimentalGenes.tsv", as.is=T)
# split all gene names by "," and then flatten it out into a single vector
experim.genes <- unlist( strsplit(t.exp$genes, ",") )
```

unlist() flattens out a nested list into a single vector

strsplit() splits a vector of strings by a custom split character (","), the results is a list of split values for each element of input vector

```
# redo the heatmap by using just the genes in the experimentally verified set
is.experimental <- rownames(values) %in% experim.genes
heatmap(values[ is.experimental, ], scale="none", col = cm.colors(256))
```

Gene clustering review

- We load in the five tables twice - first to collect gene names, then to load expression values
- Based on expression table (values) we construct a clustered heatmap first on the whole set of genes, then on a selected subset
- Go through the code, try it out it and understand it
- Try answering the following questions:
  - what is rownames(values) ?
  - why is rownames(values)[index] and t$gene giving the same output?
  - what is a difference between rownames(values) %in% experim.genes and experim.genes %in% rownames(values)

Example code:
13_geneClustering.R