

iCheck: A package checking data quality of Illumina expression data

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1 Overview of iCheck

The iCheck package provides QC pipeline and data analysis tools for high-dimensional Illumina mRNA expression data. It provides several visualization tools to help identify gene probes with outlying expression levels, arrays with low quality, batches caused technical factors, batches caused by biological factors, and gender mis-match checking, etc.

We first generate a simulated data set to illustrate the usage of iCheck functions.

```
> library(iCheck)
> if (!interactive())
+ {
+   options(rgl.useNULL = TRUE)
+ }
> # generate sample probe data
> set.seed(1234567)
> es.sim = genSimData.BayesNormal(nCpGs = 110,
+   nCases = 20, nControls = 20,
+   mu.n = -2, mu.c = 2,
+   d0 = 20, s02 = 0.64, s02.c = 1.5, testPara = "var",
+   outlierFlag = FALSE,
+   eps = 1.0e-3, applicer = lapply)
> print(es.sim)
```

```
ExpressionSet (storageMode: lockedEnvironment)
assayData: 110 features, 40 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: subj1 subj2 ... subj40 (40 total)
  varLabels: arrayID memSubj
  varMetadata: labelDescription
featureData
  featureNames: probe1 probe2 ... probe110 (110 total)
  fvarLabels: probe gene chr memGenes
```

```

    fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation:

> # create replicates
> dat=exprs(es.sim)
> dat[,1]=dat[,2]
> dat[,3]=dat[,4]
> exprs(es.sim)=dat
> es.sim$arrayID=as.character(es.sim$arrayID)
> es.sim$arrayID[1]=es.sim$arrayID[2]
> es.sim$arrayID[3]=es.sim$arrayID[4]
> es.sim$arrayID[5:8]="Hela"
> # since simulated data set does not have 'Pass_Fail',
> # 'Tissue_Descr', 'Batch_Run_Date', 'Chip_Barcode',
> # 'Chip_Address', 'Hybridization_Name', 'Subject_ID', 'gender'
> # we generate them now to illustrate the R functions in the package
>
> es.sim$Hybridization_Name = paste(es.sim$arrayID, 1:ncol(es.sim), sep="_")
> # assume the first 4 arrays are genetic control samples
> es.sim$Subject_ID = es.sim$arrayID
> es.sim$Pass_Fail = rep("pass", ncol(es.sim))
> # produce genetic control GC samples
> es.sim$Tissue_Descr=rep("CD4", ncol(es.sim))
> # assume the first 4 arrays are genetic control samples
> es.sim$Tissue_Descr[5:8]="Human HeLa Cell"
> es.sim$Batch_Run_Date = 1:ncol(es.sim)
> es.sim$Chip_Barcode = 1:ncol(es.sim)
> es.sim$Chip_Address = 1:ncol(es.sim)
> es.sim$gender=rep(1, ncol(es.sim))
> set.seed(12345)
> pos=sample(x=1:ncol(es.sim), size=ceiling(ncol(es.sim)/2), replace=FALSE)
> es.sim$gender[pos]=0
> # generate sample probe data
> es.raw = es.sim[-c(1:10),]
> print(es.raw)

ExpressionSet (storageMode: lockedEnvironment)
assayData: 100 features, 40 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: subj1 subj2 ... subj40 (40 total)
  varLabels: arrayID memSubj ... gender (10 total)
  varMetadata: labelDescription
featureData
  featureNames: probe11 probe12 ... probe110 (100 total)
  fvarLabels: probe gene chr memGenes
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation:

```

```

> # generate QC probe data
> es.QC = es.sim[c(1:10),]
> # since simulated data set does not have 'Reporter_Group_Name'
> # we created it now to illustrate the usage of 'plotQCCurves'.
> fDat=fData(es.QC)
> fDat$Reporter_Group_Name=rep("biotin", 10)
> fDat$Reporter_Group_Name[3:4]="cy3_hyb"
> fDat$Reporter_Group_Name[5:6]="housekeeping"
> fDat$Reporter_Group_Name[7:8]="low_stringency_hyb"
> fData(es.QC)=fDat
> print(es.QC)

```

```

ExpressionSet (storageMode: lockedEnvironment)
assayData: 10 features, 40 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: subj1 subj2 ... subj40 (40 total)
  varLabels: arrayID memSubj ... gender (10 total)
  varMetadata: labelDescription
featureData
  featureNames: probe1 probe2 ... probe10 (10 total)
  fvarLabels: probe gene ... Reporter_Group_Name (5 total)
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation:

>

```

2 Exclude failed arrays

The meta data variable `Pass_Fail` indicates if an array is technically failed. We first should exclude these arrays.

We first check the values of the variable `Pass_Fail`:

```

> print(table(es.raw$Pass_Fail, useNA="ifany"))

pass
 40

```

If there exist failed arrays, then we exclude them:

```

> pos<-which(es.raw$Pass_Fail != "pass")
> if(length(pos))
+ {
+   es.raw<-es.raw[, -pos]
+   es.QC<-es.QC[, -pos]
+ }

```

3 Check QC probes

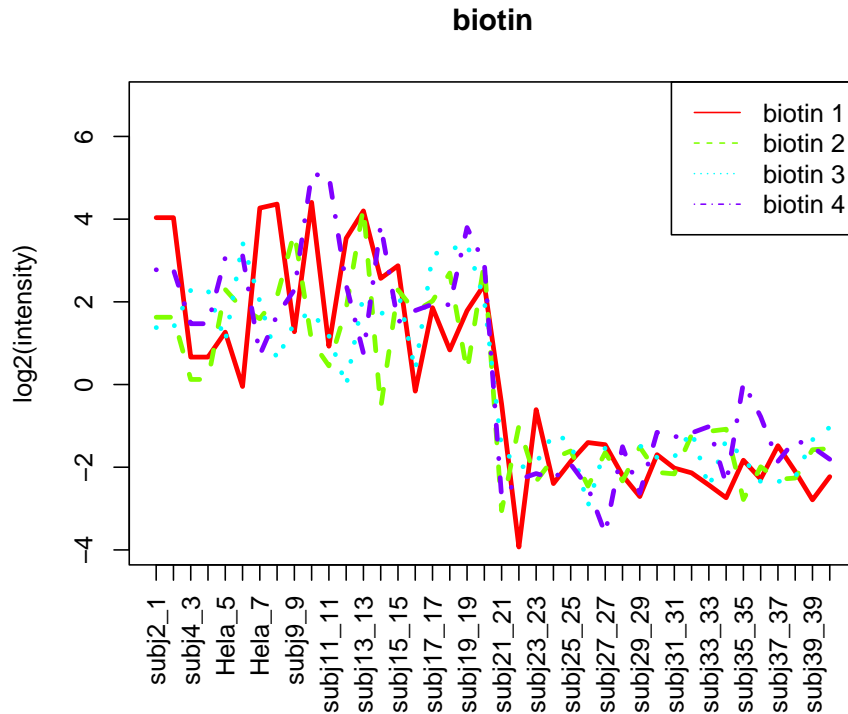
The function `plotQCCurves` shows plot of quantiles across arrays for each type of QC probes. We expect the trajectories of quantiles across arrays are horizontal lines.

To get a better view, the arrays will be sorted based on variables specified in the function argument `varSort`.

```
> plotQCCurves(  
+   esQC=es.QC,  
+   probes = c("biotin"), #"cy3_hyb", "housekeeping"),  
+   #"low_stringency_hyb"),  
+   labelVariable = "subjID",  
+   hybName = "Hybridization_Name",  
+   reporterGroupName = "Reporter_Group_Name",  
+   requireLog2 = FALSE,  
+   projectName = "test",  
+   plotOutPutFlag = FALSE,  
+   cex = 1,  
+   ylim = NULL,  
+   xlab = "",  
+   ylab = "log2(intensity)",  
+   lwd = 3,  
+   mar = c(10, 4, 4, 2) + 0.1,  
+   las = 2,  
+   cex.axis = 1,  
+   sortFlag = TRUE,  
+   varSort = c("Batch_Run_Date", "Chip_Barcode", "Chip_Address"),  
+   timeFormat = c("%m/%d/%Y", NA, NA)  
+ )
```

```
probes>>  
[1] "biotin"
```

```
***** k= 1 *****  
QC probe= biotin
```



4 Check squared correlations among genetic control (GC) arrays

Next, we draw heatmap of the squared correlations among GC arrays. We expect the squared correlations among GC arrays are high (> 0.90).

The function argument `labelVariable` indicates which meta variable will be used to label the arrays in the heatmap.

If we draw heatmap for replicated arrays, we can set the function arguments `sortFlag=TRUE`,

```
varSort=c("Subject_ID", "Hybridization_Name",
          "Batch_Run_Date", "Chip_Barcode", "Chip_Address")

and

timeFormat=c(NA, NA, "%m/%d/%Y", NA, NA)
```

so that arrays from the same subjects will be grouped together in the heatmap.

Note that although the meta variable `Batch_Run_Date` records time, it is vector of string character in R. The function `R2PlotFunc` will automatically

convert it to time variable if we set the value of the argument `timeFormat` corresponding to the variable `Batch_Run_Date` as a time format like `"%m/%d/%Y"`. Details about the time format, please see the R function `strptime`.

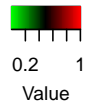
The followings show example R code to draw heatmap of GC arrays.

```
> R2PlotFunc(  
+   es=es.raw,  
+   hybName = "Hybridization_Name",  
+   arrayType = "GC",  
+   GCid = c("128115", "Hela", "Brain"),  
+   probs = seq(0, 1, 0.25),  
+   col = gplots::greenred(75),  
+   labelVariable = "subjID",  
+   outFileName = "test_R2_raw.pdf",  
+   title = "Raw Data R^2 Plot",  
+   requireLog2 = FALSE,  
+   plotOutPutFlag = FALSE,  
+   las = 2,  
+   keysize = 1,  
+   margins = c(10, 10),  
+   sortFlag = TRUE,  
+   varSort=c("Batch_Run_Date", "Chip_Barcode", "Chip_Address"),  
+   timeFormat=c("%m/%d/%Y", NA, NA)  
+ )
```

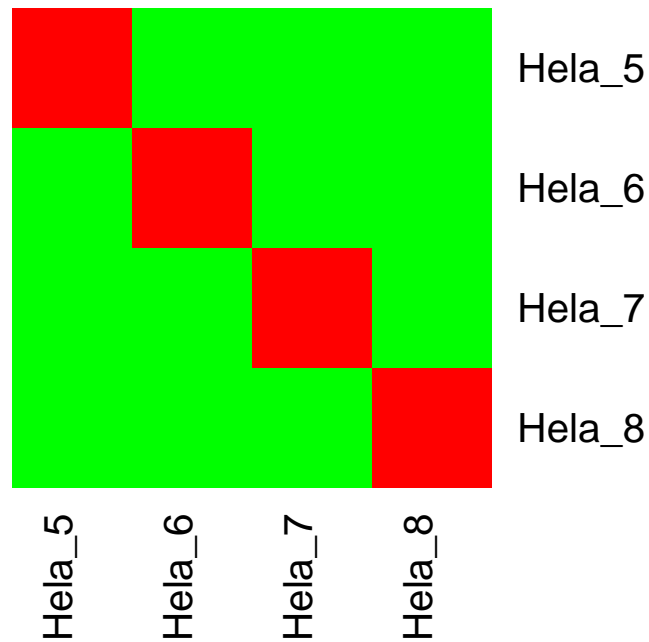
quantile of R^2>>

| | 0% | 25% | 50% | 75% | 100% |
|--|--------------|--------------|--------------|--------------|--------------|
| | 4.807015e-06 | 1.015917e-03 | 1.675295e-03 | 4.155686e-03 | 6.798879e-03 |

Color Key



Raw Data R² Plot



5 Exclude GC arrays

We next exclude GC arrays and will focus on sample arrays to check data quality.

```
> print(table(es.raw$Tissue_Descr, useNA="ifany"))
```

```
      CD4 Human HeLa Cell  
      36             4
```

```
> # for different data sets, the label for GC arrays might  
> # be different.  
> pos.del<-which(es.raw$Tissue_Descr == "Human HeLa Cell")  
> cat("No. of GC arrays=", length(pos.del), "\n")
```

```
No. of GC arrays= 4
```

```
> if(length(pos.del))  
+ {  
+   es.raw<-es.raw[,-pos.del]  
+   es.QC<-es.QC[,-pos.del]
```



```
+ print(dims(es.raw))
+ print(dims(es.QC))
+ }
```

```
      exprs
Features 100
Samples  36
      exprs
Features  10
Samples  36
```

6 Check squared correlations among replicated arrays

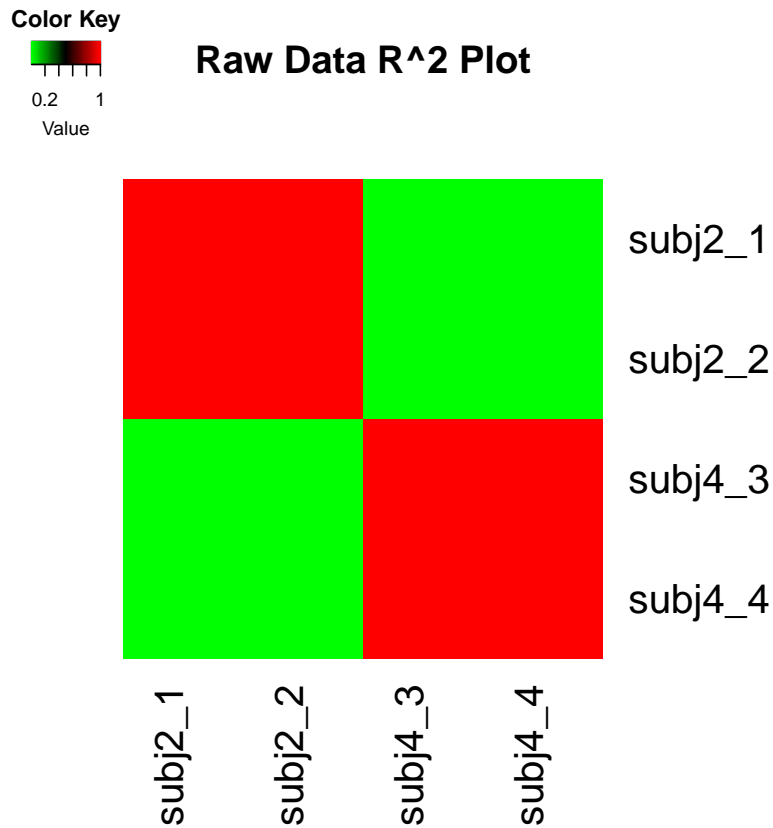
Check squared correlations among replicated arrays (excluding GC arrays). We expect within subject correlations will be high.

```
> R2PlotFunc(
+   es=es.raw,
+   arrayType = c("replicates"),
+   GCid = c("128115", "Hela", "Brain"),
+   probs = seq(0, 1, 0.25),
+   col = gplots::greenred(75),
+   labelVariable = "subjID",
+   outFileName = "test_R2_raw.pdf",
+   title = "Raw Data R^2 Plot",
+   requireLog2 = FALSE,
+   plotOutPutFlag = FALSE,
+   las = 2,
+   keysize = 1,
+   margins = c(10, 10),
+   sortFlag = TRUE,
+   varSort=c("Subject_ID", "Hybridization_Name", "Batch_Run_Date", "Chip_Barcode",
+   timeFormat=c(NA, NA, "%m/%d/%Y", NA, NA)
+ )
```

```
quantile of R^2>>
      0%      25%      50%      75%      100%
0.007151179 0.007151179 0.007151179 0.751787795 1.000000000
```

```
quantile of within-replicate R^2>>
      0% 25% 50% 75% 100%
      1  1  1  1  1
```

```
>
```



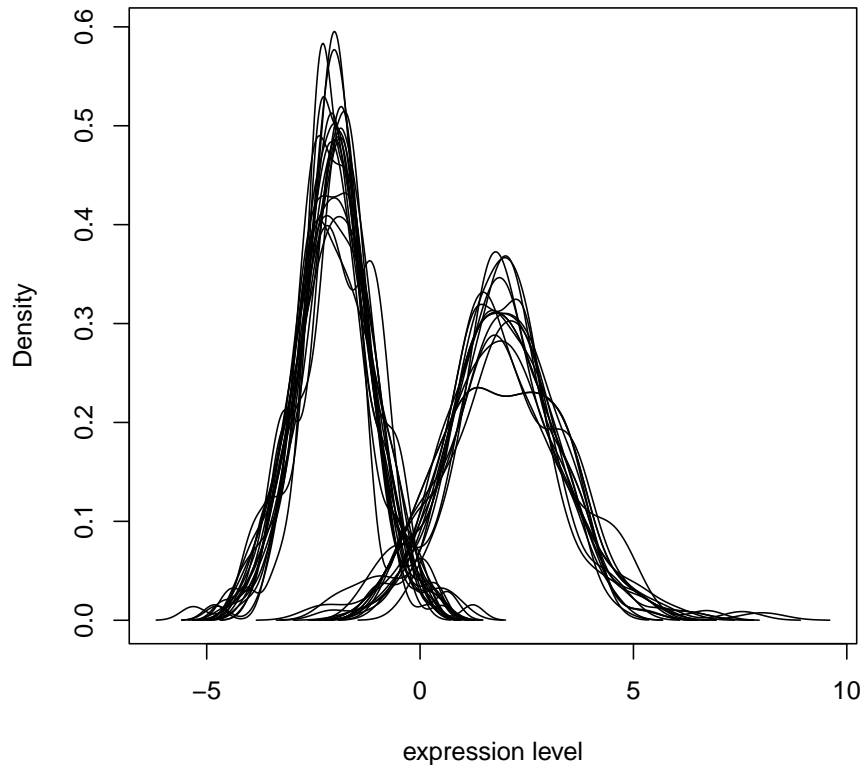
7 Obtain plot of estimated density for each array

We next draw plot of estimated density for each array. We expect the estimated densities of all arrays to be similar. However, for real data, some patterns of the estimated densities might appear indicating the existence of some batch effects.

Note that by default, the function argument `requireLog2 = TRUE`. Since the distributions of simulated data are from normal distribution, we don't need to do log2 transformation here.

```
> densityPlots(
+   es = es.raw,
+   requireLog2 = FALSE,
+   myxlab = "expression level",
+   datExtrFunc = exprs,
+   fileFlag = FALSE,
+   fileFormat = "ps",
+   fileName = "densityPlots_sim.ps")
>
```

density plots



8 Obtain plot of quantiles across arrays

We next draw plot of quantiles across sample arrays. We expect the trajectories of quantiles be horizontal. However, for real data, some patterns of the trajectories might appear indicating the existence of some batch effects.

Some times, the quantile plots can show that some probes have some outlying expression levels. In this case, we can delete those gene probes.

Note that by default, the function argument `requireLog2 = TRUE`. Hence, we need to take `log2` transformation to identify which gene probes containing outlying expression levels.

By default, we will sort the arrays by the ascending order of the median absolute deviation (MAD) to have a better view of the trajectories of quantiles.

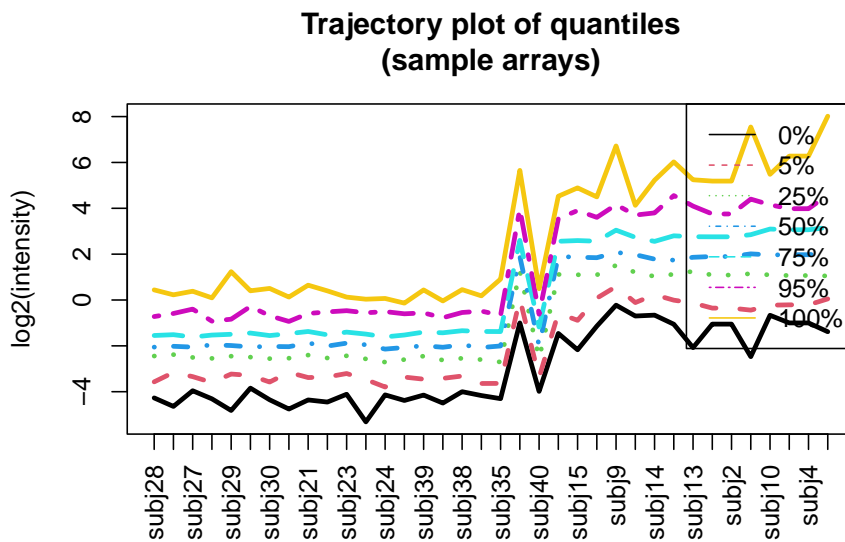
```
> quantilePlot(  
+   dat=exprs(es.raw),  
+   fileName,  
+   probs = c(0, 0.05, 0.25, 0.5, 0.75, 0.95, 1),  
+   plotOutPutFlag = FALSE,  
+   requireLog2 = FALSE,
```

```

+      sortFlag = TRUE,
+      cex = 1,
+      ylim = NULL,
+      xlab = "",
+      ylab = "log2(intensity)",
+      lwd = 3,
+      main = "Trajectory plot of quantiles\n(sample arrays)",
+      mar = c(15, 4, 4, 2) + 0.1,
+      las = 2,
+      cex.axis = 1
+    )

```

***** Arrays were sorted by MAD (median absolute deviation)!



9 Exclude gene probes with outlying expression levels

if quantile plots show some outlying expression levels, we can use the following R code to identify the gene probes with outlying expression levels.

```

> # note we need to take log2 transformation
> # if requireLog2 = TRUE.

```

```

> requireLog2 = FALSE
> if(requireLog2)
+ {
+   minVec<-apply(log2(exprs(es.raw)), 1, min, na.rm=TRUE)
+   # suppose the cutoff is 0.5
+   print(sum(minVec< 0.5))
+   pos.del<-which(minVec<0.5)
+
+   cat("Number of gene probes with outlying expression levels>>",
+       length(pos.del), "\n")
+   if(length(pos.del))
+   {
+     es.raw<-es.raw[-pos.del,]
+   }
+ }
>

```

10 Obtain plot of the ratio (p_{95}/p_{05}) of 95-th percentile to 5-th percentile across arrays

We next draw the plot of the ratio of p_{95} over p_{05} across arrays, where p_{95} (p_{05}) is the 95-th (5-th) percentile of a array. If an array with the ratio p_{95}/p_{05} is less than 6, then we regard this array as a bad array and should delete it before further analysis.

Note that we should set `requireLog2 = FALSE`.

```

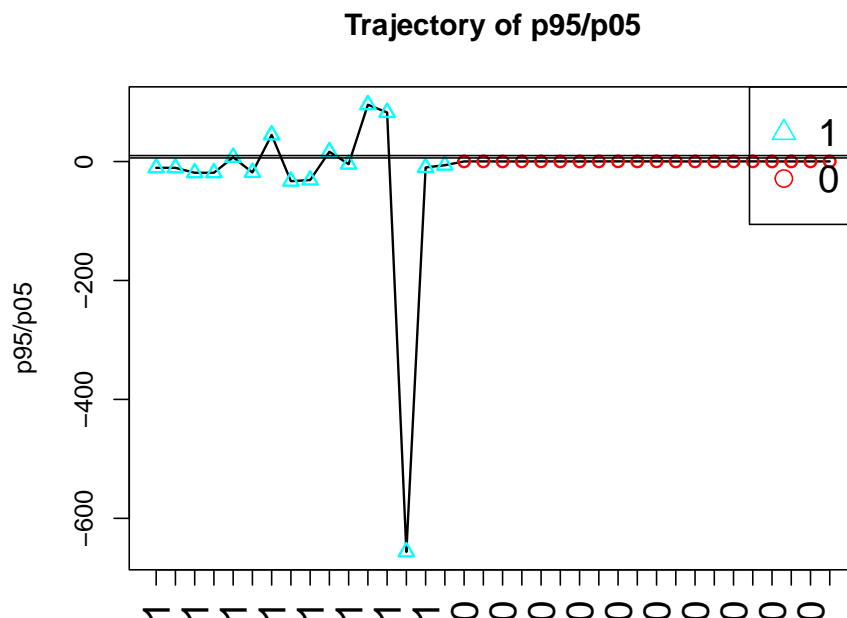
>   plotSamplep95p05(
+     es=es.raw,
+     labelVariable = "memSubj",
+     requireLog2 = FALSE,
+     projectName = "test",
+     plotOutPutFlag = FALSE,
+     cex = 1,
+     ylim = NULL,
+     xlab = "",
+     ylab = "",
+     lwd = 1.5,
+     mar = c(10, 4, 4, 2) + 0.1,
+     las = 2,
+     cex.axis=1.5,
+     title = "Trajectory of p95/p05",
+     cex.legend = 1.5,
+     cex.lab = 1.5,
+     legendPosition = "topright",
+     cut1 = 10,
+     cut2 = 6,
+     sortFlag = TRUE,
+     varSort = c("Batch_Run_Date", "Chip_Barcode", "Chip_Address"),

```

```

+       timeFormat = c("%m/%d/%Y", NA, NA),
+       verbose = FALSE)

```



11 Exclude arrays with $p_{95}/p_{05} \leq 6$

If there exist arrays with $p_{95}/p_{05} < 6$, we then need to exclude these arrays from further data analysis. The followings are example R code:

```

> p95<-quantile(exprs(es.raw), prob=0.95)
> p05<-quantile(exprs(es.raw), prob=0.05)
> r<-p95/p05
> pos.del<-which(r<6)
> print(pos.del)

```

95%

1

```

> if(length(pos.del))
+ {
+   es.raw<-es.raw[,-pos.del]

```

```

+   es.QC<-es.QC[,-pos.del]
+ }
>

```

12 Obtain Plot of principal components

We next draw pca plots to double check batch effects or treatment effects indicated by dendrogram.

The first step is to obtain principal components using the function `getPCAFunc`. For large data set, this function might be very slow.

```

>   pcaObj<-getPCAFunc(es=es.raw,
+                       labelVariable = "subjID",
+                       requireLog2 = FALSE,
+                       corFlag = FALSE
+
+
+   )
>

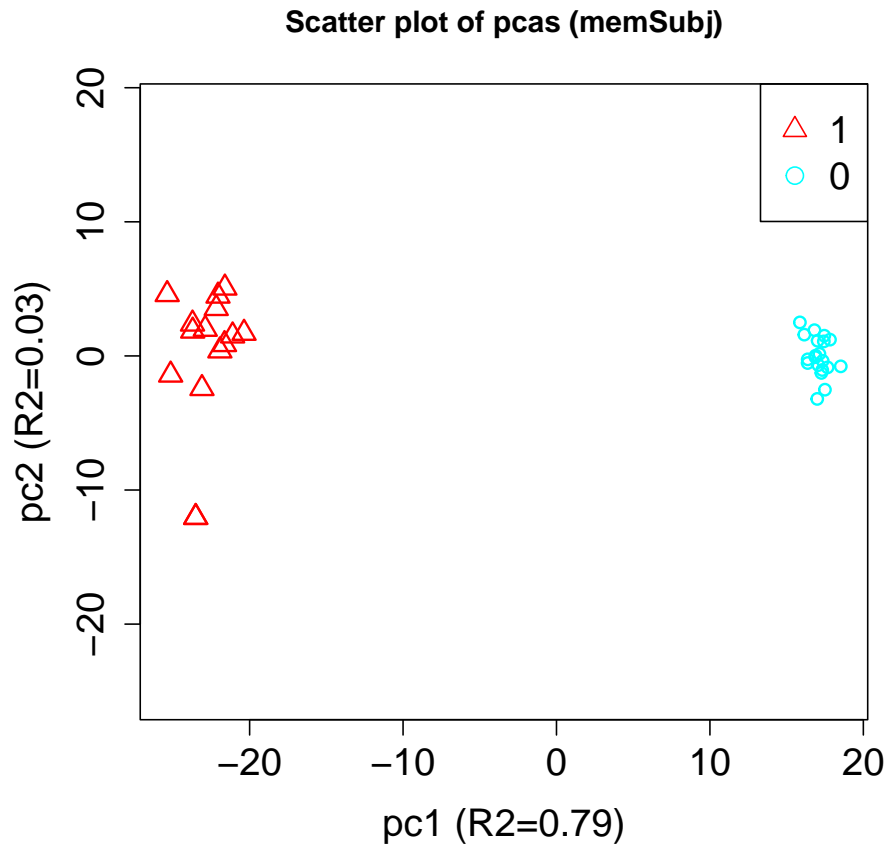
```

We then plot the first 2 or 3 principal components and label the data points by meta variables of interests, such as tissue type, study center, batch id, etc..

```

>   pca2DPlot(pcaObj=pcaObj,
+             plot.dim = c(1,2),
+             labelVariable = "memSubj",
+             outFileNames = "test_pca_raw.pdf",
+             title = "Scatter plot of pcas (memSubj)",
+             plotOutputFlag = FALSE,
+             mar = c(5, 4, 4, 2) + 0.1,
+             lwd = 1.5,
+             equalRange = TRUE,
+             xlab = NULL,
+             ylab = NULL,
+             xlim = NULL,
+             ylim = NULL,
+             cex.legend = 1.5,
+             cex = 1.5,
+             cex.lab = 1.5,
+             cex.axis = 1.5,
+             legendPosition = "topright"
+
+   )
>

```



13 Perform background correction, data transformation and normalization

```
> tt <- es.raw
> es.q<-lumiN(tt, method="quantile")
```

Perform quantile normalization ...

14 Obtain Plot of principal components for pre-processed data

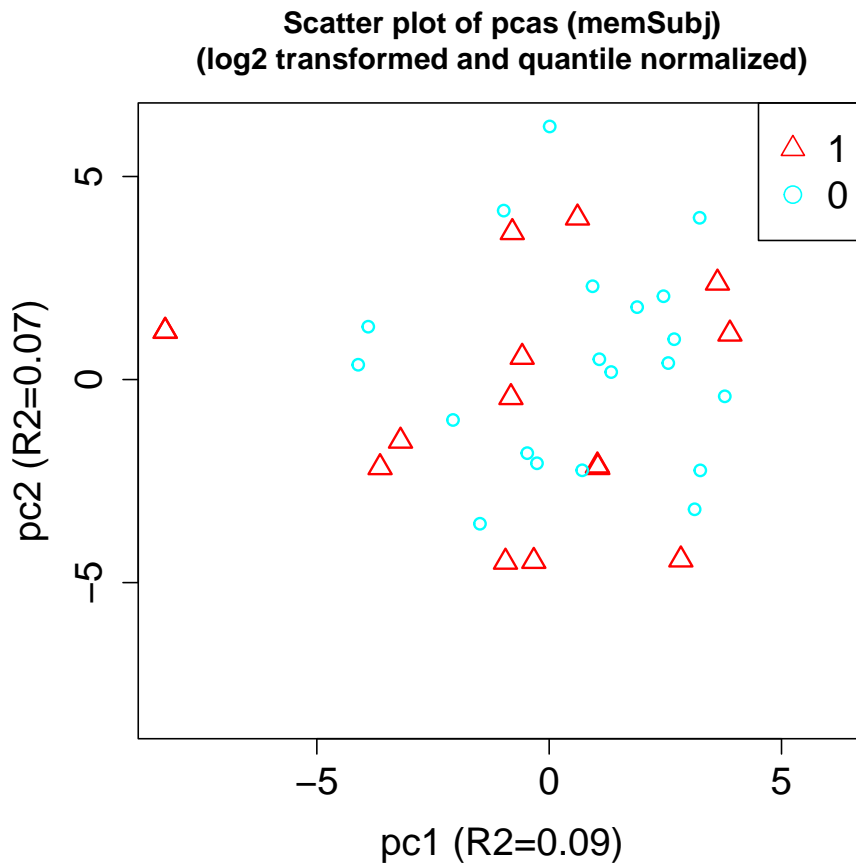
After pre-processing data, we do principal component analysis again.

Note that we should set `requireLog2 = FALSE`.

```
> pcaObj<-getPCAFunc(es=es.q,
+                   labelVariable = "subjID",
+                   requireLog2 = FALSE,
+                   corFlag = FALSE
```



```
+
+
+ )
+
+ > pca2DPlot(pcaObj=pcaObj,
+           plot.dim = c(1,2),
+           labelVariable = "memSubj",
+           outFileNames = "test_pca_raw.pdf",
+           title = "Scatter plot of pcas (memSubj)\n(log2 transformed and quantile n",
+           plotOutputFlag = FALSE,
+           mar = c(5, 4, 4, 2) + 0.1,
+           lwd = 1.5,
+           equalRange = TRUE,
+           xlab = NULL,
+           ylab = NULL,
+           xlim = NULL,
+           ylim = NULL,
+           cex.legend = 1.5,
+           cex = 1.5,
+           cex.lab = 1.5,
+           cex.axis = 1.5,
+           legendPosition = "topright"
+         )
+
+ >
```



15 Incorporate phenotype data

In addition meta data, we usually have phenotype data to describe subjects. We can now add them in.

16 Data analysis

16.1 lmFitWrapper and lmFitPaired

iCheck provide 2 limma wrapper functions `lmFitPaired` (for paired data) and `lmFitWrapper` (for unpaired data).

Note that the function argument `pos.var.interest = 1` requests the results (test statistic and p-value) for the first covariate will be print out.

If `pos.var.interest = 0`, then the results (test statistic and p-value) for the intercept will be print out.

The outcome variable must be gene probes. Can not be phenotype variables.

```
> res.limma=lmFitWrapper(
+   es=es.q,
```

```
+ formula=~as.factor(memSubj),
+ pos.var.interest = 1,
+ pvalAdjMethod="fdr",
+ alpha=0.05,
+ probeID.var="probe",
+ gene.var="gene",
+ chr.var="chr",
+ verbose=TRUE)
```

```
dim(dat)>>
[1] 100 35
```

Running lmFit...

Running eBayes...

Preparing output...

| | probeIDs | geneSymbols | chr | stats | pval | p.adj | pos |
|----|----------|-------------|-----|-----------|-------------|-----------|-----|
| 1 | probe29 | gene29 | 1 | -3.376879 | 0.001401733 | 0.1005857 | 19 |
| 2 | probe16 | gene16 | 1 | 3.254099 | 0.002011714 | 0.1005857 | 6 |
| 3 | probe92 | gene92 | 1 | 2.688895 | 0.009634725 | 0.3211575 | 82 |
| 4 | probe59 | gene59 | 1 | -2.210193 | 0.031558408 | 0.4968483 | 49 |
| 5 | probe32 | gene32 | 1 | 2.181275 | 0.033750422 | 0.4968483 | 22 |
| 6 | probe17 | gene17 | 1 | -2.143605 | 0.036806038 | 0.4968483 | 7 |
| 7 | probe103 | gene103 | 1 | -2.117636 | 0.039051322 | 0.4968483 | 93 |
| 8 | probe35 | gene35 | 1 | -2.109845 | 0.039747866 | 0.4968483 | 25 |
| 9 | probe54 | gene54 | 1 | 1.712229 | 0.092867773 | 0.8375408 | 44 |
| 10 | probe74 | gene74 | 1 | -1.703176 | 0.094560774 | 0.8375408 | 64 |
| 11 | probe40 | gene40 | 1 | 1.638282 | 0.107456092 | 0.8375408 | 30 |
| 12 | probe61 | gene61 | 1 | -1.604613 | 0.114691882 | 0.8375408 | 51 |
| 13 | probe12 | gene12 | 1 | 1.527377 | 0.132783363 | 0.8375408 | 2 |
| 14 | probe56 | gene56 | 1 | -1.521438 | 0.134263881 | 0.8375408 | 46 |
| 15 | probe90 | gene90 | 1 | -1.445861 | 0.154271925 | 0.8375408 | 80 |
| 16 | probe79 | gene79 | 1 | -1.406303 | 0.165637004 | 0.8375408 | 69 |
| 17 | probe89 | gene89 | 1 | -1.367624 | 0.177366059 | 0.8375408 | 79 |
| 18 | probe38 | gene38 | 1 | 1.360555 | 0.179576911 | 0.8375408 | 28 |
| 19 | probe96 | gene96 | 1 | -1.351534 | 0.182428796 | 0.8375408 | 86 |
| 20 | probe23 | gene23 | 1 | 1.347172 | 0.183820114 | 0.8375408 | 13 |

pvalue quantiles for intercept and covariates>>

| | (Intercept) | as.factor(memSubj)1 |
|--------|--------------|---------------------|
| min | 0.0002637651 | 0.001401733 |
| 25% | 0.0356690890 | 0.242102862 |
| median | 0.1922335675 | 0.455084256 |
| 75% | 0.4874162700 | 0.780677827 |
| max | 0.9978539142 | 0.998200667 |

formula>>

```
~as.factor(memSubj)
```

covariate of interest is as.factor(memSubj)

Number of tests= 100

```

Number of arrays= 35
Number of significant tests (raw p-value < 0.05 )= 8
Number of significant tests after p-value adjustments= 0

```

```

*****
No genes are differentially expressed!

```

```
>
```

16.2 glmWrapper

outcome variable can be phenotype variables. The function argument `family` indicates if logistic regression (`family=binomial`) used or general linear regression (`family=gaussian`) used.

```

> res.glm=glmWrapper(
+   es=es.q,
+   formula = xi~as.factor(memSubj),
+   pos.var.interest = 1,
+   family=gaussian,
+   logit=FALSE,
+   pvalAdjMethod="fdr",
+   alpha = 0.05,
+   probeID.var = "probe",
+   gene.var = "gene",
+   chr.var = "chr",
+   applier=lapply,
+   verbose=TRUE
+ )

```

| | probeIDs | geneSymbols | chr | stats | coef | pval | p.adj | pos |
|----|----------|-------------|-----|-----------|------------|-------------|-----------|-----|
| 1 | probe16 | gene16 | 1 | 3.305320 | 1.0593407 | 0.002293003 | 0.1763387 | 6 |
| 2 | probe29 | gene29 | 1 | -3.142649 | -1.3263843 | 0.003526774 | 0.1763387 | 19 |
| 3 | probe92 | gene92 | 1 | 2.508446 | 1.0489929 | 0.017219384 | 0.5739795 | 82 |
| 4 | probe59 | gene59 | 1 | -2.238212 | -0.7231024 | 0.032072229 | 0.6917895 | 49 |
| 5 | probe35 | gene35 | 1 | -2.173225 | -0.6718451 | 0.037042472 | 0.6917895 | 25 |
| 6 | probe103 | gene103 | 1 | -2.121164 | -0.7059218 | 0.041507370 | 0.6917895 | 93 |
| 7 | probe17 | gene17 | 1 | -2.029732 | -0.8040525 | 0.050510402 | 0.7215772 | 7 |
| 8 | probe32 | gene32 | 1 | 1.915186 | 1.0631079 | 0.064169227 | 0.8021153 | 22 |
| 9 | probe74 | gene74 | 1 | -1.786253 | -0.5282879 | 0.083249917 | 0.8072836 | 64 |
| 10 | probe61 | gene61 | 1 | -1.725751 | -0.4815208 | 0.093746106 | 0.8072836 | 51 |
| 11 | probe54 | gene54 | 1 | 1.633937 | 0.6301915 | 0.111777893 | 0.8072836 | 44 |
| 12 | probe40 | gene40 | 1 | 1.609611 | 0.5661679 | 0.117008899 | 0.8072836 | 30 |
| 13 | probe90 | gene90 | 1 | -1.605115 | -0.4182303 | 0.117997257 | 0.8072836 | 80 |
| 14 | probe38 | gene38 | 1 | 1.542007 | 0.3851935 | 0.132607854 | 0.8072836 | 28 |
| 15 | probe56 | gene56 | 1 | -1.464652 | -0.5487800 | 0.152478453 | 0.8072836 | 46 |
| 16 | probe99 | gene99 | 1 | 1.405098 | 0.3951053 | 0.169336626 | 0.8072836 | 89 |
| 17 | probe108 | gene108 | 1 | -1.380558 | -0.3907145 | 0.176695014 | 0.8072836 | 98 |
| 18 | probe79 | gene79 | 1 | -1.339224 | -0.5201586 | 0.189649429 | 0.8072836 | 69 |
| 19 | probe12 | gene12 | 1 | 1.333659 | 0.7656150 | 0.191448222 | 0.8072836 | 2 |

```
20 probe44      gene44    1 1.304127 0.3796869 0.201213900 0.8072836 34
```

```
pvalue quantiles for intercept and covariates>>
```

```
      pval.(Intercept) pval.as.factor(memSubj)1
min          0.0006238292          0.002293003
25%          0.0245832240          0.237742030
median       0.1952130722          0.444338213
75%          0.5048805444          0.784209263
max          0.9980082033          0.998167350
```

```
formula>>
```

```
xi ~ as.factor(memSubj)
```

```
covariate of interest is as.factor(memSubj)
```

```
Number of tests= 100
```

```
Number of arrays= 35
```

```
Number of significant tests (raw p-value < 0.05 )= 6
```

```
Number of significant tests after p-value adjustments= 0
```

```
*****
```

```
No genes are differentially expressed!
```

```
>
```

16.3 lkhrWrapper

Likelihood ratio test wrapper. Compare 2 glm models. One is reduced model. The other is full model.

```
> res.lkh=lkhrWrapper(
+   es=es.q,
+   formulaReduced = xi~as.factor(memSubj),
+   formulaFull = xi~as.factor(memSubj)+gender,
+   family=gaussian,
+   logit=FALSE,
+   pvalAdjMethod="fdr",
+   alpha = 0.05,
+   probeID.var = "probe",
+   gene.var = "gene",
+   chr.var = "chr",
+   applier=lapply,
+   verbose=TRUE
+ )
```

```
*****
```

```
Top 20 tests>>>
```

```
  probeIDs geneSymbols chr   Chisq Df      pval    p.adj pos
20 probe30      gene30   1 7.66514  1 0.005628622 0.4115445 20
34 probe44      gene44   1 6.674654  1 0.009779351 0.4115445 34
```

| | | | | | | | | |
|----|----------|---------|---|----------|---|-------------|-----------|----|
| 70 | probe80 | gene80 | 1 | 5.787806 | 1 | 0.016137715 | 0.4115445 | 70 |
| 36 | probe46 | gene46 | 1 | 5.697967 | 1 | 0.016984571 | 0.4115445 | 36 |
| 5 | probe15 | gene15 | 1 | 5.362249 | 1 | 0.020577223 | 0.4115445 | 5 |
| 78 | probe88 | gene88 | 1 | 4.471478 | 1 | 0.034465173 | 0.5722795 | 78 |
| 42 | probe52 | gene52 | 1 | 4.087700 | 1 | 0.043196384 | 0.5722795 | 42 |
| 73 | probe83 | gene83 | 1 | 3.553840 | 1 | 0.059407808 | 0.5722795 | 73 |
| 33 | probe43 | gene43 | 1 | 3.402508 | 1 | 0.065097366 | 0.5722795 | 33 |
| 86 | probe96 | gene96 | 1 | 3.352279 | 1 | 0.067112049 | 0.5722795 | 86 |
| 38 | probe48 | gene48 | 1 | 3.179674 | 1 | 0.074559584 | 0.5722795 | 38 |
| 22 | probe32 | gene32 | 1 | 3.061740 | 1 | 0.080155786 | 0.5722795 | 22 |
| 29 | probe39 | gene39 | 1 | 2.909647 | 1 | 0.088051166 | 0.5722795 | 29 |
| 94 | probe104 | gene104 | 1 | 2.901516 | 1 | 0.088496307 | 0.5722795 | 94 |
| 4 | probe14 | gene14 | 1 | 2.851523 | 1 | 0.091287395 | 0.5722795 | 4 |
| 26 | probe36 | gene36 | 1 | 2.846647 | 1 | 0.091564723 | 0.5722795 | 26 |
| 98 | probe108 | gene108 | 1 | 2.738579 | 1 | 0.097951851 | 0.5761874 | 98 |
| 52 | probe62 | gene62 | 1 | 2.504063 | 1 | 0.113553018 | 0.6308501 | 52 |
| 8 | probe18 | gene18 | 1 | 2.171881 | 1 | 0.140554180 | 0.6973549 | 8 |
| 75 | probe85 | gene85 | 1 | 2.156022 | 1 | 0.142011926 | 0.6973549 | 75 |

```
formulaReduced>>
xi ~ as.factor(memSubj)
```

```
formulaFull>>
xi ~ as.factor(memSubj) + gender
```

```
Number of tests>>> 100
```

```
Number of arrays>>> 35
```

```
Number of tests with pvalue<0.05>>> 7
```

```
Number of tests with FDR adjusted pvalue<0.05>>> 0
```

```
>
```

17 Result Visualization

Once we get analysis results, we need to check if the results are reasonable or not (e.g, were results affected by outliers?).

If the phenotype variable of interest is a binary type variable, then we can draw parallel boxplots of expression level versus the phenotype for each of top results. `iCheck` provides function `boxPlots` to do such a task.

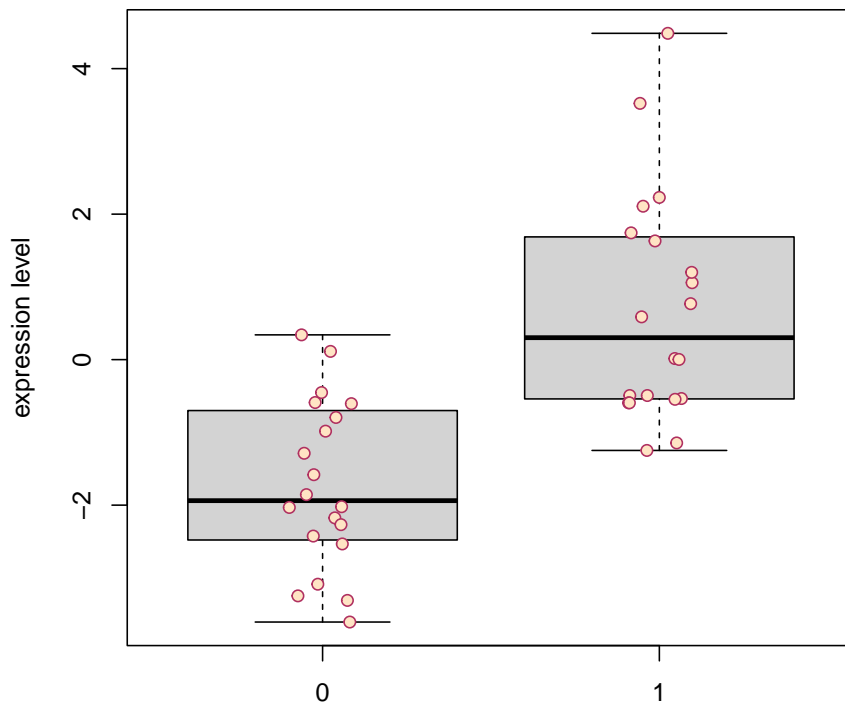
```
> boxPlots(
+   resFrame = res.limma$frame,
+   es = es.sim,
+   col.resFrame = c("probeIDs", "stats", "pval", "p.adj"),
+   var.pheno = "memSubj",
```

```

+     var.probe = "probe",
+     var.gene = "gene",
+     var.chr = "chr",
+     nTop = 20,
+     myylab = "expression level",
+     datExtrFunc = exprs,
+     fileFlag = FALSE,
+     fileFormat = "ps",
+     fileName = "boxPlots_sim.ps")
>

```

**boxplot of memSubj
probe29(gene29, chr=1)**



stat=-3.38, pval=1.4e-03, p.adj=1.0e-01

If the phenotype variable of interest is a continuous type variable, then we can draw scatter plot of expression level versus the phenotype for each of top results. `iCheck` provides function `scatterPlots` to do such a task.

```

> # regard memSubj as continuos for illustration purpose
> scatterPlots(
+   resFrame = res.limma$frame,
+   es = es.sim,
+   col.resFrame = c("probeIDs", "stats", "pval", "p.adj"),
+   var.pheno = "memSubj",
+   var.probe = "probe",

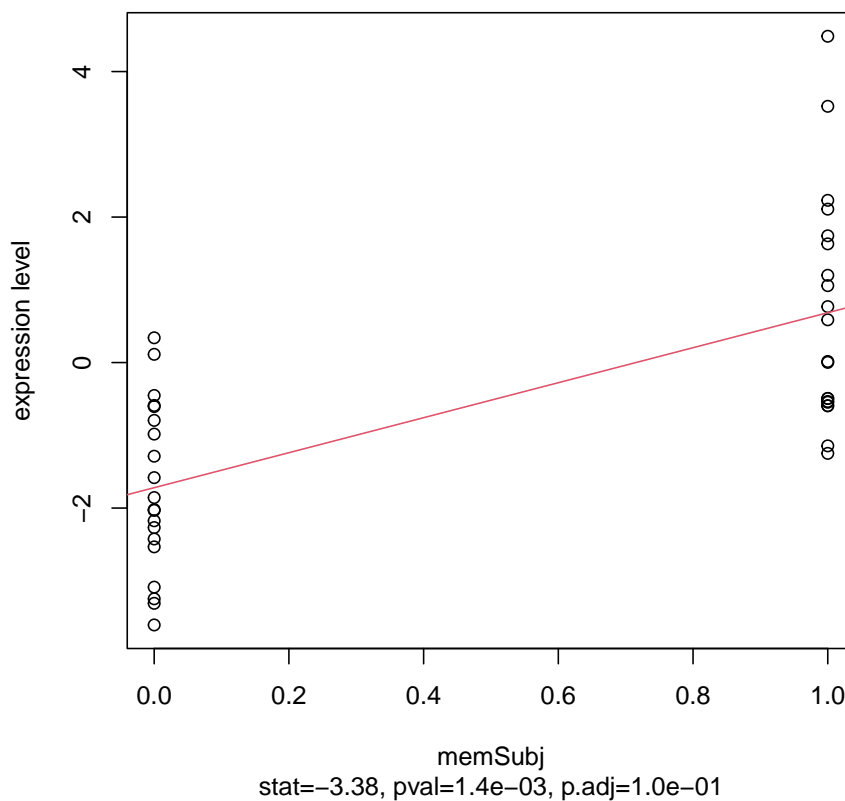
```

```

+     var.gene = "gene",
+     var.chr = "chr",
+     nTop = 20,
+     myylab = "expression level",
+     datExtrFunc = exprs,
+     fileFlag = FALSE,
+     fileFormat = "ps",
+     fileName = "scatterPlots_sim.ps")
>

```

**scatterplot of memSubj
probe29(gene29, chr=1)**



18 Session Info

Finally, we need to print out the session info so that later we can know which versions the packages are from.

```
> toLatex(sessionInfo())
```

- R version 4.1.0 (2021-05-18), x86_64-pc-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_GB, LC_COLLATE=C, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8,

LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C,
LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C

- Running under: Ubuntu 20.04.2 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.13-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.13-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: Biobase 2.52.0, BiocGenerics 0.38.0, gplots 3.1.1, iCheck 1.22.0, lumi 2.44.0
- Loaded via a namespace (and not attached): AnnotationDbi 1.54.0, BiocFileCache 2.0.0, BiocIO 1.2.0, BiocManager 1.30.15, BiocParallel 1.26.0, Biostrings 2.60.0, DBI 1.1.1, DelayedArray 0.18.0, DelayedMatrixStats 1.14.0, GEOquery 2.60.0, GeneSelectMMD 2.36.0, GenomeInfoDb 1.28.0, GenomeInfoDbData 1.2.6, GenomicAlignments 1.28.0, GenomicFeatures 1.44.0, GenomicRanges 1.44.0, HDF5Array 1.20.0, IRanges 2.26.0, KEGGREST 1.32.0, KernSmooth 2.23-20, MASS 7.3-54, Matrix 1.3-3, MatrixGenerics 1.4.0, R6 2.5.0, RColorBrewer 1.1-2, RCurl 1.98-1.3, RSQLite 2.2.7, Rcpp 1.0.6, Rhdf5lib 1.14.0, Rsamtools 2.8.0, Rttf2pt1 1.3.8, S4Vectors 0.30.0, SummarizedExperiment 1.22.0, XML 3.99-0.6, XVector 0.32.0, affy 1.70.0, affyio 1.62.0, annotate 1.70.0, askpass 1.1, assertthat 0.2.1, base64 2.0, beanplot 1.2, biomaRt 2.48.0, bit 4.0.4, bit64 4.0.5, bitops 1.0-7, blob 1.2.1, bumphunter 1.34.0, caTools 1.18.2, cachem 1.0.5, codetools 0.2-18, colorspace 2.0-1, compiler 4.1.0, crayon 1.4.1, crosstalk 1.1.1, curl 4.3.1, data.table 1.14.0, dbplyr 2.1.1, digest 0.6.27, doRNG 1.8.2, dplyr 1.0.6, ellipsis 0.3.2, extrafont 0.17, extrafontdb 1.0, fansi 0.4.2, fastmap 1.1.0, filelock 1.0.2, foreach 1.5.1, fs 1.5.0, genefilter 1.74.0, generics 0.1.0, glue 1.4.2, grid 4.1.0, gtools 3.8.2, hms 1.1.0, htmltools 0.5.1.1, htmlwidgets 1.5.3, httpuv 1.6.1, httr 1.4.2, illuminaio 0.34.0, iterators 1.0.13, jsonlite 1.7.2, knitr 1.33, later 1.2.0, lattice 0.20-44, lifecycle 1.0.0, limma 3.48.0, lmtest 0.9-38, locfit 1.5-9.4, magrittr 2.0.1, manipulateWidget 0.10.1, matrixStats 0.58.0, mclust 5.4.7, memoise 2.0.0, methylumi 2.38.0, mgcv 1.8-35, mime 0.10, minfi 1.38.0, miniUI 0.1.1.1, multtest 2.48.0, nleqslv 3.3.2, nlme 3.1-152, nor1mix 1.3-0, openssl 1.4.4, pillar 1.6.1, pkgconfig 2.0.3, pkgdown 1.6.1, plyr 1.8.6, png 0.1-7, preprocessCore 1.54.0, prettyunits 1.1.1, progress 1.2.2, promises 1.2.0.1, purrr 0.3.4, quadprog 1.5-8, randomForest 4.6-14, rappdirs 0.3.3, readr 1.4.0, reshape 0.8.8, restfulr 0.0.13, rgl 0.106.8, rhdf5 2.36.0, rhdf5filters 1.4.0, rjson 0.2.20, rlang 0.4.11, rngtools 1.5, rstudioapi 0.13, rtracklayer 1.52.0, scatterplot3d 0.3-41, scime 1.3.5, shiny 1.6.0, siggenes 1.66.0, sparseMatrixStats 1.4.0, splines 4.1.0, stats4 4.1.0, stringi 1.6.2, stringr 1.4.0, survival 3.2-11, tibble 3.1.2, tidyr 1.1.3, tidyselect 1.1.1, tools 4.1.0, utf8 1.2.1, vctrs 0.3.8, webshot 0.5.2, xfun 0.23, xml2 1.3.2, xtable 1.8-4, yaml 2.2.1, zlibbioc 1.38.0, zoo 1.8-9