

Management of genome-scale data with Bioconductor

Bioconductor Infrastructure: ExpressionSet and SummarizedExperiment

- Similar classes of objects - expressionset usually array-based experiments and summarizedexperiment usually sequencing based experiments (rows = genomic ranges)
- Expression set lives in **Biobase** library
- **GeoQuery** allows you to pull down expressionsets by name using GSE identifier. Downloads the expressionset of microarray data
- List with single element in it - access element
- Lots of metadata - **assayData**, **phenotypic data**, **experiment**, **anotations**
- First sample - column indexing
- first row or first feature - row indexing
- Index by name
- **pData** phenotypic data - information about columns of expression set
- as.numeric on factor to see how many replicates of each strain
- pData should have same # of rows as columns in the expression set
- Feature data accessible with **FData** – genes / probe sets. Gene ID, gene symbol
- Experiment data and annotation data

```
library(Biobase)

## Loading required package: BiocGenerics
## Loading required package: parallel
##
## Attaching package: 'BiocGenerics'
## The following objects are masked from 'package:parallel':
##
##   clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##   clusterExport, clusterMap, parApply, parCapply, parLapply,
##   parLapplyLB, parRapply, parSapply, parSapplyLB
## The following objects are masked from 'package:stats':
##
##   IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##   anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##   dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##   grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##   order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##   rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##   union, unique, unsplit, which.max, which.min
## Welcome to Bioconductor
##
##   Vignettes contain introductory material; view with
##   'browseVignettes()'. To cite Bioconductor, see
##   'citation("Biobase)"', and for packages 'citation("pkgname)".

library(GEOquery)

## Setting options('download.file.method.GEOquery'='auto')
```

```
## Setting options('GEOquery.inmemory.gpl'=FALSE)
geeq <- getGEO("GSE9514")      # download a microarray dataset from GEO

## Found 1 file(s)
## GSE9514_series_matrix.txt.gz
##
## -- Column specification -----
## cols(
##   ID_REF = col_character(),
##   GSM241146 = col_double(),
##   GSM241147 = col_double(),
##   GSM241148 = col_double(),
##   GSM241149 = col_double(),
##   GSM241150 = col_double(),
##   GSM241151 = col_double(),
##   GSM241152 = col_double(),
##   GSM241153 = col_double()
## )
## File stored at:
## /var/folders/cn/qxqp12fd2gx3m17yb_flfkxc0000gn/T//RtmpgeU5cJ/GPL90.soft
names(geeq)

## [1] "GSE9514_series_matrix.txt.gz"
e <- geeq[[1]]      # extract ExpressionSet
e

## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 9335 features, 8 samples
##   element names: exprs
## protocolData: none
## phenoData
##   sampleNames: GSM241146 GSM241147 ... GSM241153 (8 total)
##   varLabels: title geo_accession ... data_row_count (31 total)
##   varMetadata: labelDescription
## featureData
##   featureNames: 10000_at 10001_at ... AFFX-YFL039CM_at (9335 total)
##   fvarLabels: ID ORF ... Gene Ontology Molecular Function (17 total)
##   fvarMetadata: Column Description labelDescription
## experimentData: use 'experimentData(object)'
##   pubMedIds: 18326586
## Annotation: GPL90
# exprs gives matrix of microarray values
dim(e)      # number of features and samples in ExpressionSet

## Features  Samples
##      9335      8
ncol(e)

## Samples
##      8
```

```

nrow(e)

## Features
##      9335

exprs(e)[1:3,1:3]

##           GSM241146  GSM241147  GSM241148
## 10000_at      15.33081    9.459372    7.984865
## 10001_at      283.47190   300.729460   270.016080
## 10002_i_at    2569.45360  2382.814700  2711.814500

head(exprs(e))[,1]      # first column

##      10000_at      10001_at      10002_i_at      10003_f_at      10004_at      10005_at
##      15.330812    283.471900    2569.453600    7917.580600      4.744475    253.808090

exprs(e)[1,]           # first row

## GSM241146 GSM241147 GSM241148 GSM241149 GSM241150 GSM241151 GSM241152 GSM241153
## 15.330812  9.459372  7.984865  7.899956  8.710567  7.749938  11.783052  8.327735

exprs(e)["10000_at",]   # can also index by name

## GSM241146 GSM241147 GSM241148 GSM241149 GSM241150 GSM241151 GSM241152 GSM241153
## 15.330812  9.459372  7.984865  7.899956  8.710567  7.749938  11.783052  8.327735

rownames(e)[1]         # row names are probe sets

## [1] "10000_at"

dim(exprs(e))           # rows are features, columns are samples

## [1] 9335      8

# pData gives phenotype data (sample information)
pData(e)[1:3,1:6]

##                                     title
## GSM241146 hem1 strain grown in YPD with 250 uM ALA (08-15-06_Philpott_YG_S98_1)
## GSM241147  WT strain grown in YPD under Hypoxia (08-15-06_Philpott_YG_S98_10)
## GSM241148  WT strain grown in YPD under Hypoxia (08-15-06_Philpott_YG_S98_11)
##           geo_accession      status submission_date last_update_date
## GSM241146      GSM241146 Public on Nov 06 2007      Nov 02 2007      Aug 14 2011
## GSM241147      GSM241147 Public on Nov 06 2007      Nov 02 2007      Aug 14 2011
## GSM241148      GSM241148 Public on Nov 06 2007      Nov 02 2007      Aug 14 2011
##           type
## GSM241146  RNA
## GSM241147  RNA
## GSM241148  RNA

names(pData(e))

## [1] "title"           "geo_accession"
## [3] "status"          "submission_date"
## [5] "last_update_date" "type"
## [7] "channel_count"    "source_name_ch1"
## [9] "organism_ch1"     "characteristics_ch1"
## [11] "molecule_ch1"    "extract_protocol_ch1"
## [13] "label_ch1"        "label_protocol_ch1"

```

```

## [15] "taxid_ch1"          "hyb_protocol"
## [17] "scan_protocol"      "description"
## [19] "data_processing"    "platform_id"
## [21] "contact_name"       "contact_email"
## [23] "contact_department" "contact_institute"
## [25] "contact_address"    "contact_city"
## [27] "contact_state"      "contact_zip/postal_code"
## [29] "contact_country"    "supplementary_file"
## [31] "data_row_count"

pData(e)$characteristics_ch1 # column in GEO to describe experimental state/condition

## [1] "BY4742 hem1 delta strain" "BY4742"
## [3] "BY4742"                  "BY4742 hem1 delta strain"
## [5] "BY4742 hem1 delta strain" "BY4742 hem1 delta strain"
## [7] "BY4742 strain"           "BY4742 strain"

as.numeric(pData(e)$characteristics_ch1) # help see replicates of each state

## Warning: NAs introduced by coercion
## [1] NA NA NA NA NA NA NA NA

dim(pData(e)) # rows of pData correspond to columns of exprs

## [1] 8 31

dim(e)

## Features  Samples
##      9335      8

# fData gives feature data (probe information)
fData(e)[1:3,1:3]

##              ID      ORF SPOT_ID
## 10000_at      10000_at YLR331C
## 10001_at      10001_at YLR332W
## 10002_i_at    10002_i_at YLR333C

dim(fData(e)) # rows of fData correspond to rows of exprs

## [1] 9335 17

names(fData(e))

## [1] "ID"                  "ORF"
## [3] "SPOT_ID"             "Species Scientific Name"
## [5] "Annotation Date"     "Sequence Type"
## [7] "Sequence Source"     "Target Description"
## [9] "Representative Public ID" "Gene Title"
## [11] "Gene Symbol"         "ENTREZ_GENE_ID"
## [13] "RefSeq Transcript ID" "SGD accession number"
## [15] "Gene Ontology Biological Process" "Gene Ontology Cellular Component"
## [17] "Gene Ontology Molecular Function"

head(fData(e)$"Gene Symbol")

## [1] "" "MID2" "RPS25B" "RPS25B" "" "NUP2"

```

```

head(rownames(e))

## [1] "10000_at"   "10001_at"   "10002_i_at" "10003_f_at" "10004_at"
## [6] "10005_at"

# additional annotation tied to ExpressionSet
experimentData(e)

## Experiment data
##   Experimenter name: Olga,,Protchenko
##   Laboratory:
##   Contact information: olgap@intra.niddk.nih.gov
##   Title: Changes in gene expression in response to heme deficiency and hypoxia
##   URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9514
##   PMIDs: 18326586
##
##   Abstract: A 33 word abstract is available. Use 'abstract' method.
##   notes:
##     contact_address:
##       10 Center Dr, Bldg.10-Rm.9B-05
##     contact_city:
##       Bethesda
##     contact_country:
##       USA
##     contact_department:
##       NIDDK
##     contact_email:
##       olgap@intra.niddk.nih.gov
##     contact_institute:
##       National Institutes of health
##     contact_name:
##       Olga,,Protchenko
##     contact_state:
##       MD
##     contact_zip/postal_code:
##       20892
##     contributor:
##       Olga,,Protchenko
##   Caroline,,Philpott
##     geo_accession:
##       GSE9514
##     last_update_date:
##       Jul 01 2016
##     overall_design:
##       I.Heme depletion.The hem1 delta strain was grown for 6 hrs in YPD media su
## pplemented with 250 uM ALA. Cells were washed three times with water to re
## move excess ALA. Cells were then inoculated in duplicates into 15 ml cultu
## res of
## YPD media that was supplemented either with or without ALA. Cultur
## es were induced for 16 h before 5 ml of cells were harvested and washed
## t
## hree times in DEPC water. The culture density at the time of harvest
## was
## approximately OD600=0.5.
## II.Hypoxia. BY45742 strain was grown for 6 hrs i

```

```

## n
## YPD medium and then inoculated in 15 ml the same medium and grown
## unde
## r normoxic or hypoxic conditions for 12 hrs. To create hypoxic
## conditions
## , cultures were incubated in an anaerobic chamber using a
## BBL-GasPak (BD
## Biosciences).
##   platform_id:
##     GPL90
##   platform_taxid:
##     4932
##   pubmed_id:
##     18326586
##   relation:
##     BioProject: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA103313
##   sample_id:
##     GSM241146 GSM241147 GSM241148 GSM241149 GSM241150 GSM241151 GSM241152 GSM2
## 41153
##   sample_taxid:
##     4932
##   status:
##     Public on Nov 06 2007
##   submission_date:
##     Nov 05 2007
##   summary:
##     We addressed changes in gene expression profile in response to
## reduced ox
## ygen tension in wild type or heme depletion caused by
## deletion of heme bi
## osynthesis gene hem1
## Keywords: Response to heme depletion and hypoxia
##   supplementary_file:
##     ftp://ftp.ncbi.nlm.nih.gov/geo/series/GSE9nnn/GSE9514/suppl/GSE9514_RAW.ta
## r
##   title:
##     Changes in gene expression in response to heme deficiency and hypoxia
##   type:
##     Expression profiling by array
annotation(e)

## [1] "GPL90"

```

Assessment: ExpressionSet

```

library(GSE5859Subset)
data(GSE5859Subset)
dim(geneExpression)

```

```
## [1] 8793 24
```

```
dim(sampleInfo)
```

```
## [1] 24 4
dim(geneAnnotation)

## [1] 8793 4
identical(colnames(geneExpression), sampleInfo$filename)

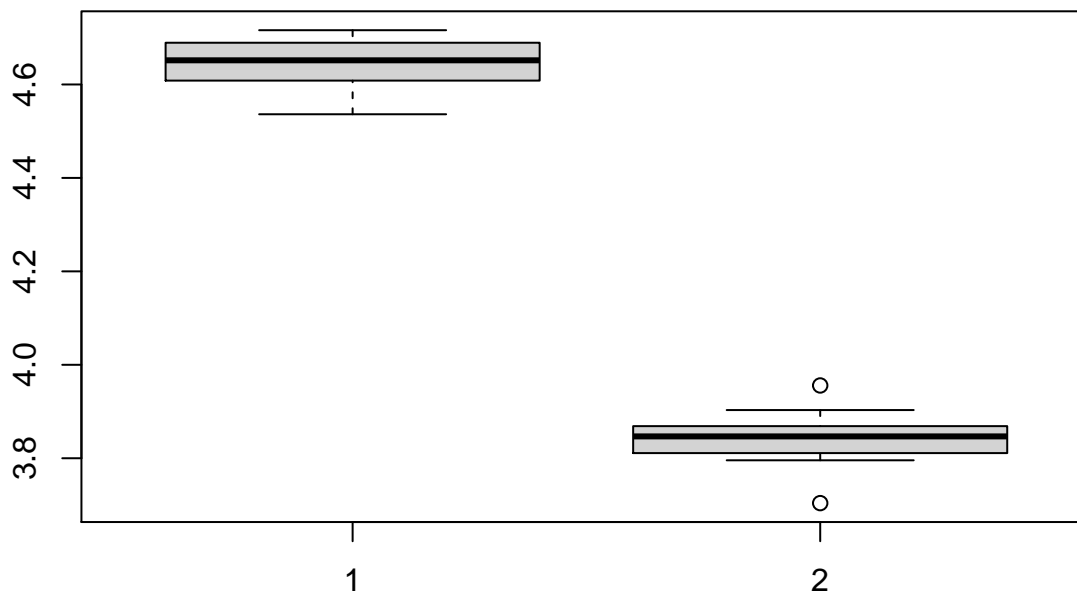
## [1] TRUE
identical(rownames(geneExpression), geneAnnotation$PROBEID)

## [1] TRUE
pd = AnnotatedDataFrame(sampleInfo)
rownames(pd) = colnames(geneExpression)
experiment_date <- pData(pd)["GSM136530.CEL.gz","date"]
paste("experiment date: ", experiment_date)

## [1] "experiment date: 2005-06-27"
varLabels(pd)

## [1] "ethnicity" "date" "filename" "group"
fd = AnnotatedDataFrame(geneAnnotation)
rownames(fd) = geneAnnotation$PROBEID
pData(fd)["204810_s_at", "CHR"]

## [1] "chr19"
eset = ExpressionSet(geneExpression, phenoData = pd, featureData = fd)
ind1 <- which(featureData(eset)$CHR == "chrY")
ind2 <- pData(eset)$group == 1
femaleY <- colMeans(exprs(eset)[ind1, ind2])
maleY <- colMeans(exprs(eset)[ind1, !ind2])
boxplot(maleY, femaleY)
```



```
median_diff=median(maleY) - median(femaleY)
```

```
paste("Difference in median expression of genes on the Y chromosome between maleY and femaleY: ", median
```

```
## [1] "Difference in median expression of genes on the Y chromosome between maleY and femaleY: 0.8049
```

Reading Microarray Raw Data: Single-Color Arrays

- loading microArray data into R: Affymetrix one-color arrays and Agilent two-color
- Affy package: parsers for reading in raw data files
- *cel* files are raw data
- *sample info* files have table describing each sample
- **pm** = perfect match probe-level intensities
- columns of probe-level data will match the rows of **pData** - each row represents a sample and column matches sample. Rownames **pData** match colnames **pm**
- Keep character which represents platform that the technology used
- Bioconductor has a package which matches gene IDs to meaningful terms
- Experiment data, sample information, third piece of information which connects us to other information which describes genes
- Last step: turn into gene-level information -> probe level. Algorithm **rma** implemented in Bioconductor to do this using quantile normalization and background correction.
- If only want gene expression data and not probe-level, you can use **justRMA** function to read and process in one shot

```
wd <- getwd()
datadir <- paste0(wd, "/rawdata-master")      # downloaded files
basedir <- paste0(datadir, "/celfiles")
setwd(basedir)
library(affy)
tab <- read.delim("sampleinfo.txt", check.names=FALSE, as.is=TRUE)
rownames(tab) <- tab$filenames
```

```
tab
```

```
##                               filenames 37777_at 684_at 1597_at
## 1521a99hpp_av06.CEL.gz 1521a99hpp_av06.CEL.gz 0.00 0.25 0.5
## 1532a99hpp_av04.CEL.gz 1532a99hpp_av04.CEL.gz 0.00 0.25 0.5
## 2353a99hpp_av08.CEL.gz 2353a99hpp_av08.CEL.gz 0.00 0.25 0.5
## 1521b99hpp_av06.CEL.gz 1521b99hpp_av06.CEL.gz 0.25 0.50 1.0
## 1532b99hpp_av04.CEL.gz 1532b99hpp_av04.CEL.gz 0.25 0.50 1.0
## 2353b99hpp_av08r.CEL.gz 2353b99hpp_av08r.CEL.gz 0.25 0.50 1.0
##                               38734_at 39058_at 36311_at 36889_at 1024_at 36202_at
## 1521a99hpp_av06.CEL.gz      1      2      4      8      16      32
## 1532a99hpp_av04.CEL.gz      1      2      4      8      16      32
## 2353a99hpp_av08.CEL.gz      1      2      4      8      16      32
## 1521b99hpp_av06.CEL.gz      2      4      8      16      32      64
## 1532b99hpp_av04.CEL.gz      2      4      8      16      32      64
## 2353b99hpp_av08r.CEL.gz      2      4      8      16      32      64
##                               36085_at 40322_at 407_at 1091_at 1708_at 33818_at
## 1521a99hpp_av06.CEL.gz      64      128 0.00      512      1024      256
## 1532a99hpp_av04.CEL.gz      64      128 0.00      512      1024      256
## 2353a99hpp_av08.CEL.gz      64      128 0.00      512      1024      256
## 1521b99hpp_av06.CEL.gz      128      256 0.25      1024      0      512
## 1532b99hpp_av04.CEL.gz      128      256 0.25      1024      0      512
## 2353b99hpp_av08r.CEL.gz      128      256 0.25      1024      0      512
##                               546_at
```



```

## 1521a99hpp_av06.CEL.gz      32
## 1532a99hpp_av04.CEL.gz      32
## 2353a99hpp_av08.CEL.gz      32
## 1521b99hpp_av06.CEL.gz      64
## 1532b99hpp_av04.CEL.gz      64
## 2353b99hpp_av08r.CEL.gz     64

fns <- list.celfiles(basedir)
fns

## [1] "1521a99hpp_av06.CEL.gz" "1521b99hpp_av06.CEL.gz"
## [3] "1532a99hpp_av04.CEL.gz" "1532b99hpp_av04.CEL.gz"
## [5] "2353a99hpp_av08.CEL.gz" "2353b99hpp_av08r.CEL.gz"

fns %in% tab[,1] ##check

## [1] TRUE TRUE TRUE TRUE TRUE TRUE

ab <- ReadAffy(phenoData=tab)

dim(pm(ab))

## Warning: replacing previous import 'AnnotationDbi::tail' by 'utils::tail' when
## loading 'hgu95acdf'

## Warning: replacing previous import 'AnnotationDbi::head' by 'utils::head' when
## loading 'hgu95acdf'

##
## [1] 201807      6

dim(pData(ab))

## [1] 6 17

rownames(ab)

##      [1] "100_g_at"          "1000_at"
##      [3] "1001_at"           "1002_f_at"
##      [5] "1003_s_at"         "1004_at"
##      [7] "1005_at"           "1006_at"
##      [9] "1007_s_at"         "1008_f_at"
##     [11] "1009_at"           "101_at"
##     [13] "1010_at"           "1011_s_at"
##     [15] "1012_at"           "1013_at"
##     [17] "1014_at"           "1015_s_at"
##     [19] "1016_s_at"         "1017_at"
##     [21] "1018_at"           "1019_g_at"
##     [23] "102_at"            "1020_s_at"
##     [25] "1021_at"           "1022_f_at"
##     [27] "1023_at"           "1024_at"
##     [29] "1025_g_at"         "1026_s_at"
##     [31] "1027_at"           "1028_at"
##     [33] "1029_s_at"         "103_at"
##     [35] "1030_s_at"         "1031_at"
##     [37] "1032_at"           "1033_g_at"
##     [39] "1034_at"           "1035_g_at"
##     [41] "1036_at"           "1037_at"

```

##	[43]	"1038_s_at"	"1039_s_at"
##	[45]	"104_at"	"1040_s_at"
##	[47]	"1041_at"	"1042_at"
##	[49]	"1043_s_at"	"1044_s_at"
##	[51]	"1045_s_at"	"1046_at"
##	[53]	"1047_s_at"	"1048_at"
##	[55]	"1049_g_at"	"105_at"
##	[57]	"1050_at"	"1051_g_at"
##	[59]	"1052_s_at"	"1053_at"
##	[61]	"1054_at"	"1055_g_at"
##	[63]	"1056_s_at"	"1057_at"
##	[65]	"1058_at"	"1059_at"
##	[67]	"106_at"	"1060_g_at"
##	[69]	"1061_at"	"1062_g_at"
##	[71]	"1063_s_at"	"1064_at"
##	[73]	"1065_at"	"1066_at"
##	[75]	"1067_at"	"1068_g_at"
##	[77]	"1069_at"	"107_at"
##	[79]	"1070_at"	"1071_at"
##	[81]	"1072_g_at"	"1073_at"
##	[83]	"1074_at"	"1075_f_at"
##	[85]	"1076_at"	"1077_at"
##	[87]	"1078_at"	"1079_g_at"
##	[89]	"108_g_at"	"1080_s_at"
##	[91]	"1081_at"	"1082_at"
##	[93]	"1083_s_at"	"1084_at"
##	[95]	"1085_s_at"	"1086_at"
##	[97]	"1087_at"	"1088_at"
##	[99]	"1089_i_at"	"109_at"
##	[101]	"1090_f_at"	"1091_at"
##	[103]	"1092_at"	"1093_at"
##	[105]	"1094_g_at"	"1095_s_at"
##	[107]	"1096_g_at"	"1097_s_at"
##	[109]	"1098_at"	"1099_s_at"
##	[111]	"110_at"	"1100_at"
##	[113]	"1101_at"	"1102_s_at"
##	[115]	"1103_at"	"1104_s_at"
##	[117]	"1105_s_at"	"1106_s_at"
##	[119]	"1107_s_at"	"1108_s_at"
##	[121]	"1109_s_at"	"111_at"
##	[123]	"1110_at"	"1111_at"
##	[125]	"1112_g_at"	"1113_at"
##	[127]	"1114_at"	"1115_at"
##	[129]	"1116_at"	"1117_at"
##	[131]	"1118_at"	"1119_at"
##	[133]	"112_g_at"	"1120_at"
##	[135]	"1121_g_at"	"1122_f_at"
##	[137]	"1123_at"	"1124_at"
##	[139]	"1125_s_at"	"1126_s_at"
##	[141]	"1127_at"	"1128_s_at"
##	[143]	"1129_at"	"113_i_at"
##	[145]	"1130_at"	"1131_at"
##	[147]	"1132_s_at"	"1133_at"
##	[149]	"1134_at"	"1135_at"

##	[151]	"1136_at"	"1137_at"
##	[153]	"1138_at"	"1139_at"
##	[155]	"114_r_at"	"1140_at"
##	[157]	"1141_at"	"1142_at"
##	[159]	"1143_s_at"	"1144_at"
##	[161]	"1145_g_at"	"1146_at"
##	[163]	"1147_at"	"1148_s_at"
##	[165]	"1149_at"	"115_at"
##	[167]	"1150_at"	"1151_at"
##	[169]	"1152_i_at"	"1153_f_at"
##	[171]	"1154_at"	"1155_at"
##	[173]	"1156_at"	"1157_s_at"
##	[175]	"1158_s_at"	"1159_at"
##	[177]	"116_at"	"1160_at"
##	[179]	"1161_at"	"1162_g_at"
##	[181]	"1163_at"	"1164_at"
##	[183]	"1165_at"	"1166_at"
##	[185]	"1167_s_at"	"1168_at"
##	[187]	"1169_at"	"117_at"
##	[189]	"1170_at"	"1171_s_at"
##	[191]	"1172_at"	"1173_g_at"
##	[193]	"1174_at"	"1175_s_at"
##	[195]	"1176_at"	"1177_at"
##	[197]	"1178_at"	"1179_at"
##	[199]	"118_at"	"1180_g_at"
##	[201]	"1181_at"	"1182_at"
##	[203]	"1183_at"	"1184_at"
##	[205]	"1185_at"	"1186_at"
##	[207]	"1187_at"	"1188_g_at"
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## [12561]	"AFFX-BioB-3_st"	"AFFX-BioB-5_at"
## [12563]	"AFFX-BioB-5_st"	"AFFX-BioB-M_at"
## [12565]	"AFFX-BioB-M_st"	"AFFX-BioC-3_at"
## [12567]	"AFFX-BioC-3_st"	"AFFX-BioC-5_at"
## [12569]	"AFFX-BioC-5_st"	"AFFX-BioDn-3_at"

```
## [12571] "AFFX-BioDn-3_st" "AFFX-BioDn-5_at"
## [12573] "AFFX-BioDn-5_st" "AFFX-CreX-3_at"
## [12575] "AFFX-CreX-3_st" "AFFX-CreX-5_at"
## [12577] "AFFX-CreX-5_st" "AFFX-DapX-3_at"
## [12579] "AFFX-DapX-5_at" "AFFX-DapX-M_at"
## [12581] "AFFX-HSAC07/X00351_3_at" "AFFX-HSAC07/X00351_3_st"
## [12583] "AFFX-HSAC07/X00351_5_at" "AFFX-HSAC07/X00351_5_st"
## [12585] "AFFX-HSAC07/X00351_M_at" "AFFX-HSAC07/X00351_M_st"
## [12587] "AFFX-hum_alu_at" "AFFX-HUMGAPDH/M33197_3_at"
## [12589] "AFFX-HUMGAPDH/M33197_3_st" "AFFX-HUMGAPDH/M33197_5_at"
## [12591] "AFFX-HUMGAPDH/M33197_5_st" "AFFX-HUMGAPDH/M33197_M_at"
## [12593] "AFFX-HUMGAPDH/M33197_M_st" "AFFX-HUMISGF3A/M97935_3_at"
## [12595] "AFFX-HUMISGF3A/M97935_5_at" "AFFX-HUMISGF3A/M97935_MA_at"
## [12597] "AFFX-HUMISGF3A/M97935_MB_at" "AFFX-HUMRGE/M10098_3_at"
## [12599] "AFFX-HUMRGE/M10098_5_at" "AFFX-HUMRGE/M10098_M_at"
## [12601] "AFFX-HUMTFRR/M11507_3_at" "AFFX-HUMTFRR/M11507_5_at"
## [12603] "AFFX-HUMTFRR/M11507_M_at" "AFFX-LysX-3_at"
## [12605] "AFFX-LysX-5_at" "AFFX-LysX-M_at"
## [12607] "AFFX-M27830_3_at" "AFFX-M27830_5_at"
## [12609] "AFFX-M27830_M_at" "AFFX-MurFAS_at"
## [12611] "AFFX-MurIL10_at" "AFFX-MurIL2_at"
## [12613] "AFFX-MurIL4_at" "AFFX-PheX-3_at"
## [12615] "AFFX-PheX-5_at" "AFFX-PheX-M_at"
## [12617] "AFFX-ThrX-3_at" "AFFX-ThrX-5_at"
## [12619] "AFFX-ThrX-M_at" "AFFX-TrpnX-3_at"
## [12621] "AFFX-TrpnX-5_at" "AFFX-TrpnX-M_at"
## [12623] "AFFX-YELO02c/WBP1_at" "AFFX-YELO18w/_at"
## [12625] "AFFX-YELO21w/URA3_at" "AFFX-YELO24w/RIP1_at"
```

```
colnames(pm(ab))
```

```
## [1] "1521a99hpp_av06.CEL.gz" "1532a99hpp_av04.CEL.gz"
## [3] "2353a99hpp_av08.CEL.gz" "1521b99hpp_av06.CEL.gz"
## [5] "1532b99hpp_av04.CEL.gz" "2353b99hpp_av08r.CEL.gz"
```

```
annotation(ab)
```

```
## [1] "hgu95a"
```

Agilent two-color Arrays

- Package **limma** for two-color arrays
- Read in sample information - pairing of files to tell you what is red channel, what is green channel. Different scanners have different way of storing.
- **lima** format is described in documentation - **readTargets**
- **read.maimages** source which tells the package what imaging software was used to produce these files
- **MA.RG** stores the log ratio of red and green
- Function to look at images

```
# datadir defined in previous video
library(limma)
```

```
##
```

```
## Attaching package: 'limma'
```

```
## The following object is masked from 'package:BiocGenerics':
```

```
##
##   plotMA
library(rafalib)
datadir <- paste0(wd, "/rawdata-master")      # downloaded files
basedir <- paste0(datadir, "/agilent")
setwd(basedir)
targets <- readTargets("TargetBeta7.txt")
RG <- read.maimages(targets$FileName, source="genepix") # red and green 23k genes

## Read 6Hs.195.1.gpr
## Read 6Hs.168.gpr
## Read 6Hs.166.gpr
## Read 6Hs.187.1.gpr
## Read 6Hs.194.gpr
## Read 6Hs.243.1.gpr

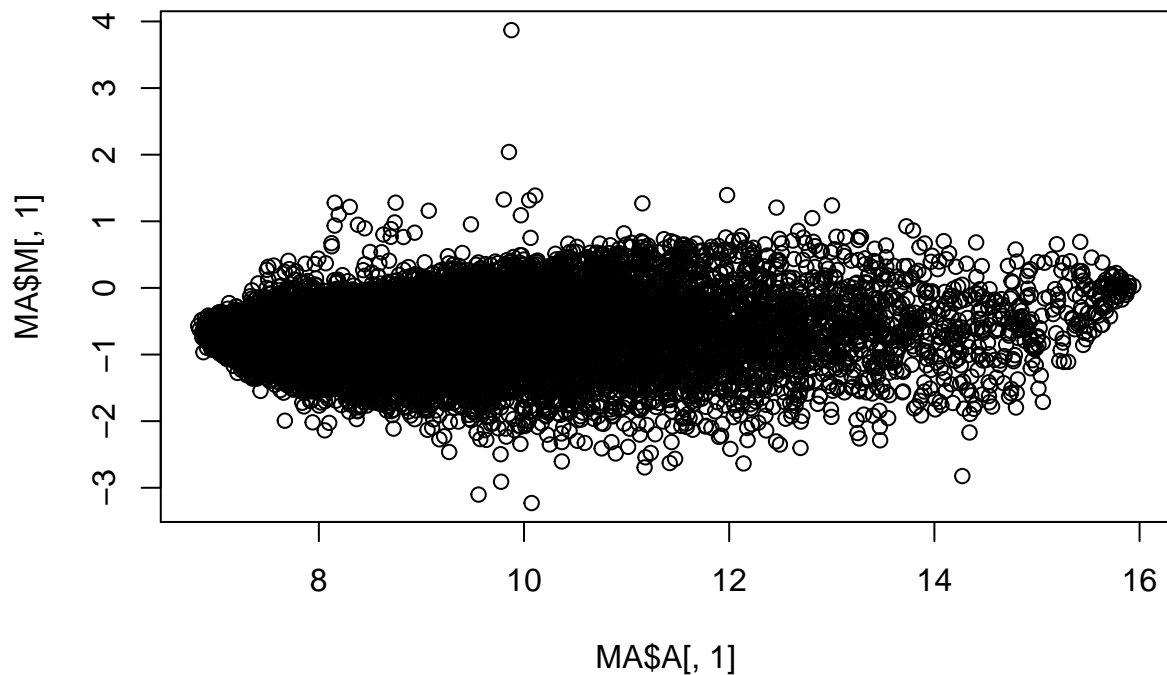
MA <- MA.RG(RG, bc.method="none") # M and A, same information but transformed
dim(RG$R)

## [1] 23184      6
dim(RG$G)

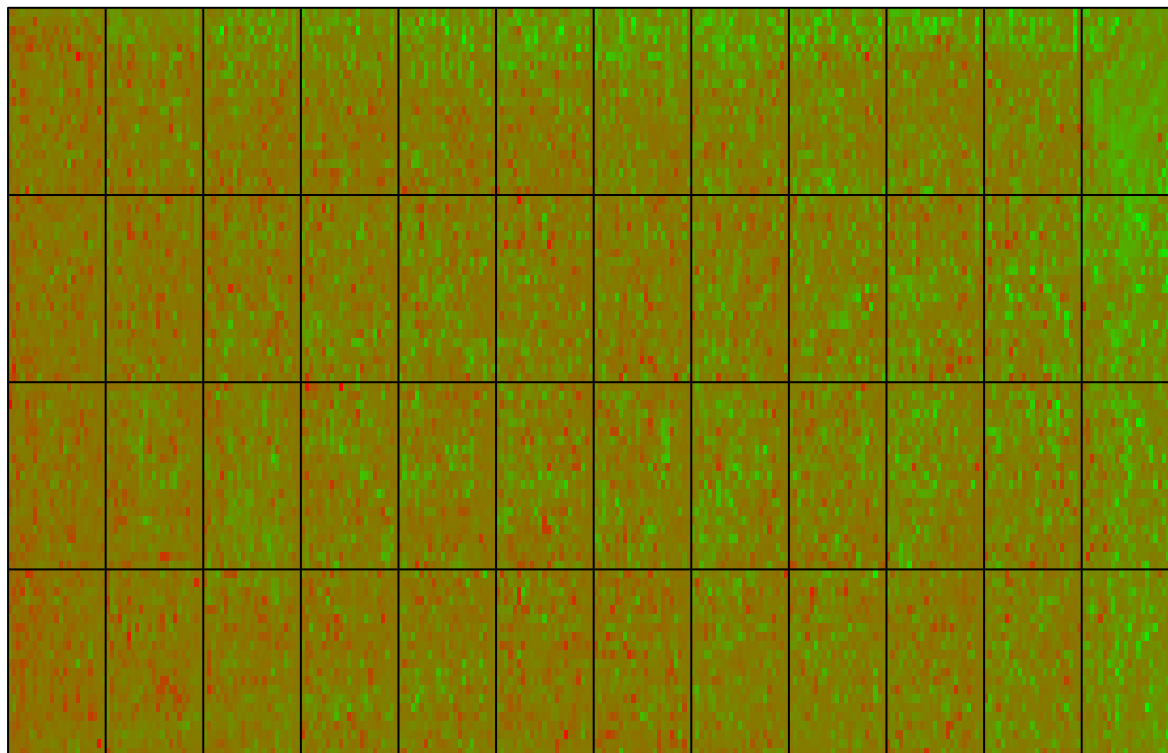
## [1] 23184      6
dim(MA$M)

## [1] 23184      6
dim(MA$A)

## [1] 23184      6
plot(MA$A[,1], MA$M[,1]) # MA plot for first sample
```



```
# microarray image
mypar(1,1)
imageplot(MA$M[,2], RG$printer, zlim=c(-3,3))
```



z-range -3 to 3.4 (saturation -3, 3)

0.5×0.5

```
dev.off()
```

```
## null device
##          1
```

Assessment: Reading microarray data

```
library(hgu95acdf)

wd <- getwd()
datadir <- paste0(wd, "/rawdata-master") # downloaded files
basedir <- paste0(datadir, "/celfiles")
setwd(basedir)
library(affy)
tab <- read.delim("sampleinfo.txt", check.names=FALSE, as.is=TRUE)
rownames(tab) <- tab$filenames
```

```
tab
```

```
##                               filenames 37777_at 684_at 1597_at
## 1521a99hpp_av06.CEL.gz 1521a99hpp_av06.CEL.gz 0.00 0.25 0.5
## 1532a99hpp_av04.CEL.gz 1532a99hpp_av04.CEL.gz 0.00 0.25 0.5
## 2353a99hpp_av08.CEL.gz 2353a99hpp_av08.CEL.gz 0.00 0.25 0.5
```



```
## 1521b99hpp_av06.CEL.gz 1521b99hpp_av06.CEL.gz 0.25 0.50 1.0
## 1532b99hpp_av04.CEL.gz 1532b99hpp_av04.CEL.gz 0.25 0.50 1.0
## 2353b99hpp_av08r.CEL.gz 2353b99hpp_av08r.CEL.gz 0.25 0.50 1.0
##
## 38734_at 39058_at 36311_at 36889_at 1024_at 36202_at
## 1521a99hpp_av06.CEL.gz 1 2 4 8 16 32
## 1532a99hpp_av04.CEL.gz 1 2 4 8 16 32
## 2353a99hpp_av08.CEL.gz 1 2 4 8 16 32
## 1521b99hpp_av06.CEL.gz 2 4 8 16 32 64
## 1532b99hpp_av04.CEL.gz 2 4 8 16 32 64
## 2353b99hpp_av08r.CEL.gz 2 4 8 16 32 64
##
## 36085_at 40322_at 407_at 1091_at 1708_at 33818_at
## 1521a99hpp_av06.CEL.gz 64 128 0.00 512 1024 256
## 1532a99hpp_av04.CEL.gz 64 128 0.00 512 1024 256
## 2353a99hpp_av08.CEL.gz 64 128 0.00 512 1024 256
## 1521b99hpp_av06.CEL.gz 128 256 0.25 1024 0 512
## 1532b99hpp_av04.CEL.gz 128 256 0.25 1024 0 512
## 2353b99hpp_av08r.CEL.gz 128 256 0.25 1024 0 512
##
## 546_at
## 1521a99hpp_av06.CEL.gz 32
## 1532a99hpp_av04.CEL.gz 32
## 2353a99hpp_av08.CEL.gz 32
## 1521b99hpp_av06.CEL.gz 64
## 1532b99hpp_av04.CEL.gz 64
## 2353b99hpp_av08r.CEL.gz 64
```

```
level_gene_spike = tab[tab$filenames == "1521a99hpp_av06.CEL.gz", "36311_at"]
paste("level gene 36311_at spiked: ", level_gene_spike)
```

```
## [1] "level gene 36311_at spiked: 4"
```

```
fns <- list.celfiles(basedir)
```

```
ab <- ReadAffy(phenoData=tab)
```

```
pn = probeNames(ab)
```

```
num_features_associated_w_gene=length(pn[pn == "36311_at"])
```

```
paste("number of features associated with gene 36311_at: ", num_features_associated_w_gene)
```

```
## [1] "number of features associated with gene 36311_at: 16"
```

```
ab_sub <- ab[,ab$filenames %in% c("1532a99hpp_av04.CEL.gz", "1532b99hpp_av04.CEL.gz")]
```

```
probe_level_intensities = pm(ab_sub)
```

```
probeset_ids = which(pn == "36085_at")
```

```
probe_level_intensities_at_probeset = probe_level_intensities[probeset_ids,]
```

```
sample_metadata = pData(ab_sub)
```

```
par(mfrow=c(1, 2))
```

```
r_1 = log2(probe_level_intensities_at_probeset[, "1532a99hpp_av04.CEL.gz"]) / log2(sample_metadata[ "1532a99hpp_av04.CEL.gz", "1532a99hpp_av04.CEL.gz"])
```

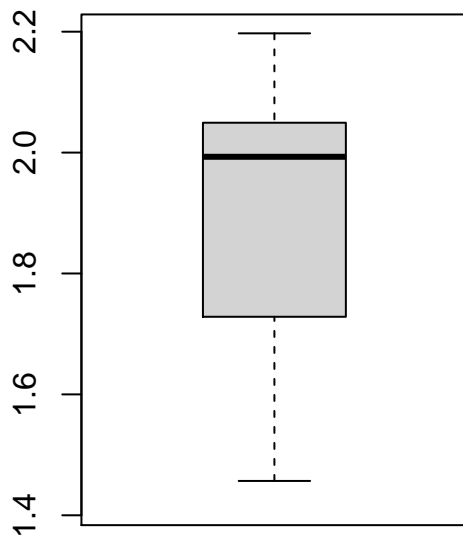
```
r_2 = log2(probe_level_intensities_at_probeset[, "1532b99hpp_av04.CEL.gz"]) / log2(sample_metadata[ "1532b99hpp_av04.CEL.gz", "1532b99hpp_av04.CEL.gz"])
```

```
lmts <- range(r_1, r_2)
```

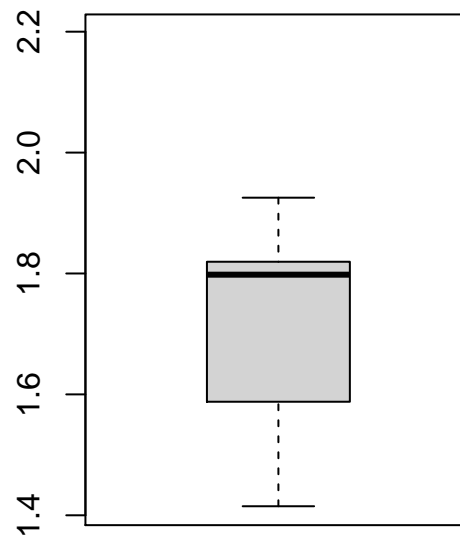
```
boxplot(r_1, main="1532a99hpp_av04.CEL.gz", ylim=lmts)
```

```
boxplot(r_2, main="1532b99hpp_av04.CEL.gz", ylim=lmts)
```

1532a99hpp_av04.CEL.gz



1532b99hpp_av04.CEL.gz



SummarizedExperiment class

- Similar to ExpressionSets but preferred for sequencing-based data
- Accomodates experiments where genomic location is important
- Most commonly used for NGS
- **assay** function to access counts
- **colData** equivalent to **pData** on ExpressionSet
- rows correspond to genes - collections of exons (GRanges list used to count RNA sequencing reads)
- **metadata** how GRanges List was constructed and from what database etc.
- **MIAME** minimal information about microarray experiment

```
library(parathyroidSE)
```

```
## Loading required package: SummarizedExperiment
## Loading required package: MatrixGenerics
## Loading required package: matrixStats
##
## Attaching package: 'matrixStats'
## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians
##
## Attaching package: 'MatrixGenerics'
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
```

```

##      colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##      colWeightedMeans, colWeightedMedians, colWeightedSds,
##      colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##      rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##      rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##      rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##      rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##      rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##      rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##      rowWeightedSds, rowWeightedVars

## The following object is masked from 'package:Biobase':
##
##      rowMedians

## Loading required package: GenomicRanges
## Loading required package: stats4
## Loading required package: S4Vectors
##
## Attaching package: 'S4Vectors'
## The following object is masked from 'package:base':
##
##      expand.grid

## Loading required package: IRanges
## Loading required package: GenomeInfoDb
## Warning: package 'GenomeInfoDb' was built under R version 4.0.4
data(parathyroidGenesSE)
se <- parathyroidGenesSE
se

## class: RangedSummarizedExperiment
## dim: 63193 27
## metadata(1): MIAME
## assays(1): counts
## rownames(63193): ENSG000000000003 ENSG000000000005 ... LRG_98 LRG_99
## rowData names(0):
## colnames: NULL
## colData names(8): run experiment ... study sample
# assay contains results of the assay
dim(se)

## [1] 63193    27
assay(se)[1:3,1:3]

##           [,1] [,2] [,3]
## ENSG000000000003 792 1064 444
## ENSG000000000005   4    1    2
## ENSG000000000419 294  282 164
dim(assay(se)) # rows = features (ranges), columns = samples

## [1] 63193    27

```

```

# colData contains sample information
colData(se)[1:3,1:6]

## DataFrame with 3 rows and 6 columns
##           run experiment patient treatment      time submission
##   <character>   <factor> <factor>  <factor> <factor>   <factor>
## 1   SRR479052   SRX140503      1   Control    24h   SRA051611
## 2   SRR479053   SRX140504      1   Control    48h   SRA051611
## 3   SRR479054   SRX140505      1     DPN      24h   SRA051611

dim(colData(se))

## [1] 27  8

names(colData(se))

## [1] "run"           "experiment" "patient"     "treatment"  "time"
## [6] "submission"  "study"      "sample"

colData(se)$treatment

## [1] Control Control DPN      DPN      OHT      OHT      Control Control DPN
## [10] DPN      DPN      OHT      OHT      OHT      Control Control DPN      DPN
## [19] OHT      OHT      Control DPN      DPN      DPN      OHT      OHT      OHT
## Levels: Control DPN OHT

as.numeric(colData(se)$treatment)

## [1] 1 1 2 2 3 3 1 1 2 2 2 3 3 3 1 1 2 2 3 3 1 2 2 2 3 3 3

# rowRanges contains feature information
rowRanges(se)[1]

## GRangesList object of length 1:
## $ENSG00000000003
## GRanges object with 17 ranges and 2 metadata columns:
##           seqnames      ranges strand | exon_id exon_name
##           <Rle>        <IRanges> <Rle> | <integer> <character>
## [1]           X 99883667-99884983     - |    664095 ENSE00001459322
## [2]           X 99885756-99885863     - |    664096 ENSE00000868868
## [3]           X 99887482-99887565     - |    664097 ENSE00000401072
## [4]           X 99887538-99887565     - |    664098 ENSE00001849132
## [5]           X 99888402-99888536     - |    664099 ENSE00003554016
## ...           ...           ...     ... |    ...      ...
## [13]          X 99890555-99890743     - |    664106 ENSE00003512331
## [14]          X 99891188-99891686     - |    664108 ENSE00001886883
## [15]          X 99891605-99891803     - |    664109 ENSE00001855382
## [16]          X 99891790-99892101     - |    664110 ENSE00001863395
## [17]          X 99894942-99894988     - |    664111 ENSE00001828996
## -----
## seqinfo: 580 sequences (1 circular) from an unspecified genome

class(rowRanges(se))

## [1] "CompressedGRangesList"
## attr(,"package")
## [1] "GenomicRanges"

```

```

length(rowRanges(se))      # number of genes

## [1] 63193

length(rowRanges(se)[[1]])  # number of exons for first gene

## [1] 17

head(rownames(se))

## [1] "ENSG000000000003" "ENSG000000000005" "ENSG000000000419" "ENSG000000000457"
## [5] "ENSG000000000460" "ENSG000000000938"

metadata(rowRanges(se))

## $genomeInfo
## $genomeInfo$`Db type`
## [1] "TranscriptDb"
##
## $genomeInfo$`Supporting package`
## [1] "GenomicFeatures"
##
## $genomeInfo$`Data source`
## [1] "BioMart"
##
## $genomeInfo$Organism
## [1] "Homo sapiens"
##
## $genomeInfo$`Resource URL`
## [1] "www.biomart.org:80"
##
## $genomeInfo$`BioMart database`
## [1] "ensembl"
##
## $genomeInfo$`BioMart database version`
## [1] "ENSEMBL GENES 72 (SANGER UK)"
##
## $genomeInfo$`BioMart dataset`
## [1] "hsapiens_gene_ensembl"
##
## $genomeInfo$`BioMart dataset description`
## [1] "Homo sapiens genes (GRCh37.p11)"
##
## $genomeInfo$`BioMart dataset version`
## [1] "GRCh37.p11"
##
## $genomeInfo$`Full dataset`
## [1] "yes"
##
## $genomeInfo$`miRBase build ID`
## [1] NA
##
## $genomeInfo$transcript_nrow
## [1] "213140"
##
## $genomeInfo$exon_nrow

```

```
## [1] "737783"
##
## $genomeInfo$cds_nrow
## [1] "531154"
##
## $genomeInfo$`Db created by`
## [1] "GenomicFeatures package from Bioconductor"
##
## $genomeInfo$`Creation time`
## [1] "2013-07-30 17:30:25 +0200 (Tue, 30 Jul 2013)"
##
## $genomeInfo$`GenomicFeatures version at creation time`
## [1] "1.13.21"
##
## $genomeInfo$`RSQLite version at creation time`
## [1] "0.11.4"
##
## $genomeInfo$DBSCHEMAVERSION
## [1] "1.0"

# additional metadata, including sample information
metadata(se)$MIAME

## Experiment data
##   Experimenter name: Felix Haglund
##   Laboratory: Science for Life Laboratory Stockholm
##   Contact information: Mikael Huss
##   Title: DPN and Tamoxifen treatments of parathyroid adenoma cells
##   URL: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE37211
##   PMIDs: 23024189
##
##   Abstract: A 251 word abstract is available. Use 'abstract' method.

#abstract(metadata(se)$MIAME)
```

Assessment: SummarizedExperiment

```
library(airway)
data(airway)
metadata(airway)
```

```
## [[1]]
## Experiment data
##   Experimenter name: Himes BE
##   Laboratory: NA
##   Contact information:
##   Title: RNA-Seq transcriptome profiling identifies CRISPLD2 as a glucocorticoid responsive gene tha
##   URL: http://www.ncbi.nlm.nih.gov/pubmed/24926665
##   PMIDs: 24926665
##
##   Abstract: A 226 word abstract is available. Use 'abstract' method.

dim(airway)
```

```
## [1] 64102      8
```

```

sample_metadata <- colData(airway)
sample_cell_line = sample_metadata["SRR1039509","cell"]
paste("Cell line associated with sample: ", sample_cell_line)

## [1] "Cell line associated with sample:  N61311"

index_min = which.min(sample_metadata$avgLength)
sample_name <- rownames(sample_metadata[index_min,])
paste("Sample with shortest average read length: ", sample_name)

## [1] "Sample with shortest average read length:  SRR1039513"

num_genes=length(rowRanges(airway))
paste("number of genes in dataset: ", num_genes)

## [1] "number of genes in dataset:  64102"

g100_md <- rowRanges(airway)[[100]]
num_exons_g100 <- length(g100_md)
paste("number of exons at 100th gene: ", num_exons_g100)

## [1] "number of exons at 100th gene:  55"

total_bp_long_including_introns <- sum(width(range(g100_md)))
paste("number of bases long of 100th gene (including introns): ", total_bp_long_including_introns)

## [1] "number of bases long of 100th gene (including introns):  78739"

tssgr <- resize(range(rowRanges(airway)[[100]]), 1)
tss <- start(tssgr)
paste("transcription start site of 100th gene: ", tss)

## [1] "transcription start site of 100th gene:  87109751"

expr <- assay(airway)["ENSG00000103196",]
mean_expr <- mean(expr)
paste("mean expression of ENSG00000103196 across all samples: ", mean_expr)

## [1] "mean expression of ENSG00000103196 across all samples:  2865.625"

samples_treated_w_dex <- rownames(sample_metadata[which(sample_metadata$dex == "trt"),])
expr_treated <- subset(expr, names(expr) %in% samples_treated_w_dex)
mean_expr_treated <- mean(expr_treated)
paste("mean expression of ENSG00000103196 across all samples treated with dex: ", mean_expr_treated)

## [1] "mean expression of ENSG00000103196 across all samples treated with dex:  4842.75"

expr_untreated <- subset(expr, !names(expr) %in% samples_treated_w_dex)
mean_expr_untreated <- mean(expr_untreated)
paste("mean expression of ENSG00000103196 across all samples not treated with dex: ", mean_expr_untreated)

## [1] "mean expression of ENSG00000103196 across all samples not treated with dex:  888.5"

log_ratio_trt_untrt <- log2(mean_expr_treated / mean_expr_untreated)
paste("log ratio of mean expression between treated and untreated: ", log_ratio_trt_untrt)

## [1] "log ratio of mean expression between treated and untreated:  2.44638284643983"

```

Importing NGS data in R

- Two main Bioconductor packages for working with NGS data:
 - **Rsamtools** - low-level functions for reading and parsing raw NGS stored in standard formats. Interface to samtools (implemented in C)
 - **GenomicAlignments** - high-level functions and classes for reading and organizing NGS data as bioconductor objects
- BAM files - compressed format of SAM. Contains information about alignment of NGS reads to reference genome. Produced by alignment software which takes: fastq files, genomic index created from reference genome.
- Alignment software is application specific - i.e. RNA-seq programs different from genomic DNA sequencing

How to import NGS data using Rsamtools

Example from pasillaBamSubset package

```
library(pasillaBamSubset)
library(Rsamtools)

## Loading required package: Biostrings
## Loading required package: XVector
##
## Attaching package: 'Biostrings'
## The following object is masked from 'package:base':
##
##      strsplit

filename <- untreated1_chr4()
# create bamfile object which allows other functions to know how to process
bf <- BamFile(filename) # information about chromosomes which are declared in header of the BAM file
seqinfo(bf)

## Seqinfo object with 8 sequences from an unspecified genome:
##   seqnames seqlengths isCircular genome
##   chr2L      23011544         NA    <NA>
##   chr2R      21146708         NA    <NA>
##   chr3L      24543557         NA    <NA>
##   chr3R      27905053         NA    <NA>
##   chr4        1351857         NA    <NA>
##   chrM         19517         NA    <NA>
##   chrX      22422827         NA    <NA>
##   chrYHet     347038         NA    <NA>

s1 <- seqlengths(bf)

quickBamFlagSummary(bf) # summary of the kind of alignments in the file

##
##               group |    nb of |    nb of | mean / max
##               of   | records | unique  | records per
##               records | in group | QNAMEs  | unique QNAME
## All records..... A |   204355 | 190770  | 1.07 / 10
```



```
## o template has single segment.... S | 204355 | 190770 | 1.07 / 10
## o template has multiple segments. M | 0 | 0 | NA / NA
## - first segment..... F | 0 | 0 | NA / NA
## - last segment..... L | 0 | 0 | NA / NA
## - other segment..... O | 0 | 0 | NA / NA
##
## Note that (S, M) is a partitioning of A, and (F, L, O) is a partitioning of M.
## Indentation reflects this.
##
## Details for group S:
## o record is mapped..... S1 | 204355 | 190770 | 1.07 / 10
## - primary alignment..... S2 | 204355 | 190770 | 1.07 / 10
## - secondary alignment..... S3 | 0 | 0 | NA / NA
## o record is unmapped..... S4 | 0 | 0 | NA / NA
```

Specifying: what and which

- Number of functions in Rsamtools take argument param which expects **ScanBamParam** specification
- Two important options:
 - what - what kind of information to extract
 - which - which ranges of alignments to extract?
- BAM files often paired with index file to quickly pull out info about reads from a particular genomic range

```
# count the number of records (reads) on chromosome 4:
gr <- GRanges("chr4", IRanges(1, s1["chr4"]))
countBam(bf, param = ScanBamParam(which=gr))
```

```
## space start end width file records nucleotides
## 1 chr4 1 1351857 1351857 untreated1_chr4.bam 204355 15326625
```

```
reads <- scanBam(BamFile(filename, yieldSize = 5)) # limit number of reads which will be extracted to .
```

Examining the output of scanBam

- **reads** object is a list of lists - outer list indexes over the ranges in the which command. Inner list contains info from the bam file - since we didn't specify what we get everything

```
class(reads)
```

```
## [1] "list"
```

```
names(reads[[1]])
```

```
## [1] "qname" "flag" "rname" "strand" "pos" "qwidth" "mapq" "cigar"
## [9] "mrnm" "mpos" "isize" "seq" "qual"
```

```
reads[[1]]$pos # aligned start position
```

```
## [1] 892 919 924 936 949
```

```
reads[[1]]$rname # chromosome
```

```
## [1] chr4 chr4 chr4 chr4 chr4
```

```
## Levels: chr2L chr2R chr3L chr3R chr4 chrM chrX chrYHet
```

```

reads[[1]]$strand # the strand

## [1] - - + + +
## Levels: + - *

reads[[1]]$qwidth # the width of the read

## [1] 75 75 75 75 75

reads[[1]]$seq # the sequence of the read

## DNAStringSet object of length 5:
##      width seq
## [1]    75 CTGTGGTGACCAACACCACAGAATGGTTCGGGCC...GGGTTCCTGCCCTTTCCTGGCTAGGTTGTCC
## [2]    75 TCGGGCCCAATTAGAGGGTTCCTGCCCTTTC...TTGTCCGCTAGCTCATTTCCTGGGCTGTTGTTG
## [3]    75 CCCAATTAGAGGATTCTCTGCCCTTTCCTGGCT...CGGTAGCTCATTTCCTGGGATGTTGTTGTGTCC
## [4]    75 GTTCTCTGCCCTTTCCTGGCTAGGTTGTCCGCT...TCCCGAGATGTTGTTGTGTCCCGGACCCACCT
## [5]    75 TTCCTGGCTAGGTTGTCCGCTAGCTCATTTCCT...TTGTGTCCCGGACACACCTTATTGTGAGTTTG

# Example specifying what and which to specify what parameters to extract over which ranges

gr <- GRanges("chr4", IRanges(500000, 700000))
reads <- scanBam(bf, params = ScanBamParam(what=c("pos", "strand"), which=gr))

```

GenomicAlignments package

- Containers for storing and manipulating short genomic alignments. Counting, computing coverage, junction detection, working with nucleotide content of the alignments
- Two most important functions in **GenomicAlignments** are:
 - **readGAlignments()** - read data from BAM files
 - **summarizeOverlaps()** - simplifies counting reads in genomic ranges across one or more files

```

library(GenomicAlignments)
ga <- readGAlignments(bf)
length(ga)

## [1] 204355

granges(ga[1]) # extract GRanges object within GAlignments object

## GRanges object with 1 range and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle> <IRanges> <Rle>
## [1]    chr4    892-966      -
## -----
##      seqinfo: 8 sequences from an unspecified genome

# GenomicRanges functions that work on GAlignments

gr <- GRanges("chr4", IRanges(700000, 800000))
(fo <- findOverlaps(ga, gr))

## Hits object with 6465 hits and 0 metadata columns:
##      queryHits subjectHits
##      <integer> <integer>
## [1]    127157           1

```

```
##      [2]      127238          1
##      [3]      127240          1
##      [4]      127242          1
##      [5]      127243          1
##      ...      ...      ...
## [6461]      144660          1
## [6462]      144661          1
## [6463]      144662          1
## [6464]      144663          1
## [6465]      144664          1
## -----
## queryLength: 204355 / subjectLength: 1
```

```
countOverlaps(gr, ga)
```

```
## [1] 6465
```

```
table(ga %over% gr)
```

```
##
## FALSE TRUE
## 197890 6465
```

Assessment: Importing NGS data

- pasillaBamSubset package - BAM file containing a subset of reads from single-end RNA-seq experiment in fruit fly, aligning to chromosome 4

```
library(pasillaBamSubset)
library(Rsamtools)
filename <- untreated1_chr4()
bf <- BamFile(filename)
gr <- GRanges("chr4", IRanges(440000, 470000))
num_read_aligns <- countBam(bf, param = ScanBamParam(which=gr))["records"]
paste("number of read aligns between chromosomes 440k - 470k: ", num_read_aligns)
```

```
## [1] "number of read aligns between chromosomes 440k - 470k: 1665"
```

```
rs_dna_string_set <- scanBam(bf, params = ScanBamParam(what="seq", which=gr))
seq_data <- rs_dna_string_set[[1]]$seq
gc_content <- (vcountPattern("C", seq_data) + vcountPattern("G", seq_data)) / width(seq_data)
mean_gc_content <- mean(gc_content)
paste("average proportion of reads which align to chromosome 4 between positions 440k and 470k: ", mean_gc_content)
```

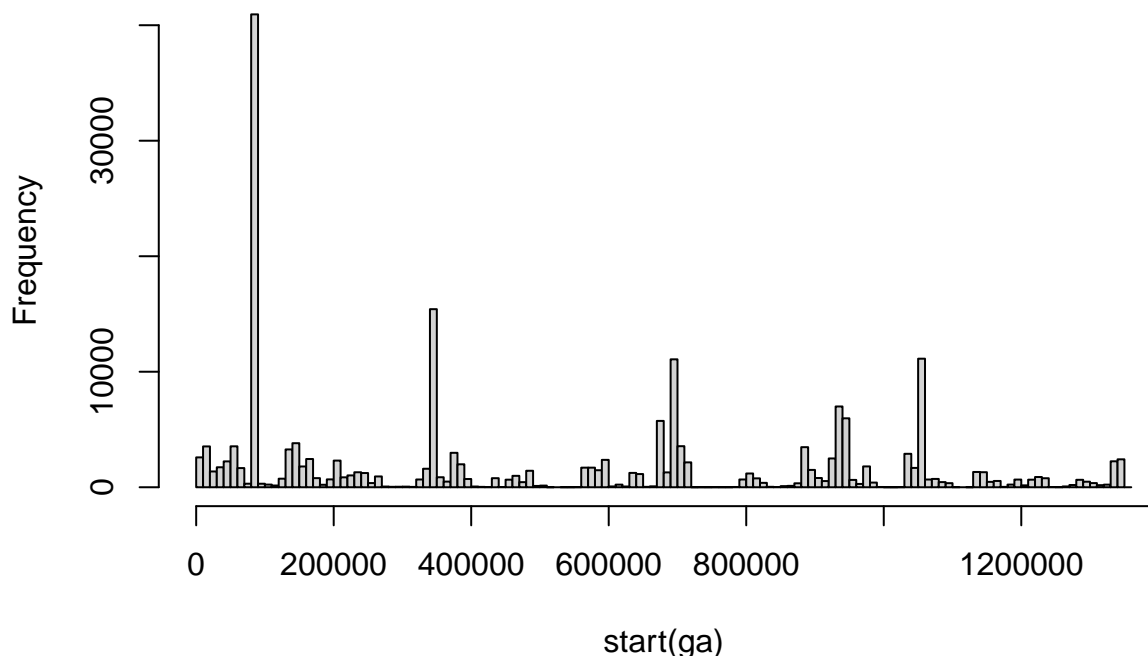
```
## [1] "average proportion of reads which align to chromosome 4 between positions 440k and 470k: 0.4280098"
```

```
mean(letterFrequency(seq_data, "GC", as.prob=TRUE))
```

```
## [1] 0.4280098
```

```
library(GenomicAlignments)
ga <- readGAlignments(BamFile(filename))
hist(start(ga), breaks=100) # genes are spread out on the chromosome
```

Histogram of start(ga)



```
library(TxDb.Dmelanogaster.UCSC.dm3.ensGene)

## Loading required package: GenomicFeatures
## Warning: package 'GenomicFeatures' was built under R version 4.0.4
## Loading required package: AnnotationDbi
g <- genes(TxDb.Dmelanogaster.UCSC.dm3.ensGene)      # GRanges corresponding to genes

g2 <- g[g %over% GRanges("chr4", IRanges(200000, 300000))]

num_overlaps <- sum(countOverlaps(ga, g2["FBgn0039890"]))
paste("negative strand reads overlapping the FBgn0039890 gene: ", num_overlaps)

## [1] "negative strand reads overlapping the FBgn0039890 gene: 1085"
```

Creating a count table from a BAM file

- How to count sequencing reads which fall into genomic features
- End up with summarized experiment object - count matrix with rows corresponding to genomic ranges and columns corresponding to different samples
- Mapped to DM3 genome
- If can't find transcript database you are interested in you can use the makeTranscriptDbFromGFF file function
- Files contain transcripts and exons which make up the gene

```
library(pasillaBamSubset)
library(TxDb.Dmelanogaster.UCSC.dm3.ensGene) # transcript database
txdb <- TxDb.Dmelanogaster.UCSC.dm3.ensGene
```

```
gr1 <- exonsBy(txdb, by="gene") # for each gene, pull out the exons
gr1[100] # GRangesList of exons for 100th gene
```

```
## GRangesList object of length 1:
## $FBgn0000286
## GRanges object with 8 ranges and 2 metadata columns:
##      seqnames      ranges strand | exon_id exon_name
##      <Rle>        <IRanges> <Rle> | <integer> <character>
## [1] chr2L 4876890-4879196 - | 8515 <NA>
## [2] chr2L 4877289-4879196 - | 8516 <NA>
## [3] chr2L 4880294-4880472 - | 8517 <NA>
## [4] chr2L 4880378-4880472 - | 8518 <NA>
## [5] chr2L 4881215-4882492 - | 8519 <NA>
## [6] chr2L 4882865-4883113 - | 8520 <NA>
## [7] chr2L 4882889-4883113 - | 8521 <NA>
## [8] chr2L 4882889-4883341 - | 8522 <NA>
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

```
gr1[[100]] # GRanges with exons of 100th gene
```

```
## GRanges object with 8 ranges and 2 metadata columns:
##      seqnames      ranges strand | exon_id exon_name
##      <Rle>        <IRanges> <Rle> | <integer> <character>
## [1] chr2L 4876890-4879196 - | 8515 <NA>
## [2] chr2L 4877289-4879196 - | 8516 <NA>
## [3] chr2L 4880294-4880472 - | 8517 <NA>
## [4] chr2L 4880378-4880472 - | 8518 <NA>
## [5] chr2L 4881215-4882492 - | 8519 <NA>
## [6] chr2L 4882865-4883113 - | 8520 <NA>
## [7] chr2L 4882889-4883113 - | 8521 <NA>
## [8] chr2L 4882889-4883341 - | 8522 <NA>
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

```
gr1[[100]][1] # first exon of 100th gene
```

```
## GRanges object with 1 range and 2 metadata columns:
##      seqnames      ranges strand | exon_id exon_name
##      <Rle>        <IRanges> <Rle> | <integer> <character>
## [1] chr2L 4876890-4879196 - | 8515 <NA>
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
```

```
# paths to BAM files
```

```
fl1 <- untreated1_chr4()
```

```
fl2 <- untreated3_chr4()
```

```
library(Rsamtools)
```

```
library(GenomicRanges)
```

```
library(GenomicAlignments)
```

```
# specify files with BamFileList - container for two files
```

```
fls <- BamFileList(c(fl1, fl2)) # can specify yieldSize for number of reads
```

```
names(fls) <- c("first", "second")
```

```

# find reads that overlap exons. Different counting modes possible
# inter.feature: if read overlaps multiple features, it should not be counted which is the default
# singleEnd: files have single-end reads instead of paired-end reads
# fragments: if you are counting reads in paired end, you also want to count where only one of two read
so1 <- summarizeOverlaps(features=gr1, reads=fls, ignore.strand=TRUE)
so1

```

```

## class: RangedSummarizedExperiment
## dim: 15682 2
## metadata(0):
## assays(1): counts
## rownames(15682): FBgn0000003 FBgn0000008 ... FBgn0264726 FBgn0264727
## rowData names(0):
## colnames(2): first second
## colData names(0):

```

```

# examine count matrix - assay functions pulls the first matrix from the assay slot
head(assay(so1))

```

```

##           first second
## FBgn0000003      0      0
## FBgn0000008      0      0
## FBgn0000014      0      0
## FBgn0000015      0      0
## FBgn0000017      0      0
## FBgn0000018      0      0

```

```

colSums(assay(so1)) # number of reads which align uniquely to these features

```

```

## first second
## 156469 122872

```

```

# examine rest of the SummarizedExperiment components
rowRanges(so1)

```

```

## GRangesList object of length 15682:
## $FBgn0000003
## GRanges object with 1 range and 2 metadata columns:
##           seqnames      ranges strand | exon_id exon_name
##           <Rle>        <IRanges> <Rle> | <integer> <character>
## [1] chr3R 2648220-2648518      + |      45123      <NA>
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
##
## $FBgn0000008
## GRanges object with 13 ranges and 2 metadata columns:
##           seqnames      ranges strand | exon_id exon_name
##           <Rle>        <IRanges> <Rle> | <integer> <character>
## [1] chr2R 18024494-18024531      + |      20314      <NA>
## [2] chr2R 18024496-18024713      + |      20315      <NA>
## [3] chr2R 18024938-18025756      + |      20316      <NA>
## [4] chr2R 18025505-18025756      + |      20317      <NA>
## [5] chr2R 18039159-18039200      + |      20322      <NA>
## ...      ...      ...      ...      ...      ...
## [9] chr2R 18058283-18059490      + |      20326      <NA>
## [10] chr2R 18059587-18059757      + |      20327      <NA>

```

```
## [11] chr2R 18059821-18059938 + | 20328 <NA>
## [12] chr2R 18060002-18060339 + | 20329 <NA>
## [13] chr2R 18060002-18060346 + | 20330 <NA>
## -----
## seqinfo: 15 sequences (1 circular) from dm3 genome
##
## ...
## <15680 more elements>
```

```
colData(so1)
```

```
## DataFrame with 2 rows and 0 columns
```

```
colData(so1)$sample <- c("one", "two")
colData(so1)
```

```
## DataFrame with 2 rows and 1 column
```

```
##           sample
##      <character>
## first          one
## second         two
```

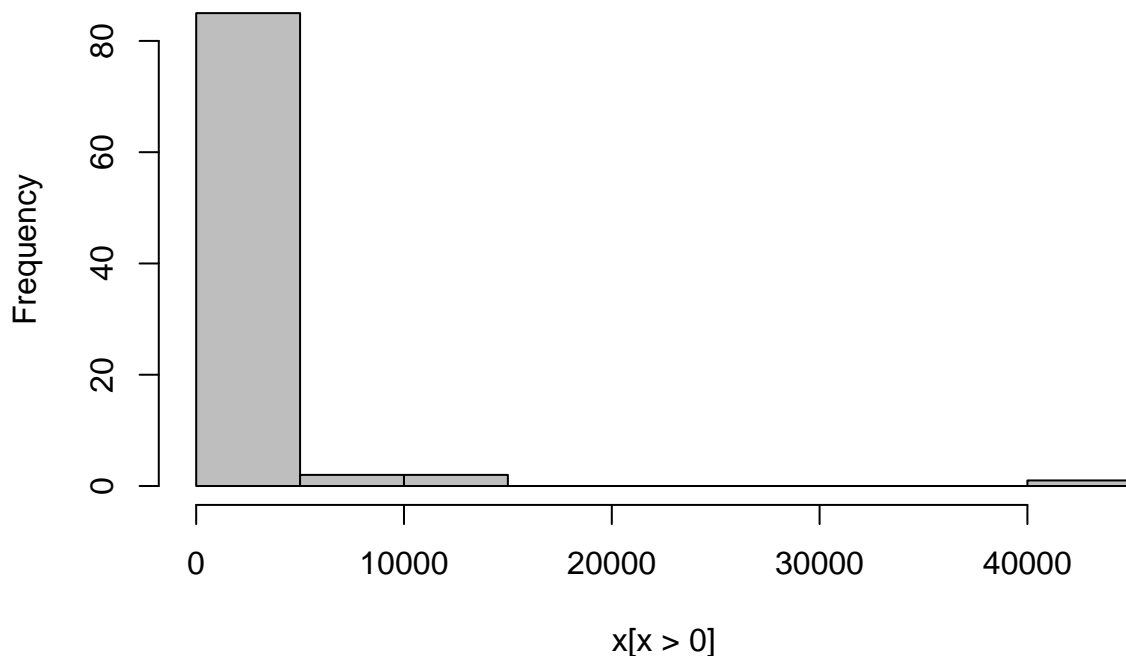
```
metadata(rowRanges(so1))
```

```
## $genomeInfo
## $genomeInfo$`Db type`
## [1] "TxDb"
##
## $genomeInfo$`Supporting package`
## [1] "GenomicFeatures"
##
## $genomeInfo$`Data source`
## [1] "UCSC"
##
## $genomeInfo$Genome
## [1] "dm3"
##
## $genomeInfo$Organism
## [1] "Drosophila melanogaster"
##
## $genomeInfo$`Taxonomy ID`
## [1] "7227"
##
## $genomeInfo$`UCSC Table`
## [1] "ensGene"
##
## $genomeInfo$`Resource URL`
## [1] "http://genome.ucsc.edu/"
##
## $genomeInfo$`Type of Gene ID`
## [1] "Ensembl gene ID"
##
## $genomeInfo$`Full dataset`
## [1] "yes"
##
## $genomeInfo$`miRBase build ID`
```

```
## [1] NA
##
## $genomeInfo$transcript_nrow
## [1] "29173"
##
## $genomeInfo$exon_nrow
## [1] "76920"
##
## $genomeInfo$cds_nrow
## [1] "62135"
##
## $genomeInfo$`Db created by`
## [1] "GenomicFeatures package from Bioconductor"
##
## $genomeInfo$`Creation time`
## [1] "2015-10-07 18:15:53 +0000 (Wed, 07 Oct 2015)"
##
## $genomeInfo$`GenomicFeatures version at creation time`
## [1] "1.21.30"
##
## $genomeInfo$`RSQLite version at creation time`
## [1] "1.0.0"
##
## $genomeInfo$DBSCHEMAVERSION
## [1] "1.1"
```

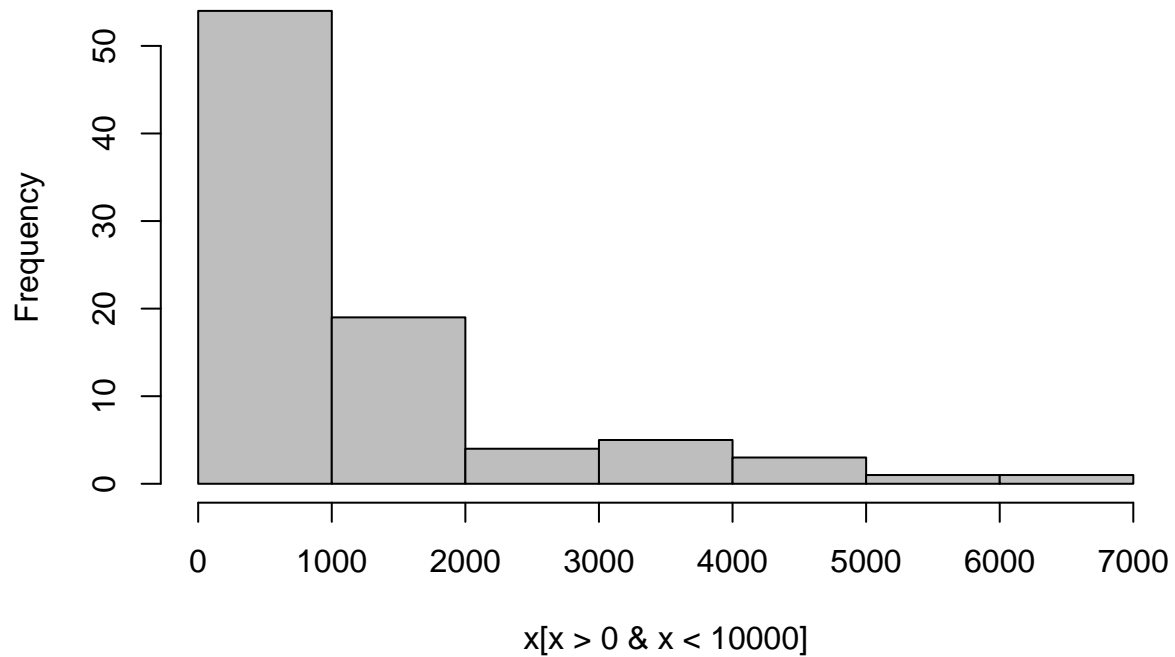
```
# exploratory data analysis of counts
x <- assay(so1)[,1]
hist(x[x > 0], col="grey")
```

Histogram of $x[x > 0]$

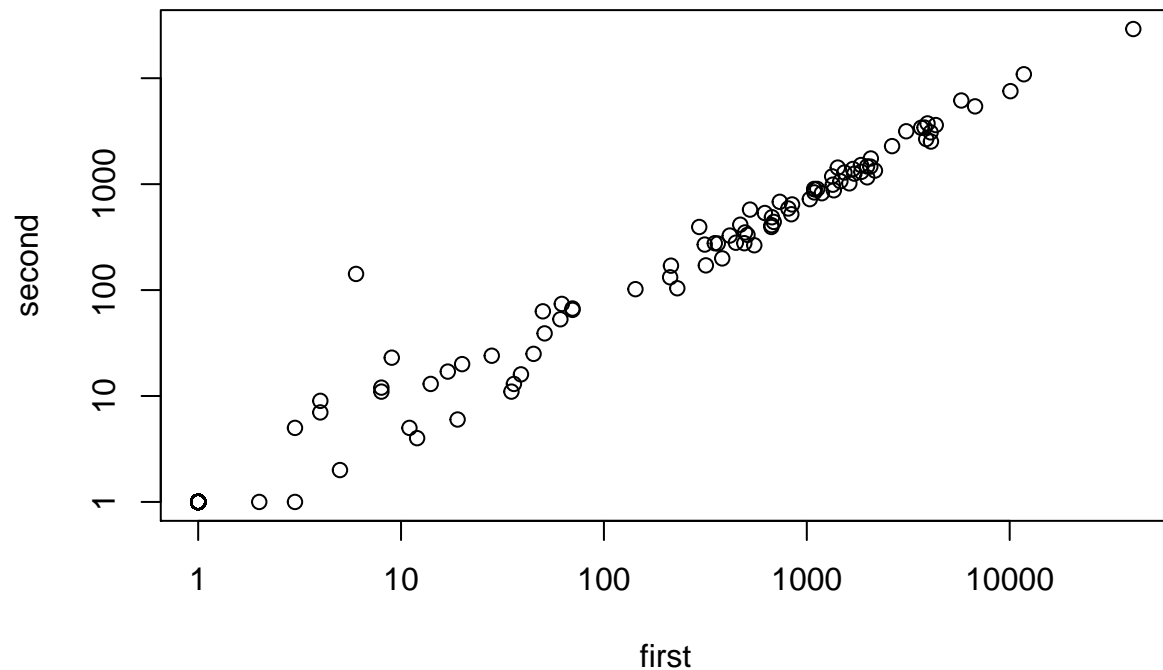



```
hist(x[x > 0 & x < 10000], col="grey")
```

Histogram of $x[x > 0 \ \& \ x < 10000]$



```
plot(assay(so1) + 1, log="xy") # highly correlated samples
```



```
# count second file as paired-end reads
```

```
fls <- BamFileList(fl2)
so2 <- summarizeOverlaps(features=gr1,
```

```

reads=fls,
ignore.strand=TRUE,
singleEnd=FALSE,
fragments=TRUE
)

colSums(assay(so2))

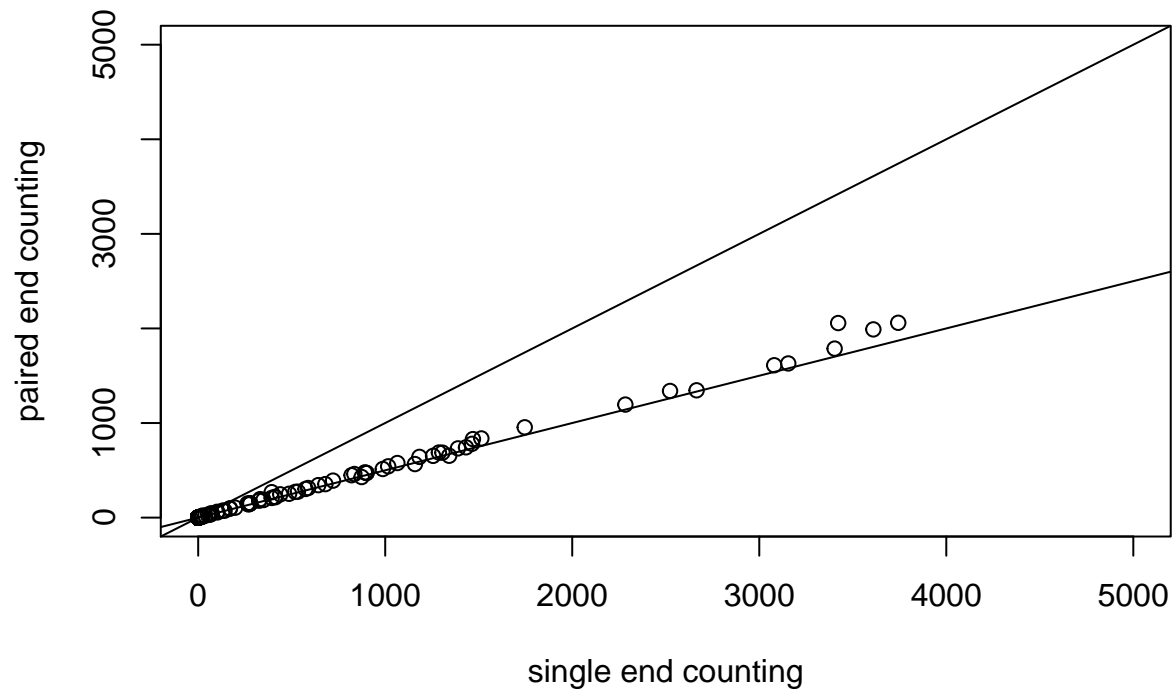
## untreated3_chr4.bam
##           65591

colSums(assay(so1))

## first second
## 156469 122872

plot(assay(so1)[,2], assay(so2)[,1], xlim=c(0,5000), ylim=c(0,5000),
      xlab="single end counting", ylab="paired end counting")
abline(0,1)
abline(0,0.5)

```



- rowData - information about the genes
- colData: information about the samples
- metadata on the features (rowData) - how the rowData was generated