GSE32866, Genome-scale DNA methylation profiling of lung adenocarcinoma:

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Introduction

Load the following packages:

```
library(rmarkdown) # paged table
library(GEOquery) #geo query access
library(dplyr)
library(devtools)
library(ggplot2)
library(limma)
library(Glimma)
library(edgeR)
library(kableExtra)
library(ggrepel)
library(tinytex)
library(purrr)
Sys.setenv(VROOM_CONNECTION_SIZE = 25600000)
```

Importing the data

```
my_id <- "GSE32866"
gse <- getGEO(my_id)</pre>
```

Some datasets on GEO may be derived from different microarray platforms. Therefore the object gse is a list of different datasets. You can find out how many were used by checking the length of the gse object. Usually there will only be one platform and the dataset we want to analyse will be the first object in the list (gse[[1]]).

```
length(gse)
```

[1] 1

Extract the data

```
gse <- gse[[1]]
gse
```

```
ExpressionSet (storageMode: lockedEnvironment)
assayData: 27578 features, 55 samples
element names: exprs
protocolData: none
phenoData
sampleNames: GSM813533 GSM813534 ... GSM813587 (55 total)
varLabels: title geo_accession ... tissue:ch1 (58 total)
varMetadata: labelDescription
featureData
featureNames: cg00000292 cg00002426 ... cg27665659 (27578 total)
fvarLabels: ID Name ... ORF (38 total)
fvarMetadata: Column Description labelDescription
experimentData: use 'experimentData(object)'
pubMedIds: 22613842
Annotation: GPL8490
```

Exploratory analysis

The exprs function can retrieve the expression values as a data frame; with one column per-sample and one row per-gene.

```
pdata= pData(gse) #sample information
edata= exprs(gse) #expression data
fdata = fData(gse) #gene annotation
```

Make sure dimensions match up

The number of rows of the feature data should match the number of rows of the genomic data (both are the number of genes). The number of rows of the phenotype data should match the number of columns of the genomic data (both are the number of samples).

Look at overall distributions

For visualisation and statistical analysis, we will inspect the data to discover what scale the data are presented in. The methods we will use assume the data are on a log2 scale; typically in the range of 0 to 16.

The summary function can then be used to print the distributions.

```
## exprs get the expression levels as a data frame and get the distribution
dat <- summary(exprs(gse))
m <- matrix(1:ncol(dat), 5)
for (i in 1:ncol(m)) {
  cat(kbl(dat[, m[, i]], 'latex', booktabs=TRUE), "\newline")
}</pre>
```

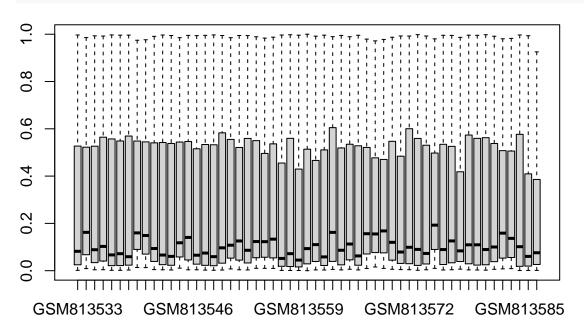
GSM813533	GSM813534	GSM813535	GSM813536	GSM813537
Min. :0.00158 1st Qu.:0.02499 Median :0.08185 Mean :0.26751 3rd Qu.:0.52701	Min. :0.0088 1st Qu.:0.0675 Median :0.1621 Mean :0.2951 3rd Qu.:0.5223	Min. :0.003852 1st Qu.:0.034014 Median :0.088613 Mean :0.270265 3rd Qu.:0.526548	Min. :0.00412 1st Qu.:0.04078 Median :0.10260 Mean :0.28832 3rd Qu.:0.56454	Min. :0.001593 1st Qu.:0.021340 Median :0.066667 Mean :0.274648 3rd Qu.:0.557164
Max. :0.99686 NA's :118		Max. :0.993288 NA's :23	Max. :0.99236 NA's :66	Max. :0.997155 NA's :1
 GSM813538	GSM813539	GSM813540	GSM813541	GSM813542
Min. :0.001563 1st Qu.:0.021631 Median :0.071831 Mean :0.272946 3rd Qu.:0.548465	Min. :0.002816 1st Qu.:0.023452 Median :0.05912 Mean :0.272262 3rd Qu.:0.569431	0 Median :0.15982 Mean :0.30666	Mean $:0.29943$	Mean $:0.27430$
Max. :0.996068 NA's :4	Max. :0.996306 NA's :3	Max. :0.97459 NA's :25	Max. :0.97656 NA's :16	Max. :0.99097 NA's :36
GSM813543	GSM813544	GSM813545	GSM813546	GSM813547
Min. :0.003748 1st Qu.:0.025443 Median :0.066108 Mean :0.269724 3rd Qu.:0.542025	Min. :0.002923 1st Qu.:0.022825 Median :0.06055 Mean :0.263932 3rd Qu.:0.538074	8 Median :0.11796 Mean :0.28764	Mean $:0.29780$	Mean $:0.25911$
Max. :0.996605 NA's :1	Max. :0.994622 NA's :5	Max. :0.98549 NA's :6	Max. :0.99514 NA's :42	Max. :0.99406 NA's :2
GSM813548	GSM813549	GSM813550	GSM813551	GSM813552
Min. :0.002475 1st Qu.:0.022601 Median :0.074436 Mean :0.271670 3rd Qu.:0.533615	Min. :0.001802 1st Qu.:0.021012 Median :0.05929 Mean :0.264499 3rd Qu.:0.532461	4 Median :0.09664 Mean :0.29360 3rd Qu.:0.58296	Mean :0.286343 3rd Qu.:0.55510	Median :0.12566 Mean :0.28056 6 3rd Qu.:0.52114
Max. :0.995359 NA	Max. :0.997065 NA's :6	Max. :0.99442 NA's :96	Max. :0.985281 NA's :3	Max. :0.99471 NA's :208

GSM813553	GSM813554	GSM813555	GSM813556	GSM813557
Min. :0.003055 1st Qu.:0.032660 Median :0.086232 Mean :0.280567 3rd Qu.:0.559251	Min. :0.01007 1st Qu.:0.05484 Median :0.12322 Mean :0.28903 3rd Qu.:0.55005	Min. :0.0099 1st Qu.:0.0568 Median :0.1227 Mean :0.2733 3rd Qu.:0.4960	1st Qu.:0.05408 Median :0.13335 Mean :0.28789	Min. :0.001198 1st Qu.:0.018511 Median :0.051788 Mean :0.239002 3rd Qu.:0.455191
Max. :0.994143 NA	Max. :0.98923 NA's :51	Max. :0.9836 NA's :453		Max. :0.996707 NA's :16
GSM813558	GSM813559	GSM813560	GSM813561	GSM813562
Min. :0.000755 1st Qu.:0.017597 Median :0.072014 Mean :0.277990 3rd Qu.:0.559703 Max. :0.997774 NA's :8	Min. :0.000893 1st Qu.:0.015929 Median :0.044893 Mean :0.230163 3rd Qu.:0.429716 Max. :0.996079 NA's :19	Mean $: 0.26941$	Min. :0.00329 1st Qu.:0.03900 Median :0.11016 Mean :0.26246 3rd Qu.:0.46627 Max. :0.99187 NA's :102	Min. :0.00288 1st Qu.:0.02200 Median :0.05827 Mean :0.25957 3rd Qu.:0.51087 Max. :0.99605 NA's :49
GSM813563	GSM813564	GSM813565	GSM813566	GSM813567
Min. :0.005434 1st Qu.:0.039251 Median :0.162052 Mean :0.322312 3rd Qu.:0.604959 Max. :0.989720 NA's :1	Min. :0.003316 1st Qu.:0.024736 Median :0.086374 Mean :0.271873 3rd Qu.:0.518953 Max. :0.995040 NA's :1	Mean $:0.28444$	Min. :0.003185 1st Qu.:0.023066 Median :0.06245 Mean :0.269006 3rd Qu.:0.528240 Max. :0.995625 NA's :2	3 Median :0.156346 Mean :0.297500
GSM813568	GSM813569	GSM813570	GSM813571	GSM813572
Min. :0.01552 1st Qu.:0.07648 Median :0.15532 Mean :0.28663 3rd Qu.:0.47719	Min. :0.00985 1st Qu.:0.07542 Median :0.16813 Mean :0.28440 3rd Qu.:0.47053	Min. :0.006009 1st Qu.:0.044466 Median :0.119853 Mean :0.290716 3rd Qu.:0.547162	Min. :0.004533 1st Qu.:0.030680 Median :0.078872 Mean :0.256968 3rd Qu.:0.484131	Min. :0.003998 1st Qu.:0.029502 Median :0.098945 Mean :0.302563 3rd Qu.:0.600460
Max. :0.97192 NA's :4	Max. :0.97799 NA's :51	Max. :0.987253 NA's :1	Max. :0.992672 NA's :3	Max. :0.992380 NA's :1
GSM813573	GSM813574	GSM813575	GSM813576	GSM813577
Min. :0.001719 1st Qu.:0.022253 Median :0.090038 Mean :0.283277 3rd Qu.:0.559240	Min. :0.003869 1st Qu.:0.028332 Median :0.072875 Mean :0.270009 3rd Qu.:0.530568	Min. :0.00984 1st Qu.:0.08979 Median :0.19254 Mean :0.30278 3rd Qu.:0.49747	Min. :0.002911 1st Qu.:0.026097 Median :0.08930 Mean :0.274118 3rd Qu.:0.534446	1 Median :0.125818 Mean :0.286136
· ·			Max. :0.995558	

GSM813578	GSM813579	GSM813580	GSM813581	GSM813582
Min. :0.00654 1st Qu.:0.03869 Median :0.08380 Mean :0.24641 3rd Qu.:0.41828	Min. :0.003284 1st Qu.:0.029137 Median :0.109404 Mean :0.294090 3rd Qu.:0.573597	Min. :0.002743 1st Qu.:0.023585 Median :0.109505 Mean :0.289157 3rd Qu.:0.559674	Min. :0.002231 1st Qu.:0.025043 Median :0.089401 Mean :0.282871 3rd Qu.:0.562494	Min. :0.005838 1st Qu.:0.038127 Median :0.100111 Mean :0.280244 3rd Qu.:0.537907
Max. :0.98813 NA's :6	Max. :0.995953 NA	Max. :0.995473 NA's :4	Max. :0.997839 NA's :16	Max. :0.991527 NA's :4
GSM813583	GSM813584	GSM813585	GSM813586	GSM813587
Min. :0.00773 1st Qu.:0.05315 Median :0.15874 Mean :0.28648 3rd Qu.:0.50774	Min. :0.00872 1st Qu.:0.05574 Median :0.13669 Mean :0.28092 3rd Qu.:0.50595	Min. :0.000538 1st Qu.:0.018411 Median :0.101503 Mean :0.294703 3rd Qu.:0.576664	Min. :0.001063 1st Qu.:0.019389 Median :0.060440 Mean :0.228942 3rd Qu.:0.409377	Min. :0.0012 1st Qu.:0.0262 Median :0.0759 Mean :0.2264 3rd Qu.:0.3859

A boxplot can also be generated to see if the data have been normalised. If so, the distributions of each sample should be highly similar.

boxplot(edata,outline=FALSE)



kable(table(pdata\$characteristics_ch1.1), booktabs = T)

Var1	Freq
tissue: Lung tumor	28
tissue: Normal lung	27

	tissue: Lung tumor	tissue: Normal lung
gender: Female	14	13
gender: Male	11	11
gender: NA	3	3

Inspect the clinical variables

Data submitted to GEO contain sample labels assigned by the experimenters, and some information about the processing protocol. All these data can be extracted by the pData function.

```
sampleInfo <- pData(gse)

## source_name_ch1 and characteristics_ch1.1 seem to contain factors we might need for the analysis
sampleInfo <- select(sampleInfo, source_name_ch1,characteristics_ch1.1)

#rename to more convenient column names
sampleInfo <- rename(sampleInfo, patient=characteristics_ch1.1, group = source_name_ch1)
kable(sampleInfo, longtable = T, booktabs = T, caption = "SampleInfo") %>%
kable_styling(latex_options = c("repeat_header"))
```

Table 1: SampleInfo

_				
		group		patient
	GSM813533	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813534	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813535	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813536	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813537	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813538	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813539	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813540	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813541	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813542	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813543	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813544	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813545	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813546	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813547	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813548	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813549	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813550	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813551	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813552	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813553	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813554	Fresh frozen macrodisse	ected tissue	tissue: Normal lung
	GSM813555	Fresh frozen macrodisse	ected tissue	tissue: Normal lung

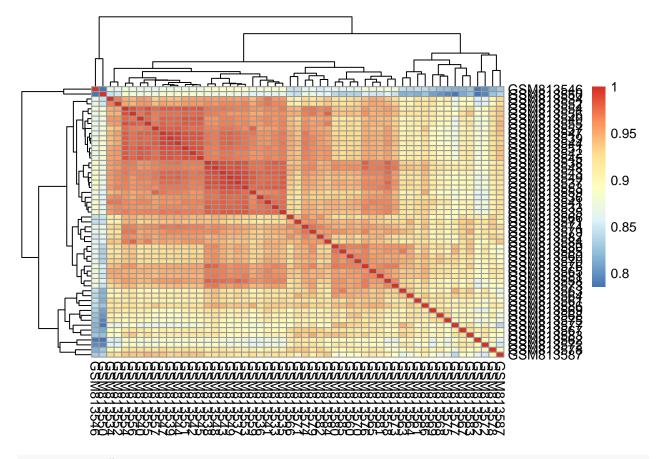
Table 1: SampleInfo (continued)

	group	patient
GSM813556	Fresh frozen macrodissected tissue	tissue: Normal lung
$\operatorname{GSM}813557$	Fresh frozen macrodissected tissue	tissue: Normal lung
GSM813558	Fresh frozen macrodissected tissue	tissue: Normal lung
GSM813559	Fresh frozen macrodissected tissue	tissue: Normal lung
GSM813560	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813561	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813562	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813563	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813564	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813565	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813566	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813567	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813568	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813569	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813570	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813571	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813572	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813573	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813574	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813575	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813576	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813577	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813578	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813579	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813580	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813581	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813582	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813583	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813584	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813585	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813586	Fresh frozen macrodissected tissue	tissue: Lung tumor
GSM813587	Fresh frozen macrodissected tissue	tissue: Lung tumor

Sample clustering and Principal Components Analysis

Unsupervised analysis is a good way to get an understanding of the sources of variation in the data. It can also identify potential outlier samples.

```
library(pheatmap)
corMatrix <- cor(exprs(gse), use="c")
pheatmap(corMatrix)</pre>
```



session_info()

- Session info ------

setting value

version R version 4.0.5 (2021-03-31)

os macOS Big Sur 10.16 system x86_64, darwin17.0

ui X11 language (EN)

collate en_GB.UTF-8
ctype en_GB.UTF-8
tz Europe/London
date 2022-02-23

pandoc 2.11.4 @ /Applications/RStudio.app/Contents/MacOS/pandoc/ (via rmarkdown)

- Packages ------

	0					
!	package	*	version	date (UTC)	lib	source
	annotate		1.68.0	2020-10-27	[2]	Bioconductor
	AnnotationDbi		1.52.0	2020-10-27	[2]	Bioconductor
	assertthat		0.2.1	2019-03-21	[2]	CRAN (R 4.0.2)
	Biobase	*	2.50.0	2020-10-27	[2]	Bioconductor
	BiocGenerics	*	0.36.1	2021-04-16	[2]	Bioconductor
	BiocParallel		1.24.1	2020-11-06	[2]	Bioconductor
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	bit64		4.0.5	2020-08-30	[2]	CRAN (R 4.0.2)
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                          0.13
  rvest
                          1.0.2
                                     2021-10-16 [2] CRAN (R 4.0.2)
  S4Vectors
                          0.28.1
                                     2020-12-09 [2] Bioconductor
  scales
                          1.1.1
                                     2020-05-11 [2] CRAN (R 4.0.2)
  sessioninfo
                          1.2.2
                                     2021-12-06 [2] CRAN (R 4.0.2)
                          1.7.6
                                     2021-11-29 [2] CRAN (R 4.0.2)
  stringi
                                     2019-02-10 [?] CRAN (R 4.0.2)
P stringr
                          1.4.0
  SummarizedExperiment
                          1.20.0
                                     2020-10-27 [2] Bioconductor
  survival
                          3.2-13
                                     2021-08-24 [2] CRAN (R 4.0.2)
                                     2022-02-03 [2] CRAN (R 4.0.5)
  svglite
                          2.1.0
                          1.0.4
                                     2022-02-11 [2] CRAN (R 4.0.5)
  systemfonts
  testthat
                          3.1.2
                                     2022-01-20 [2] CRAN (R 4.0.5)
  tibble
                          3.1.6
                                     2021-11-07 [2] CRAN (R 4.0.2)
P tidyr
                          1.2.0
                                     2022-02-01 [?] CRAN (R 4.0.5)
  tidyselect
                          1.1.2
                                     2022-02-21 [2] CRAN (R 4.0.5)
                                     2022-02-16 [2] CRAN (R 4.0.5)
                        * 0.37
  tinytex
  tzdb
                          0.2.0
                                     2021-10-27 [2] CRAN (R 4.0.2)
                        * 2.1.5
                                     2021-12-09 [2] CRAN (R 4.0.2)
  usethis
  utf8
                          1.2.2
                                     2021-07-24 [2] CRAN (R 4.0.2)
                          0.3.8
                                     2021-04-29 [2] CRAN (R 4.0.2)
  vctrs
                          0.4.0
                                     2021-04-13 [2] CRAN (R 4.0.2)
  viridisLite
                          1.5.7
                                     2021-11-30 [2] CRAN (R 4.0.2)
  vroom
                          0.5.2
                                     2019-11-22 [2] CRAN (R 4.0.2)
  webshot
  withr
                          2.4.3
                                     2021-11-30 [2] CRAN (R 4.0.2)
                                     2021-12-14 [2] CRAN (R 4.0.2)
  xfun
                          0.29
                          3.99-0.8
  XML
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  xm12
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                                     2021-11-30 [2] CRAN (R 4.0.2)
                                     2019-04-21 [2] CRAN (R 4.0.2)
                          1.8-4
  xtable
  XVector
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                                     2020-10-28 [2] Bioconductor
                                     2022-02-21 [2] CRAN (R 4.0.5)
  yaml
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  zlibbioc
                          1.36.0
                                     2020-10-28 [2] Bioconductor
```

^{[1] /}Users/paulyna/Documents/Github/Gene Analysis/renv/library/R-4.0/x86_64-apple-darwin17.0

^{[2] /}Library/Frameworks/R.framework/Versions/4.0/Resources/library

P -- Loaded and on-disk path mismatch.
