

Introduction to R

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1 Introduction

This lab introduces basic *R* operations by inputing and manipulating data describing a microarray experiment involving 128 individuals with acute lymphoblastic leukemia. Covariates include measures such as age, sex, type, stage of the disease, etc., and are provided as a comma separated file `pData.csv`. Our goal in this exercise is to

- Read the covariates into *R*.
- Perform data manipulations such as tabulation and subsetting
- Visualize some of the data using the *lattice* package.

2 Loading tabular data into *R*

Here we load the microarray experiment covariates from a ‘csv’ (comma-separated value) file. The file is located in the `extdata` folder of the *IWB2011* package.

R provides several functions such as `read.table` for reading in meta data files into a `data.frame` with appropriate column and row names from the header information provided in the file. Convenience functions such as `count.fields` are also available for discovering problems in files (such as certain rows in the file having different number of fields) when using the `read.table` function.

Exercise 1

- Start R, and load the IWB2011 package.
- Use the `system.file` to locate the path to the files `exprsMat.csv` and `pData.csv`
- Make use of the `count.fields` function on the `pData.csv` file to ensure that the file has the same number of fields in each line of the file.
- Use the `read.table` function to read in the experimental meta data into an R variable `pdOrig`.
- View the first few records of the data using `head`.
- Obtain a brief summary of the data using `summary`.
- Tabulate the number of males and females in the study by selecting the `sex` column and using `table`.

Solution:

```
> library(IWB2011)
> phenoPath <- system.file( "extdata", "pData.csv", package="IWB2011")
> pdOrig <- read.table(phenoPath)
> names(pdOrig)
```

[1]	"cod"	"diagnosis"	"sex"
[4]	"age"	"BT"	"remission"
[7]	"CR"	"date.cr"	"t.4.11."
[10]	"t.9.22."	"cyto.normal"	"citog"
[13]	"mol.biol"	"fusion.protein"	"mdr"
[16]	"kinet"	"ccr"	"relapse"
[19]	"transplant"	"f.u"	"date.last.seen"

```
> head(pdOrig)
```

	cod	diagnosis	sex	age	BT	remission	CR	date.cr
01005	1005	5/21/1997	M	53	B2		CR	8/6/1997
01010	1010	3/29/2000	M	19	B2		CR	6/27/2000
03002	3002	6/24/1998	F	52	B4		CR	8/17/1998
04006	4006	7/17/1997	M	38	B1		CR	9/8/1997
04007	4007	7/22/1997	M	57	B2		CR	9/17/1997
04008	4008	7/30/1997	M	17	B1		CR	9/27/1997

	t.4.11.	t.9.22.	cyto.normal		citog	mol.biol
01005	FALSE	TRUE	FALSE	t(9;22)	BCR/ABL	
01010	FALSE	FALSE	FALSE	simple alt.	NEG	
03002	NA	NA	NA	<NA>	BCR/ABL	
04006	TRUE	FALSE	FALSE	t(4;11)	ALL1/AF4	
04007	FALSE	FALSE	FALSE	del(6q)	NEG	
04008	FALSE	FALSE	FALSE	complex alt.	NEG	
	fusion.protein	mdr	kinet	ccr	relapse	transplant
01005	p210	NEG	dyploid	FALSE	FALSE	TRUE
01010	<NA>	POS	dyploid	FALSE	TRUE	FALSE
03002	p190	NEG	dyploid	FALSE	TRUE	FALSE
04006	<NA>	NEG	dyploid	FALSE	TRUE	FALSE
04007	<NA>	NEG	dyploid	FALSE	TRUE	FALSE
04008	<NA>	NEG	hyperd.	FALSE	TRUE	FALSE
	f.u	date.last.seen				
01005	BMT / DEATH IN CR	<NA>				
01010	REL	8/28/2000				
03002	REL	10/15/1999				
04006	REL	1/23/1998				
04007	REL	11/4/1997				
04008	REL	12/15/1997				

> summary(pdOrig)

	cod	diagnosis	sex	age
10005	: 1	1/15/1997 : 2	F :42	Min. : 5.00
1003	: 1	1/29/1997 : 2	M :83	1st Qu.:19.00
1005	: 1	11/15/1997: 2	NA's: 3	Median :29.00
1007	: 1	2/10/1998 : 2		Mean :32.37
1010	: 1	2/10/2000 : 2		3rd Qu.:45.50
11002	: 1	(Other) :116		Max. :58.00
(Other):122	NA's	: 2		NA's : 5.00
	BT	remission		CR
B2	:36	CR :99	CR	:96
B3	:23	REF :15	DEATH IN CR	: 3
B1	:19	NA's:14	DEATH IN INDUCTION:	7
T2	:15		REF	:15
B4	:12		NA's	: 7
T3	:10			
(Other):13				
	date.cr	t.4.11.	t.9.22.	
11/11/1997:	3	Mode :logical	Mode :logical	
1/21/1998 :	2	FALSE:86	FALSE:67	
10/18/1999:	2	TRUE :7	TRUE :26	
12/7/1998 :	2	NA's :35	NA's :35	
1/17/1997 :	1			

```

(Other)      :87
NA's         :31
cyto.normal  citog      mol.biol
Mode :logical normal    :24  ALL1/AF4:10
FALSE:69      simple alt. :15  BCR/ABL :37
TRUE :24      t(9;22)    :12  E2A/PBX1: 5
NA's :35      t(9;22)+other:11 NEG      :74
              complex alt. :10 NUP-98   : 1
              (Other)      :21 p15/p16  : 1
              NA's         :35
fusion.protein mdr      kinet      ccr
p190           :17      NEG :101    dyploid:94 Mode :logical
p190/p210: 8      POS : 24    hyperd.:27 FALSE:74
p210           : 8      NA's: 3    NA's   : 7    TRUE :26
NA's           :95                      NA's :28

```

```

relapse      transplant      f.u
Mode :logical Mode :logical REL      :61
FALSE:35      FALSE:91      CCR      :23
TRUE :65      TRUE :9       BMT / DEATH IN CR: 4
NA's :28      NA's :28      BMT / CCR   : 3
                        DEATH IN CR : 2
                        (Other)     : 7
                        NA's        :28

```

```

date.last.seen
1/7/1998 : 2
12/15/1997: 2
12/31/2002: 2
3/29/2001 : 2
7/11/1997 : 2
(Other) :83
NA's :35

```

```
> table(pdOrig$sex)
```

```

 F  M
42 83

```

3 Subset data

The `pdOrig` variable is a `data.frame` with columns representing the various co-variates that describe the experiment (age, sex, etc.). The row names correspond

to the sample Id's. The column BT is a factor indicating the tumour cell type (B1, B2, T1 ,T2 etc. with B indicating B-cell type and T indicating T-cell type). Similarly the column mol biol is a factor indicating the molecular biology of the cancer. (BCR/ABL, NEG, E2A/PBX1 etc.) The mol biol column can be accessed using `pdOrig[["mol biol"]]`.

In this section, we make use of indexing, subsetting, factors etc. to modify `pdOrig` to select a subset of samples. We are specifically interested in B-cell tumours with molecular biology type "NEG" or "BCR/ABL".

Exercise 2

- Identify the samples that are "NEG" or "BCR/ABL" molecular biology type using the column `mol biol`, the `%in%` function and the `which` functions in R.
- Use the `grep` function on the BT column in the `pdOrig data.frame` to identify the B cell tumours.
- Identify samples that are both B cell tumours and are "BCR/ABL" or "NEG" using the `intersect` function on the indices that we have previously computed.
- Subset the phenotypic data `pdOrig` to create a new variable `psubData`.
Note: The rows of the `data.frame` represent samples.

Solution:

```
> types <- c("NEG", "BCR/ABL")
> moltyp <- which(as.character(pdOrig$mol.biol) %in% types)
> bcell <- grep("^B", as.character(pdOrig$BT))
> indx <- intersect(bcell, moltyp)
> psubData <- pdOrig[indx,]
```

4 Recodig factor levels

The covariate data for some variables, for example BT, is represented using a variable of type factor. The 'levels' are the distinct categorical values of a factor. Subsetting a factor leaves the levels of the variable unchanged. In this exercise, we take a look at the levels of the factor variables that we have just subsetted, and then update the levels.

Exercise 3

- Observe the levels for the `mol.biol` and `moltyp` variables Do you notice any problem ?.
- Recode the factor levels for the `mol.biol` and `moltyp` variables using the `factor` function.

Solution:

```
> levels(psubData$BT)

[1] "B"  "B1" "B2" "B3" "B4" "T"  "T1" "T2" "T3" "T4"

> psubData$BT <- factor(psubData$BT)
> levels(psubData$BT)

[1] "B"  "B1" "B2" "B3" "B4"

> psubData$mol.biol <- factor(psubData$mol.biol)
> levels(psubData$mol.biol)

[1] "BCR/ABL" "NEG"
```

5 Compute summary statistics

R includes several functions that allows you to do a lot while writing only few lines of code. A good example is the `aggregate` function that splits the data into subsets, computes summary statistics for each subset, and returns the result in a convenient form. For more details regarding this function, please type in `help("aggregate")` into an R session. We will be making use of the `formula` and the `data.frame` methods for the `aggregate` function in this example. Another useful function for creating contingency tables that we will be using is the `xtabs` function.

We proceed to create some summary statistics on the variable `psubData` using the `aggregate` and `xtabs` functions.

Exercise 4

- Find the average age of male and females in our subsetting metadata variable `psubData` for the NEG and BCR/ABL groups using the `data.frame` interface for the `aggregate` function. The table generated by using the `aggregate` should look similar to the one found below. Hint: Try passing `na.rm = TRUE` to the `aggregate` function

	sex	molBiol	age
1	F	BCR/ABL	39.94
2	M	BCR/ABL	40.50
3	F	NEG	29.75
4	M	NEG	24.86

- Recalculate the average age of male and females in our subsetting metadata variable `psubData` for the NEG and BCR/ABL groups, this time using the `formula` interface for the `aggregate` function. Make sure that the results are identical to the one from the previous step.

- The column `relapse` in `psubData` is a logical vector indicating whether the patient had a relapse or not. Create a contingency table of the number of subjects that have had a relapse for the samples included in `psubData` using the `xtabs` function and the covariates `relapse`, `mol.biol` and `sex`. The table generated by using the `xtabs` function should look similar to the one found below.

	BCR/ABL	NEG
F	7.00	3.00
M	9.00	18.00

Solution:

```
> aggregate(psubData[, "age", drop = FALSE],
+           by= list(sex= psubData$sex, molBiol= psubData[["mol.biol"]]),
+           FUN = mean, na.rm = TRUE )

  sex molBiol      age
1  F BCR/ABL 39.93750
2  M BCR/ABL 40.50000
3  F      NEG 29.75000
4  M      NEG 24.85714

> aggregate(age ~ sex + mol.biol, data = psubData, FUN = mean)

  sex mol.biol      age
1  F BCR/ABL 39.93750
2  M BCR/ABL 40.50000
3  F      NEG 29.75000
4  M      NEG 24.85714

> xtabs(relapse ~ sex + mol.biol, data = psubData)

      mol.biol
sex BCR/ABL NEG
F          7   3
M          9  18
```

6 Data Visualization

Base *R* can produce many different types of statistical visualizations. Additional packages such as *lattice* or *ggplot2* extend this functionality. We will explore the *lattice* package. A typical call to the *lattice* function `xyplot` is

```
> xyplot(y ~ x | c, data, groups = g)
```

The arguments to a lattice function can be summarized in terms of

1. lattice function: A lattice plotting function such as `xyplot`, `dotplot` etc.
2. formula: The first argument to a lattice method is a formula. The formula for our example is `y ~ x | c`. If the lattice method takes only a single vector as input, the formula can be expressed as `~ x | c`.
 - primary variables: Variables `y` (Y axis of the plot) and `x` (X axis of the plot) that defines the lattice display separated by the `~` character.
 - conditioning variable: Variable `c` in the example separated from the primary variables by the character `|`. The conditioning variable divides the plot into separate panels.
3. grouping variable: The variable `g` in the example. The grouping variable segregates data into subgroups within each panel.
4. data: A *data.frame* with column names corresponding to the variables `y`, `x`, `c` and `g`.

Exercise 5

- Load the lattice package. Use the `bwplot` function to create a box-and-whiskers plot of `age` as a function of `sex`, conditioning on `mol.biol`.

Solution:

```
> library(lattice)
> plt <- bwplot(age ~ sex | mol.biol, psubData)
> print(plt)
```

7 Session information

- R version 2.12.1 (2010-12-16), i386-pc-mingw32
- Locale: C
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: ALL 1.4.7, AnnotationDbi 1.12.0, BSgenome 1.18.3, BSgenome.Scerevisiae.UCSC.sacCer2 1.3.16, Biobase 2.10.0, Biostrings 2.18.2, DBI 0.2-5, DESeq 1.2.1, GenomicFeatures 1.2.3, GenomicRanges 1.2.3, IRanges 1.8.8, IWB2011 0.0.2, RSQLite 0.9-4, Rsamtools 1.2.3, ShortRead 1.8.2, akima 0.5-4, edgeR 2.0.3, genefilter 1.32.0, hgu95av2.db 2.4.5, lattice 0.19-17, locfit 1.5-6, org.Hs.eg.db 2.4.6, org.Sc.sgd.db 2.4.6

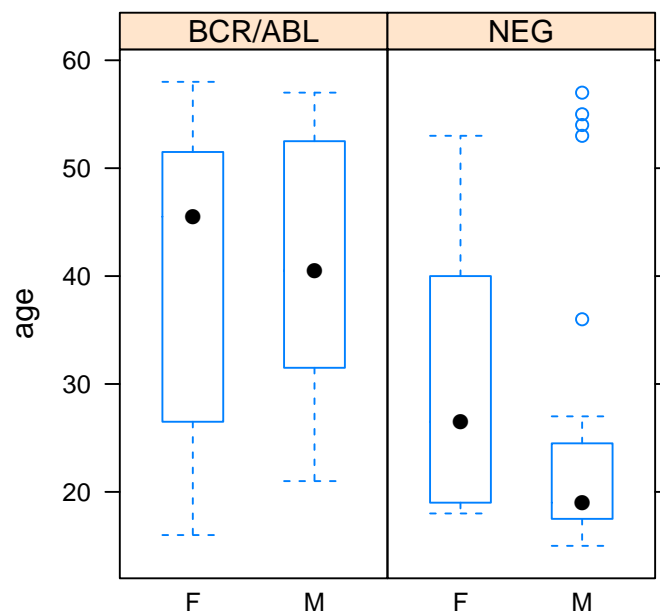


Figure 1: Box and whiskers plot summarizing age as a function of sex, conditioned on molecular biology.

- Loaded via a namespace (and not attached): RColorBrewer 1.0-2, RCurl 1.5-0.1, XML 3.2-0.2, annotate 1.28.0, biomaRt 2.6.0, geneplotter 1.28.0, grid 2.12.1, hwriter 1.3, limma 3.6.9, rtracklayer 1.10.6, splines 2.12.1, survival 2.36-2, tools 2.12.1, xtable 1.5-6