Transcriptomic preprocessing and Differential Expression analysis of GSE25724 of Type 2 Diabetes

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```
library(GEOquery)
```

```
## Loading required package: Biobase

## Loading required package: BiocGenerics

##
## Attaching package: ’BiocGenerics’

## The following objects are masked from ’package:stats’:
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from ’package:base’:
##
##     anyDuplicated, aperm, 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")’.

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

## Setting options(’GEOquery.inmemory.gpl’=FALSE)
```

```
library(affy)
library(limma)
```

```
##
## Attaching package: ’limma’
## The following object is masked from ’package:BiocGenerics’:
##
##     plotMA
```

```r
library(sva)
```

```
## Loading required package: mgcv
```

```
## Loading required package: nlme
```

```
## This is mgcv 1.9-1. For overview type ’help("mgcv-package")’.
```

```
## Loading required package: genefilter
```

```
## Loading required package: BiocParallel
```

```r
library(WGCNA)
```

```
## Loading required package: dynamicTreeCut
```

```
## Loading required package: fastcluster
```

```
##
## Attaching package: ’fastcluster’
```

```
## The following object is masked from ’package:stats’:
##
##     hclust
```

```
##
```

```
##
## Attaching package: ’WGCNA’
```

```
## The following object is masked from ’package:stats’:
##
##     cor
```

```r
library(ensembldb)
```

```
## Loading required package: GenomicRanges
```

```
## Loading required package: stats4
```

```
## Loading required package: S4Vectors
```

```
##
## Attaching package: ’S4Vectors’
## The following object is masked from 'package:utils':
##
##     findMatches

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: IRanges

##
## Attaching package: 'IRanges'

## The following object is masked from 'package:nlme':
##
##     collapse

## Loading required package: GenomeInfoDb

## Loading required package: GenomicFeatures

## Loading required package: AnnotationDbi

## Loading required package: AnnotationFilter

##
## Attaching package: 'ensembldb'

## The following object is masked from 'package:stats':
##
##     filter
```

```r
library(biomaRt)
library(arrayQualityMetrics)
```

```r
# Download GSE25724 data ()
gse_T2DM <- getGEO("GSE25724", GSEMatrix =TRUE,getGPL=FALSE)
```

```
## Found 1 file(s)

## GSE25724_series_matrix.txt.gz
```

```r
datMeta_T2DM <- pData(gse_T2DM[[1]])
rownames(datMeta_T2DM) <- datMeta_T2DM$geo_accession
```

```r
# Read GSE25724 data
setwd("/Users/lorandacalderonzamora/GSE25724/")
data.affy_T2DM <- ReadAffy(celfile.path = "./")
datExpr_T2DM <- exprs(data.affy_T2DM)
```

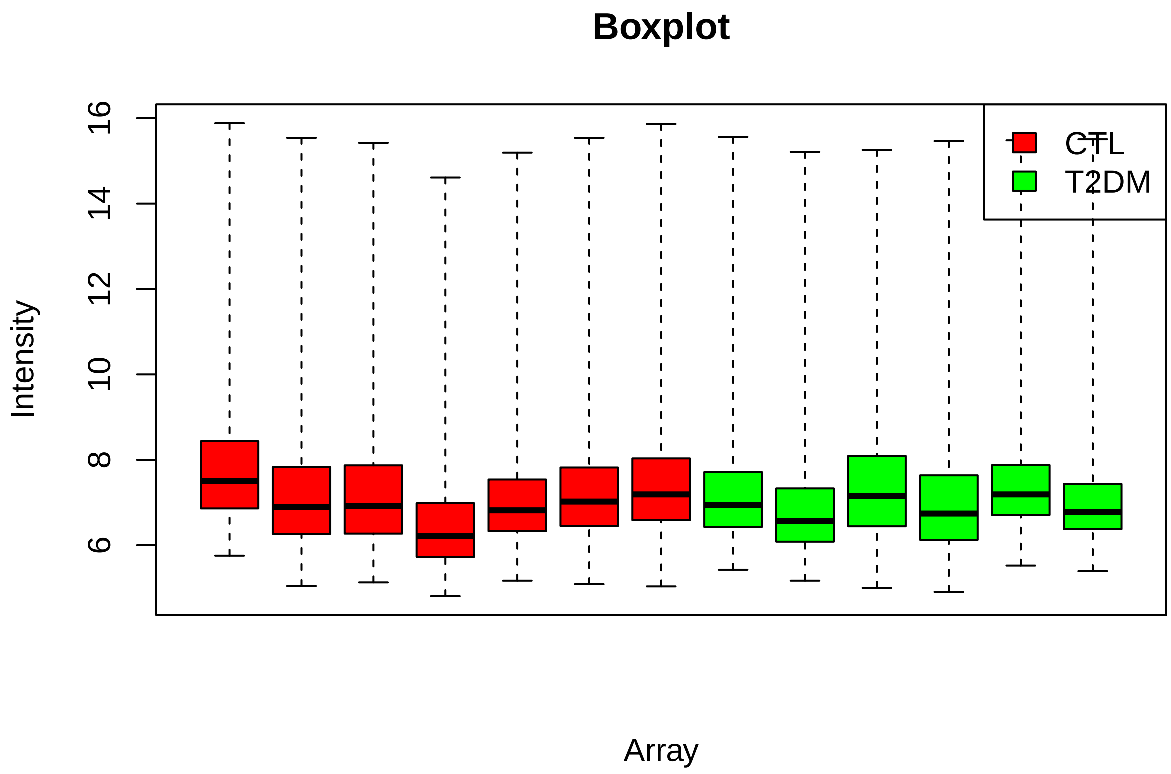
```r
# Align datMeta_T2DM and datExpr_T2DM by sample identifiers
GSM_T2DM <- rownames(pData(data.affy_T2DM))
GSM_T2DM <- substr(GSM_T2DM, 1, 9)
idx_T2DM <- match(GSM_T2DM, datMeta_T2DM$geo_accession)
datMeta_T2DM <- datMeta_T2DM[idx_T2DM, ]
colnames(datExpr_T2DM) <- rownames(datMeta_T2DM)
```

```r
# Cleaning and formatting of GSE25724 metadata
datMeta_T2DM <- datMeta_T2DM[,-c(3:7,14:36)]
colnames(datMeta_T2DM)[2] <- c("Dx")
datMeta_T2DM$Dx[rownames(datMeta_T2DM) %in% c("GSM631755", "GSM631756", "GSM631757", "GSM631758", "GSM63
datMeta_T2DM$Dx[rownames(datMeta_T2DM) %in% c("GSM631762", "GSM631763", "GSM631764", "GSM631765", "GSM63
datMeta_T2DM$Dx <- as.factor(datMeta_T2DM$Dx)
```

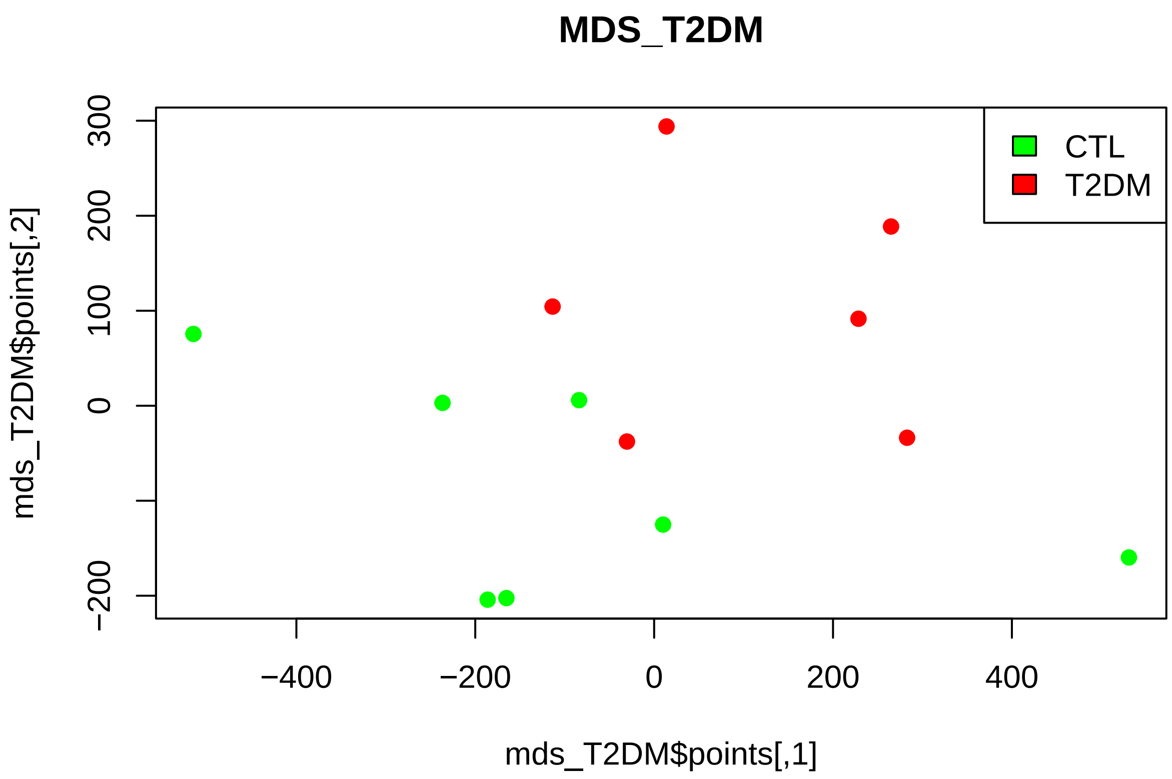
```r
# Preprocessing and quality assessment of GSE25724 raw expression data
datExpr_T2DM <- log2(datExpr_T2DM)
dim(datExpr_T2DM)
```

```
## [1] 506944     13
```

```r
# Exploratory visualization of GSE25724 raw data
boxplot(datExpr_T2DM,range=0, col=c('red', 'green')[as.numeric(datMeta_T2DM$Dx)], xaxt='n', xlab = "Arra
legend("topright",legend = levels(datMeta_T2DM$Dx),fill = c('red', 'green')[as.numeric(as.factor(levels
```



```
mds_T2DM = cmdscale(dist(t(datExpr_T2DM)),eig=TRUE)
plot(mds_T2DM$points,col=c('green', 'red')[as.numeric(datMeta_T2DM$Dx)],pch=19,main="MDS_T2DM")
legend("topright",legend = levels(datMeta_T2DM$Dx),fill =c('green', 'red')[as.numeric(as.factor(levels(
```

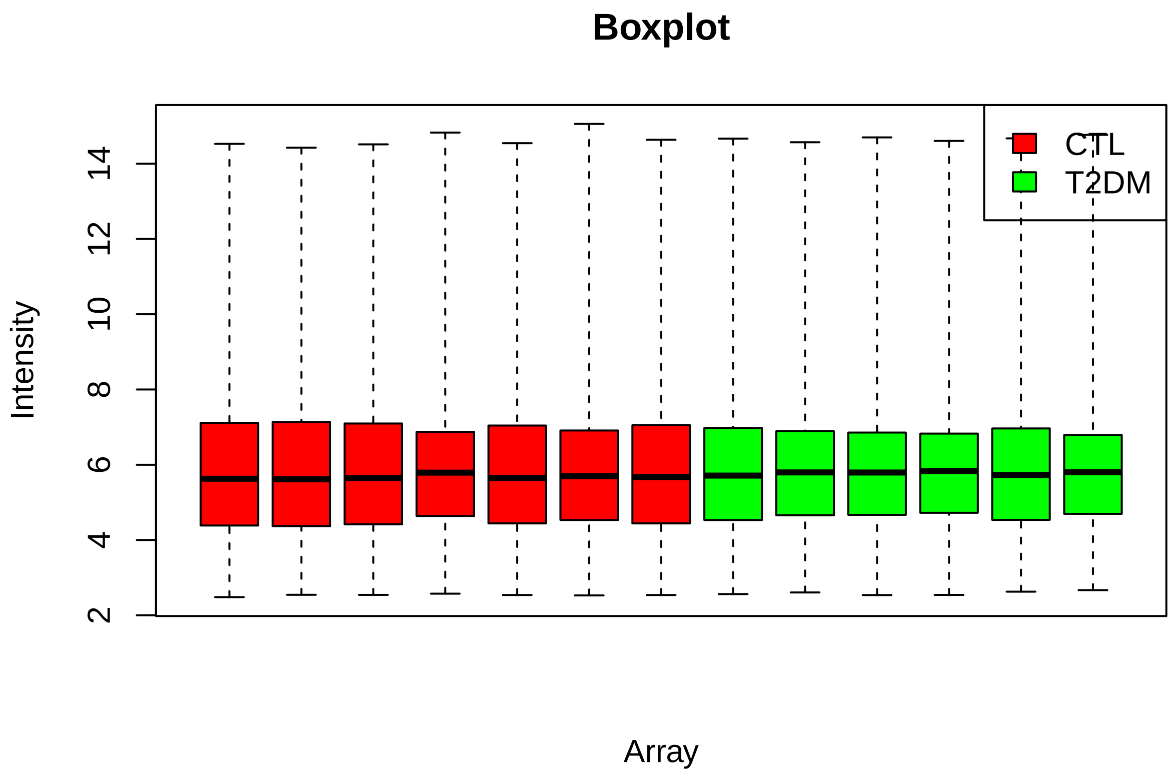


```
# Normalization using RMA
datExpr_T2DM <- rma(data.affy_T2DM, background=T, normalize=T, verbose=T)
```

```
## Warning: replacing previous import ’AnnotationDbi::tail’ by ’utils::tail’ when
## loading ’hgu133acdf’

## Warning: replacing previous import ’AnnotationDbi::head’ by ’utils::head’ when
## loading ’hgu133acdf’

##

## Background correcting
## Normalizing
## Calculating Expression
```

```
datExpr_T2DM <- exprs(datExpr_T2DM)
```

```r
# Exploratory visualization of GSE25724 normalized data
boxplot(datExpr_T2DM,range=0, col=c('red', 'green')[as.numeric(datMeta_T2DM$Dx)], xaxt='n', xlab = "Arra
legend("topright",legend = levels(datMeta_T2DM$Dx),fill = c('red', 'green')[as.numeric(as.factor(levels
```



```r
# QC analysis with arrayQualityMetrics
datMeta_proc_T2DM <- new("AnnotatedDataFrame", data = datMeta_T2DM)
colnames(datExpr_T2DM) <- rownames(datMeta_T2DM)
eset_T2DM <- new("ExpressionSet", exprs = datExpr_T2DM, phenoData = datMeta_proc_T2DM)

arrayQualityMetrics(expressionset = eset_T2DM,
                    outdir = "/Users/lorandacalderonzamora/Downloads/QC_GSE25724_Report",
                    force = TRUE,
                    do.logtransform = FALSE)
```

```
## The report will be written into directory '/Users/lorandacalderonzamora/Downloads/QC_GSE25724_Report
```

```
## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value
## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value
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## name(s): subscripts, group.number, group.value
## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value
## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value
## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value
```

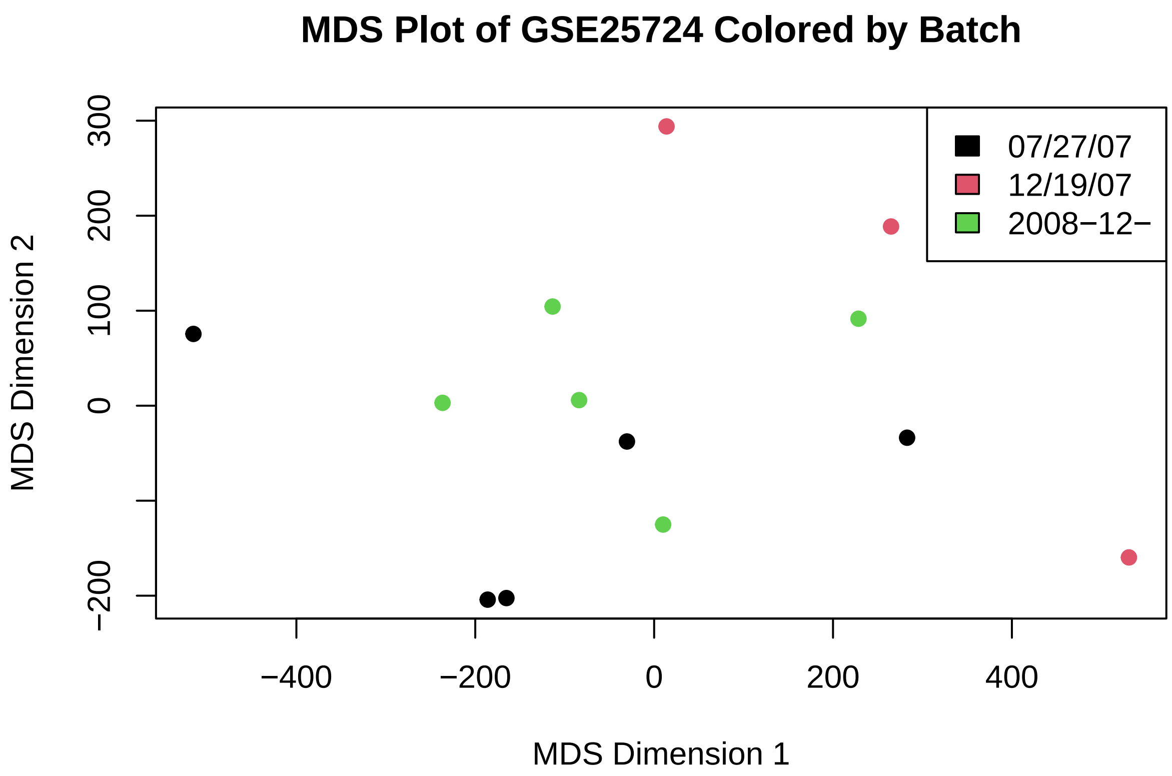
```
## (loaded the KernSmooth namespace)
```

```
# Extract ScanDate from GSE25724 for batch effect correction
batch_T2DM <- protocolData(data.affy_T2DM)$ScanDate
batch_T2DM <- substr(batch_T2DM,1,8)
batch_T2DM <- as.factor(batch_T2DM)
table(batch_T2DM)
```

```
## batch_T2DM
## 07/27/07 12/19/07 2008-12-
##        5        3        5
```

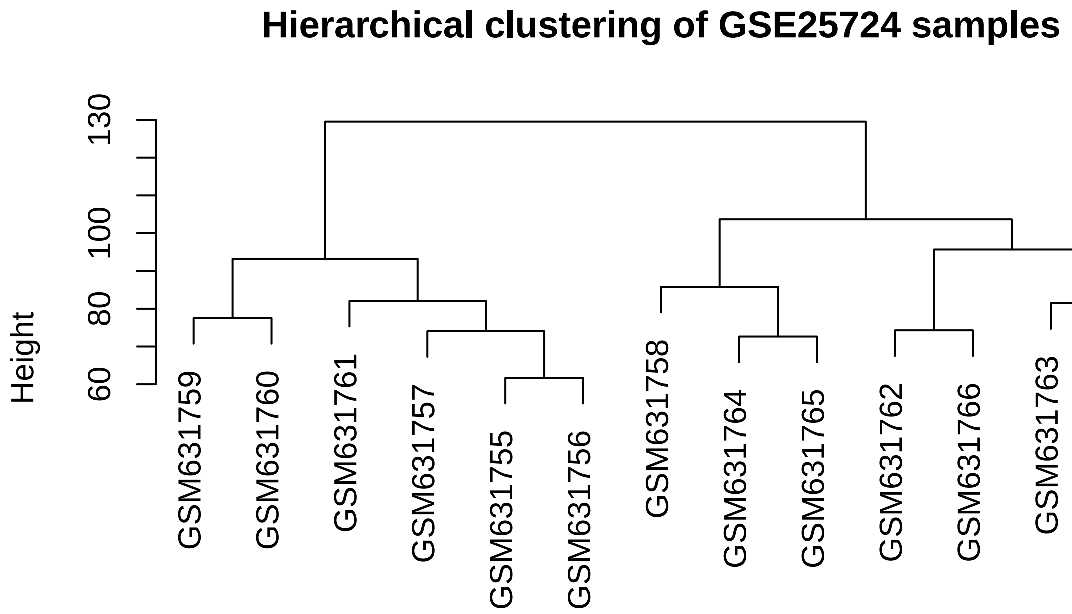
```
datMeta_T2DM$Batch <- batch_T2DM
```

```
# Visualization of ScanDate metadata from GSE25724 to identify potential batch effects
plot(mds_T2DM$points,col = as.numeric(datMeta_T2DM$Batch),pch=19,main="MDS Plot of GSE25724 Colored by B
legend("topright",legend = levels(datMeta_T2DM$Batch),fill = as.numeric(as.factor(levels(datMeta_T2DM$Ba
```



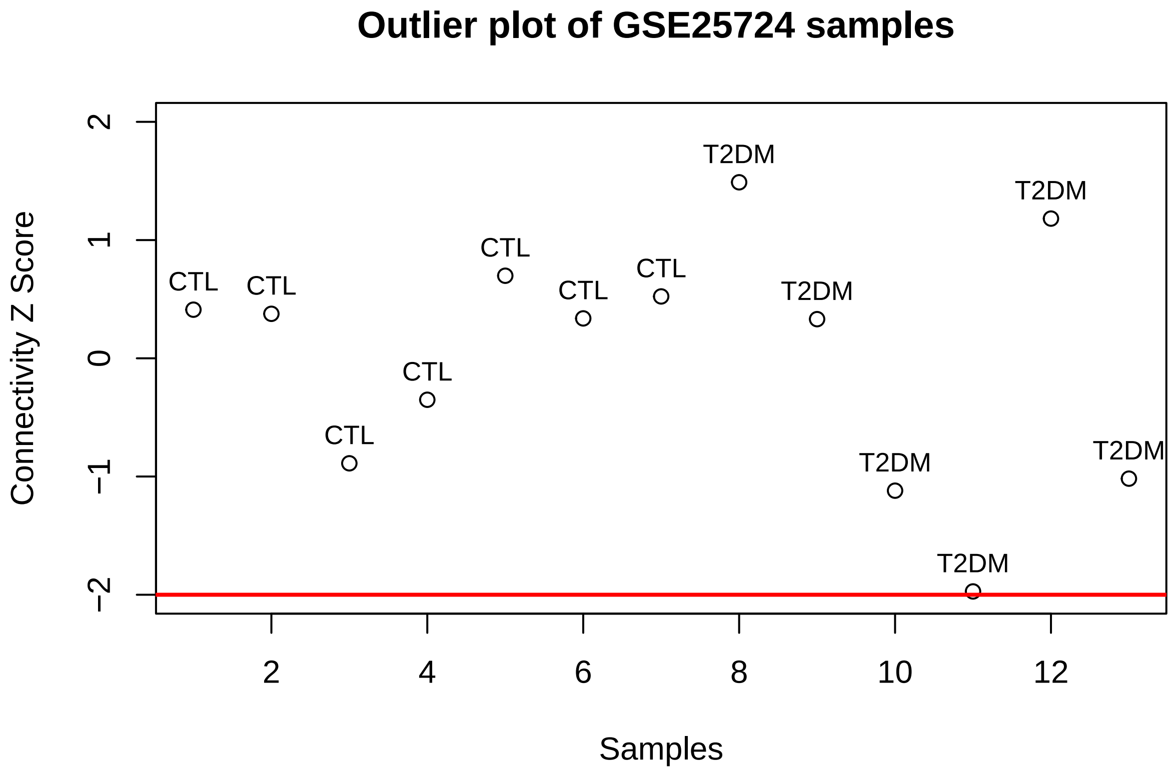
```r
# Create ExpressionSet object after Batch effect assessment
datMeta_T2DM$Batch <- batch_T2DM
datMeta_proc_T2DM <- new("AnnotatedDataFrame", data = datMeta_T2DM)
colnames(datExpr_T2DM) <- rownames(datMeta_T2DM)
datAll_T2DM <- new("ExpressionSet", exprs = datExpr_T2DM, phenoData = datMeta_proc_T2DM)
# No singular batch was detected in the GSE25724 dataset.
# Therefore, batch correction with ComBat is technically feasible.
# However, as no evident batch effect was observed in exploratory analyses (MDS),
# ComBat was not applied, and no batch removal was necessary.
```

```r
# Sample Clustering and outlier detection
tree_T2DM <- hclust(dist(t(exprs(datAll_T2DM))), method = "average")
plot(tree_T2DM, main = "Hierarchical clustering of GSE25724 samples", xlab = "", sub = "")
```



```
normadj_T2DM <- (0.5 + 0.5*bicor(exprs(datAll_T2DM)))^2
netsummary_T2DM <- fundamentalNetworkConcepts(normadj_T2DM)
C_T2DM <- netsummary_T2DM$Connectivity
Z.C_T2DM <- (C_T2DM - mean(C_T2DM)) / sqrt(var(C_T2DM))

datLabel_T2DM <- pData(datAll_T2DM)$Dx
plot(1:length(Z.C_T2DM),Z.C_T2DM,main="Outlier plot of GSE25724 samples ",xlab = "Samples",ylab="Connec
text(1:length(Z.C_T2DM),Z.C_T2DM,label=datLabel_T2DM,pos=3,cex=0.8)
abline(h= -2, col="red", lwd = 2)
```



```
# Identify and remove potential outlier from GSE25724 samples based on connectivity Z-score
# No samples exceeded the threshold (Z < -2), so none were removed
to_keep_T2DM <- abs(Z.C_T2DM) < 2
table(to_keep_T2DM)
```

```
## to_keep_T2DM
## TRUE
##   13
```

```
colnames(exprs(datAll_T2DM))[!to_keep_T2DM]
```

```
## character(0)
```

```
datAll_T2DM <- datAll_T2DM[, to_keep_T2DM]
```

```
# Annotating Probes using Ensembl
ensembl <- useEnsembl(biomart = "ensembl", dataset = "hsapiens_gene_ensembl")
```

```
# Annotating Probes for GSE25724 dataset
identifier <- "affy_hg_u133a_2"
getinfo <- c("affy_hg_u133a_2", "ensembl_gene_id", "entrezgene_id", "external_gene_name")
geneDat_T2DM <- getBM(attributes = getinfo,
                      filters = identifier,
                      values = rownames(exprs(datAll_T2DM)),
                      mart = ensembl)
idx_T2DM <- match(rownames(exprs(datAll_T2DM)), geneDat_T2DM$affy_hg_u133a_2)
geneDat_T2DM <- geneDat_T2DM[idx_T2DM, ]
table(is.na(geneDat_T2DM$ensembl_gene_id))
```

```
##
## FALSE  TRUE
## 20259  2024
```

```
to_keep_T2DM <- !is.na(geneDat_T2DM$ensembl_gene_id)
geneDat_T2DM <- geneDat_T2DM[to_keep_T2DM, ]
datAll_T2DM <- datAll_T2DM[to_keep_T2DM, ]
```

```
# Collapse Rows for GSE25724 by Ensembl Gene ID
table(duplicated(geneDat_T2DM$affy_hg_u133a_2))
```

```
##
## FALSE
## 20259
```

```
table(duplicated(geneDat_T2DM$ensembl_gene_id))
```

```
##
## FALSE  TRUE
## 13366  6893
```

```
CR_T2DM <- collapseRows(exprs(datAll_T2DM),
                        rowGroup = geneDat_T2DM$ensembl_gene_id,
                        rowID = geneDat_T2DM$affy_hg_u133a_2)
CRdata_T2DM <- CR_T2DM$datETcollapsed
idx_T2DM <- match(CR_T2DM$group2row[,"selectedRowID"], geneDat_T2DM$affy_hg_u133a_2)
geneDat_T2DM <- geneDat_T2DM[idx_T2DM, ]
rownames(geneDat_T2DM) <- geneDat_T2DM$ensembl_gene_id
```

```
# Differential Expression Analysis from GSE25724
mod_T2DM <- model.matrix(~pData(datAll_T2DM)$Dx)
fit_T2DM <- lmFit(CR_T2DM$datETcollapsed,mod_T2DM)
fit_T2DM <- eBayes(fit_T2DM)
tt_T2DM <- topTable(fit_T2DM,coef = 2,n = Inf,genelist = geneDat_T2DM)
head(tt_T2DM)
```

```
##                  affy_hg_u133a_2 ensembl_gene_id entrezgene_id
## ENSG00000147642       218692_at ENSG00000147642         55638
## ENSG00000171109     207098_s_at ENSG00000171109         55669
## ENSG00000156413     211465_x_at ENSG00000156413          2528
## ENSG00000143575       201145_at ENSG00000143575         10456
## ENSG00000187735     216241_s_at ENSG00000187735          6917
## ENSG00000086619       220012_at ENSG00000086619         56605
##                  external_gene_name      logFC  AveExpr         t      P.Value
## ENSG00000147642               SYBU -1.7852973 7.456118 -8.898821 3.975337e-07
## ENSG00000171109               MFN1 -2.0197137 5.854147 -8.708803 5.146010e-07
## ENSG00000156413               FUT6  0.9909830 8.111291  7.684775 2.221989e-06
## ENSG00000143575               HAX1 -0.9649762 8.280549 -7.464098 3.096722e-06
## ENSG00000187735              TCEA1 -1.9923078 8.559339 -7.444819 3.188778e-06
## ENSG00000086619              ERO1B -2.4723116 7.598684 -7.394610 3.442341e-06
##                  adj.P.Val        B
## ENSG00000147642 0.003439078 6.555426
## ENSG00000171109 0.003439078 6.335236
## ENSG00000156413 0.007096584 5.061263
## ENSG00000143575 0.007096584 4.766488
## ENSG00000187735 0.007096584 4.740382
## ENSG00000086619 0.007096584 4.672123
```