Expression Analysis of Breast Cancer Cell-lines (E-GEOD-18494, GSE47533 and GSE41491)

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HIF1a, !VHL & !O2
p53, !Mdm2
Mdm2, p53 & !VHL
VHL, HIF1a & !p53
p300, ((p53 & HIF1a) & !VHL) | (!(p53 & HIF1a) & VHL)
BIM, !MCL_1 & !BCLXL & !BCL2
BAD, p53
BID, (!HIF1a & (p53 & VHL)) | (!MCL_1 & !BCLXL & !BCL2)
BIK, !MCL_1 & !BCLXL & !BCL2
MCL_1, HIF1a
BCLXL, HIF1a & !(p53 & VHL) & ((!Casp3 & !BAD) | (!Casp3 & BCL2))
BCL2, HIF1a & !(p53 & VHL) & ((MCL_1 & !BIM & !BIK & !BAD) | (!BIM & !BIK & BCLXL & !BAD))
IAPs, !DIABLO
BAX, (BIM & !BCLXL) | (BIK & !BCLXL & !BCL2) | (BID & !BCLXL & !BCL2) | (BIM & BID) | (BIM & BIK) | (BIM & !BCL2) | (!MCL_1 & BIM)
BAK, (!MCL_1 & BIM & !BCLXL) | (BID & !BCL2) | (BID & !BCLXL) | (!MCL_1 & BID) | (!MCL_1 & BIK & !BCLXL) | (BIM & BID) | (BIK & BID)
DIABLO, BAX | BAK
Cyto_C, BAX | BAK
Casp9, Casp3 | (!IAPs & Cyto_C)
Casp3, !IAPs & Casp9

BCLXL ?

No VHL in GSE41491

```
# Selected genes from HIF Axis
#hif.symbols <- c("HIF1A", "TP53", "MDM2", "VHL", "EP300","TMBIM1","TMBIM4", "TMBIM6","BAD","BIK","MCL1
hif.symbols <- c("HIF1A", "TP53", "MDM2", "VHL", "EP300")

hif.probes <- anno.EGEOD18494$probes[anno.EGEOD18494$symbol %in% hif.symbols]

# Select the probes and genes
# EGEOD18494
expr.EGEOD18494.hif <- as.data.frame(expr.EGEOD18494) %>%
  rownames_to_column('probes') %>%
  filter(probes %in% hif.probes) %>%
  merge(anno.EGEOD18494[anno.EGEOD18494$symbol %in% hif.symbols, c("probes","symbol")], by = "probes") %
  mutate(., symbol=ifelse(symbol %in% c("TMBIM1","TMBIM4", "TMBIM6"), "BIM", symbol)) %>%
  mutate(., symbol=ifelse(symbol %in% c("BIRC2", "BIRC3", "BIRC5", "BIRC6", "BIRC7"), "IAPs", symbol)) %
  group_by(symbol) %>%
  summarise_at(vars(-probes), funs(mean(., na.rm=TRUE)))  %>%
  column_to_rownames(var = "symbol") %>%
  dplyr::select(c(data.EGEOD18494$codes[data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"]))
```

```
## Warning: `funs()` is deprecated as of dplyr 0.8.0.
## Please use a list of either functions or lambdas:
##
##   # Simple named list:
##   list(mean = mean, median = median)
##
##   # Auto named with `tibble::lst()`:
##   tibble::lst(mean, median)
##
##   # Using lambdas
##   list(~ mean(., trim = .2), ~ median(., na.rm = TRUE))
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_warnings()` to see where this warning was generated.
```

```r
expr.EGEOD18494.hif <- expr.EGEOD18494.hif[c("HIF1A", "TP53", "MDM2", "VHL", "EP300"),]

hif.probes <- anno.GSE47533$probes[anno.GSE47533$symbol %in% hif.symbols]

# GSE47533
expr.GSE47533.hif <- as.data.frame(expr.GSE47533) %>%
  rownames_to_column('probes') %>%
  filter(probes %in% hif.probes) %>%
  merge(anno.GSE47533[anno.GSE47533$symbol %in% hif.symbols, c("probes","symbol")], by = "probes") %>%
  mutate(., symbol=ifelse(symbol %in% c("TMBIM1","TMBIM4", "TMBIM6"), "BIM", symbol)) %>%
  mutate(., symbol=ifelse(symbol %in% c("BIRC2", "BIRC3", "BIRC5", "BIRC6", "BIRC7"), "IAPs", symbol)) %
  group_by(symbol) %>%
  summarise_at(vars(-probes), funs(mean(., na.rm=TRUE)))  %>%
  column_to_rownames(var = "symbol")

expr.GSE47533.hif <- expr.GSE47533.hif[c("HIF1A", "TP53", "MDM2", "VHL", "EP300"),]

hif.probes <- anno.GSE41491$probes[anno.GSE41491$symbol %in% hif.symbols]

# GSE41491
expr.GSE41491.hif <- as.data.frame(expr.GSE41491) %>%
  rownames_to_column('probes') %>%
  filter(probes %in% hif.probes) %>%
  merge(anno.GSE41491[anno.GSE41491$symbol %in% hif.symbols, c("probes","symbol")], by = "probes") %>%
  mutate(., symbol=ifelse(symbol %in% c("TMBIM1","TMBIM4", "TMBIM6"), "BIM", symbol)) %>%
  mutate(., symbol=ifelse(symbol %in% c("BIRC2", "BIRC3", "BIRC5", "BIRC6", "BIRC7"), "IAPs", symbol)) %
  group_by(symbol) %>%
  summarise_at(vars(-probes), funs(mean(., na.rm=TRUE)))  %>%
  column_to_rownames(var = "symbol")
```

```r
write.table(expr.GSE47533.hif,"expr.GSE47533.txt", sep ="\t")
write.table(expr.EGEOD18494.hif, "expr.EGEOD18494.txt", sep ="\t")
write.table(expr.GSE41491.hif, "expr.GSE41491.txt", sep ="\t")

expr.EGEOD18494.tdm <- TDM::tdm_transform(ref_file = "expr.GSE47533.txt", file = "expr.EGEOD18494.txt")
```

```
##
## Attaching package: 'data.table'

## The following objects are masked from 'package:dplyr':
##
```

```
##     between, first, last

## The following object is masked from 'package:purrr':
##
##     transpose

##
## Attaching package: 'scales'

## The following objects are masked from 'package:psych':
##
##     alpha, rescale

## The following object is masked from 'package:purrr':
##
##     discard

## The following object is masked from 'package:readr':
##
##     col_factor
```

```
expr.GSE41491.tdm <- TDM::tdm_transform(ref_file = "expr.GSE47533.txt", file = "expr.GSE41491.txt")

symbols <-  expr.EGEOD18494.tdm$gene
expr.EGEOD18494.tdm$gene  <- NULL

expr.EGEOD18494.tdm <- as.data.frame(matrix(as.numeric(unlist(expr.EGEOD18494.tdm)),
                                            nrow = dim(expr.EGEOD18494.tdm)[1],
                                            ncol = dim(expr.EGEOD18494.tdm)[2]))
colnames(expr.EGEOD18494.tdm) <- colnames(expr.EGEOD18494.hif)

rownames(expr.EGEOD18494.tdm) <- symbols
```

```
require(BiTrinA)
```

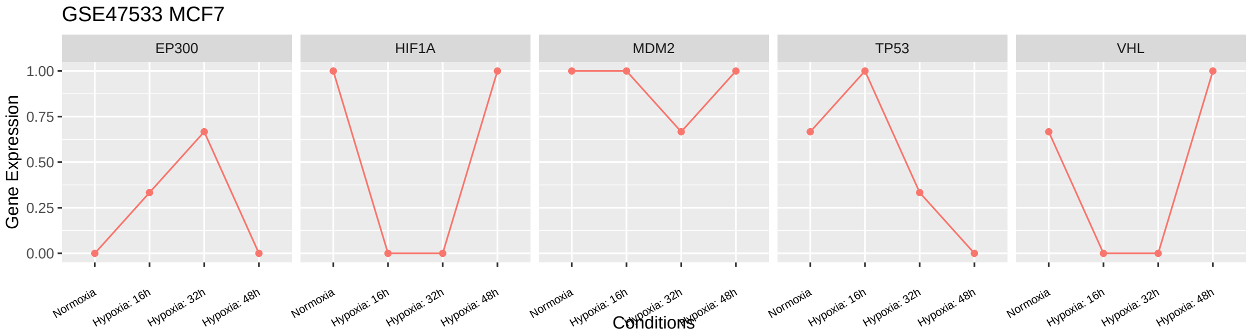
```
## Loading required package: BiTrinA

## Loading required package: diptest
```

```
expr.GSE47533.hif.bin <- binarizeMatrix(expr.GSE47533.hif,
               method = c("BASCA"),
               adjustment = "none")

expr.GSE47533.hif.bin$symbol <- row.names(expr.GSE47533.hif.bin)

expr.GSE47533.hif.bin <- expr.GSE47533.hif.bin[, c(as.character(data.GSE47533$codes), c("threshold", "p

names(expr.GSE47533.hif.bin) <- c(paste0(substr(data.GSE47533$condition,1,4),".", data.GSE47533$time, "

head(expr.GSE47533.hif.bin) %>%
  knitr::kable(.)
```

	Norm.0.1	Norm.0.2	Norm.0.3	Hypo.16h.1	Hypo.16h.2	Hypo.16h.3	Hypo.32h.1	Hypo.32h.2	Hypo.32h.3	Hypo.48h.1	Hypo.48h.2	Hypo.48h.3	threshold	pvalue	symbol
HIF1A	1	1	1	0	0	0	0	0	0	1	1	1	8.699331	0.001	HIF1A

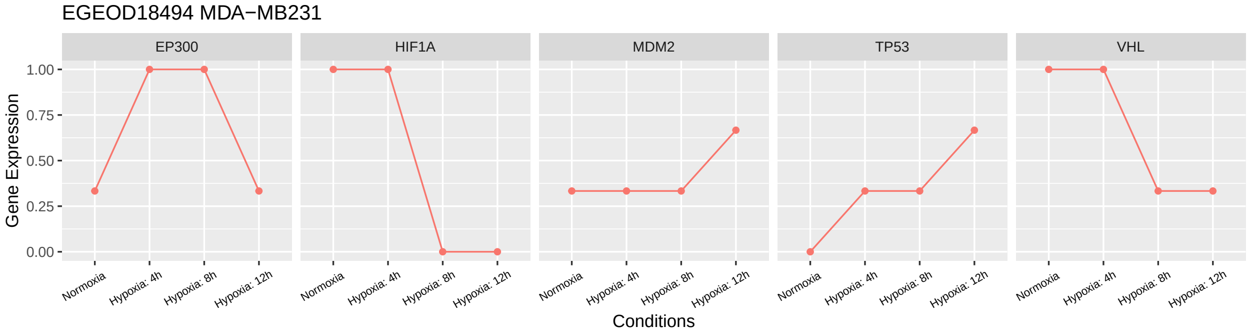
	Norm.01	Norm.02	Norm.03	Hypo.16h.1	Hypo.16h.2	Hypo.16h.3	Hypo.32h.1	Hypo.32h.2	Hypo.32h.3	Hypo.48h.1	Hypo.48h.2	Hypo.48h.3	threshold	pvalue	symbol
TP53	0	1	1	1	1	1	0	0	1	0	0	0	9.531254	0.001	TP53
MDM2	1	1	1	1	1	1	1	0	1	1	1	1	6.619755	1.000	MDM2
VHL	1	1	0	0	0	0	0	0	0	1	1	1	9.558766	0.780	VHL
EP300	0	0	0	1	0	0	0	1	1	0	0	0	9.133766	0.374	EP300

```
expr.GSE47533.hif.mean <- expr.GSE47533.hif.bin %>%
 mutate(Norm = rowMeans(dplyr::select(., starts_with("Norm"))),
        Hypo.16h = rowMeans(dplyr::select(., starts_with("Hypo.16h"))),
        Hypo.32h = rowMeans(dplyr::select(., starts_with("Hypo.32h"))),
        Hypo.48h = rowMeans(dplyr::select(., starts_with("Hypo.48h"))))  %>%
  dplyr::select(., -ends_with(c(".1",".2", ".3")))



expr.GSE47533.hif.pivot <- expr.GSE47533.hif.mean %>%
  group_by(symbol) %>%
  pivot_longer(cols = starts_with(c("Norm","Hypo")), names_to = "codes", values_to = "value")

expr.GSE47533.hif.pivot$codes <- factor(expr.GSE47533.hif.pivot$codes,  levels =  c("Norm", "Hypo.16h"

expr.GSE47533.hif.pivot$time <- as.numeric(expr.GSE47533.hif.pivot$codes)

# hif.symbols <- c("HIF1A", "TP53", "MDM2", "VHL", "EP300","TMBIM1","TMBIM4", "TMBIM6","BAD","BIK","MCL

p.MCF7 <- ggplot(aes(x = factor(time), y = value, group = symbol, color="red"),
           #data = expr.GSE47533.hif.pivot[expr.GSE47533.hif.pivot$symbol %in% c("HIF1A", "TP53", "MDM2
           data = expr.GSE47533.hif.pivot[expr.GSE47533.hif.pivot$symbol %in% c("HIF1A", "TP53", "MDM2"
  geom_point() +
  geom_line() +
  scale_x_discrete(breaks = c(1, 2, 3, 4),
                labels = c("Normoxia", "Hypoxia: 16h" , "Hypoxia: 32h" , "Hypoxia: 48h")) +
  xlab("Conditions") + ylab("Gene Expression") +
  ggtitle("GSE47533 MCF7") +
  theme(legend.position = "none", axis.text.x=element_text(color = "black", size=7, angle=30, vjust=0.5
  #geom_line(aes(linetype=Symbol, color=Symbol)) +
  facet_wrap(~ symbol, nrow = 1)

p.MCF7
```

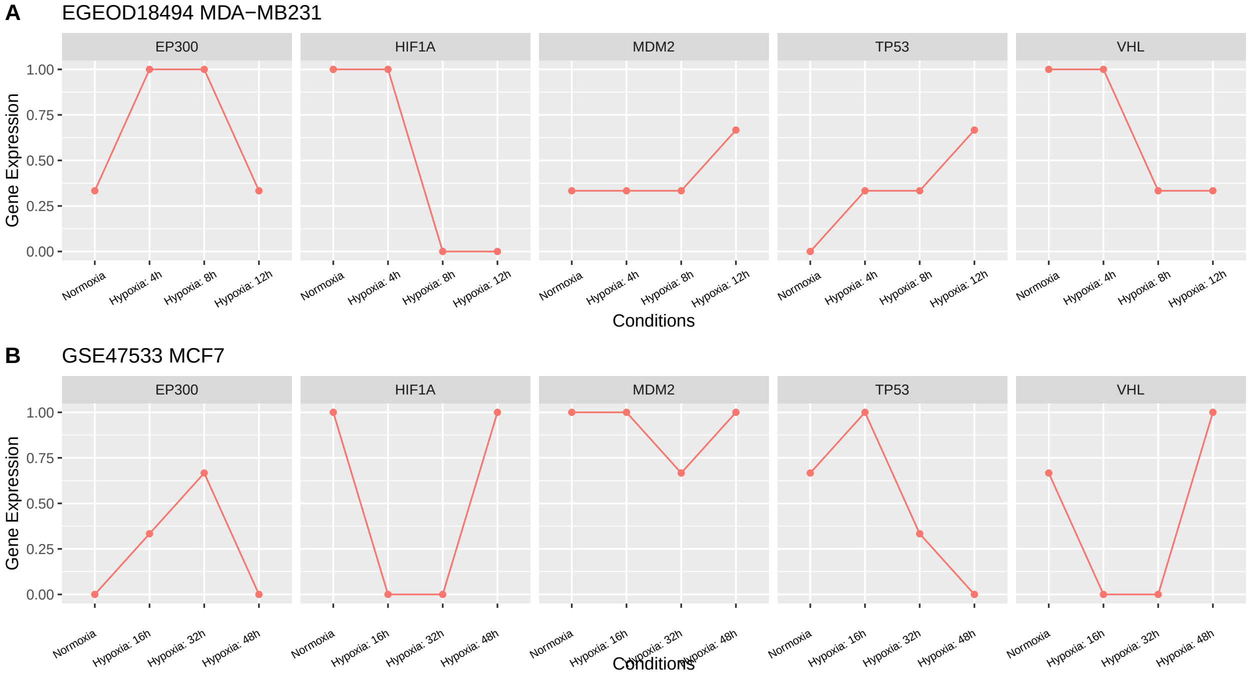


EGEOD18494

```
# expr.EGEOD18494.hif.bin <- binarizeMatrix(expr.EGEOD18494.tdm,
#                 method = c("BASCA"),
#                 tau = 0.15,
#                 #sigma = 0.9,
#                 adjustment = "none")

expr.EGEOD18494.hif.bin <- binarizeMatrix(expr.EGEOD18494.hif)

#expr.EGEOD18494.hif.bin <- expr.EGEOD18494.tdm

expr.EGEOD18494.hif.bin$symbol <- row.names(expr.EGEOD18494.hif.bin)

row <- data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"
expr.EGEOD18494.hif.bin <- expr.EGEOD18494.hif.bin[, c(as.character(data.EGEOD18494$codes[row]), c("thr

names(expr.EGEOD18494.hif.bin) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18

#names(expr.EGEOD18494.hif.bin) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD1

# head(expr.EGEOD18494.hif.bin) %>%
#   knitr::kable(.)


expr.EGEOD18494.hif.mean <- expr.EGEOD18494.hif.bin %>%
 mutate(norm = rowMeans(dplyr::select(., starts_with("norm"))),
       hypo.4h = rowMeans(dplyr::select(., starts_with("hypo.4h"))),
       hypo.8h = rowMeans(dplyr::select(., starts_with("hypo.8h"))),
       hypo.12h = rowMeans(dplyr::select(., starts_with("hypo.12h"))))  %>%
  dplyr::select(., -ends_with(c(".1",".2", ".3")))



expr.EGEOD18494.hif.pivot <- expr.EGEOD18494.hif.mean %>%
  group_by(symbol) %>%
  pivot_longer(cols = starts_with(c("Norm","Hypo")), names_to = "codes", values_to = "value")

expr.EGEOD18494.hif.pivot$codes <- factor(expr.EGEOD18494.hif.pivot$codes,  levels =  c("norm", "hypo.4

expr.EGEOD18494.hif.pivot$time <- as.numeric(expr.EGEOD18494.hif.pivot$codes)

# hif.symbols <- c("HIF1A", "TP53", "MDM2", "VHL", "EP300","TMBIM1","TMBIM4", "TMBIM6","BAD","BIK","MCL

p.MDA <- ggplot(aes(x = factor(time), y = value, group = symbol, color="red"),
           #data = expr.EGEOD18494.hif.pivot[expr.EGEOD18494.hif.pivot$symbol %in% c("HIF1A", "TP53", "
           data = expr.EGEOD18494.hif.pivot[expr.EGEOD18494.hif.pivot$symbol %in% c("HIF1A", "TP53", "M
  geom_point() +
  geom_line() +
  scale_x_discrete(breaks = c(1, 2, 3, 4),
                 labels = c("Normoxia", "Hypoxia: 4h" , "Hypoxia: 8h" , "Hypoxia: 12h")) +
  xlab("Conditions") + ylab("Gene Expression") +
  ggtitle("EGEOD18494 MDA-MB231") +
  theme(legend.position = "none", axis.text.x=element_text(color = "black", size=7, angle=30, vjust=.8,
  #geom_line(aes(linetype=Symbol, color=Symbol)) +
  facet_wrap(~ symbol, nrow = 1)

p.MDA
```



```
library(cowplot)

plot_grid(p.MDA, p.MCF7, labels = c('A', 'B'), ncol = 1)
```



Heatmaps - EGEOD18494

Multivariate Shapiro-Wilk normality test

From the output, the p-value > 0.05 implying that the distribution of the data are not significantly different from normal distribution. In other words, we can assume the normality.

```
#library(rstatix)

#rstatix::mshapiro_test(expr.EGEOD18494.hif)
```

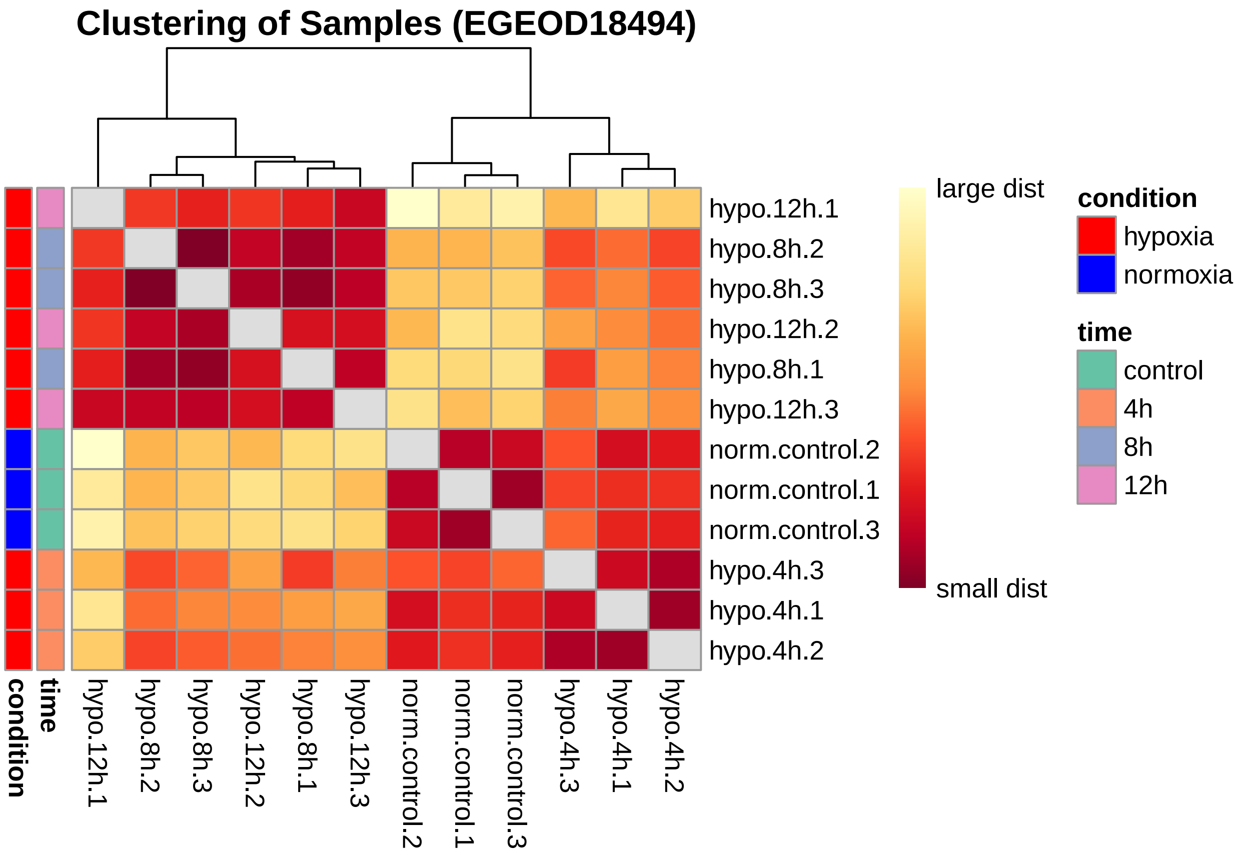
```r
library("pheatmap")
library("ComplexHeatmap")
```

```
## ========================================
## ComplexHeatmap version 2.4.3
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
## Github page: https://github.com/jokergoo/ComplexHeatmap
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
##
## If you use it in published research, please cite:
## Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
##   genomic data. Bioinformatics 2016.
##
## This message can be suppressed by:
##   suppressPackageStartupMessages(library(ComplexHeatmap))
## ========================================
```

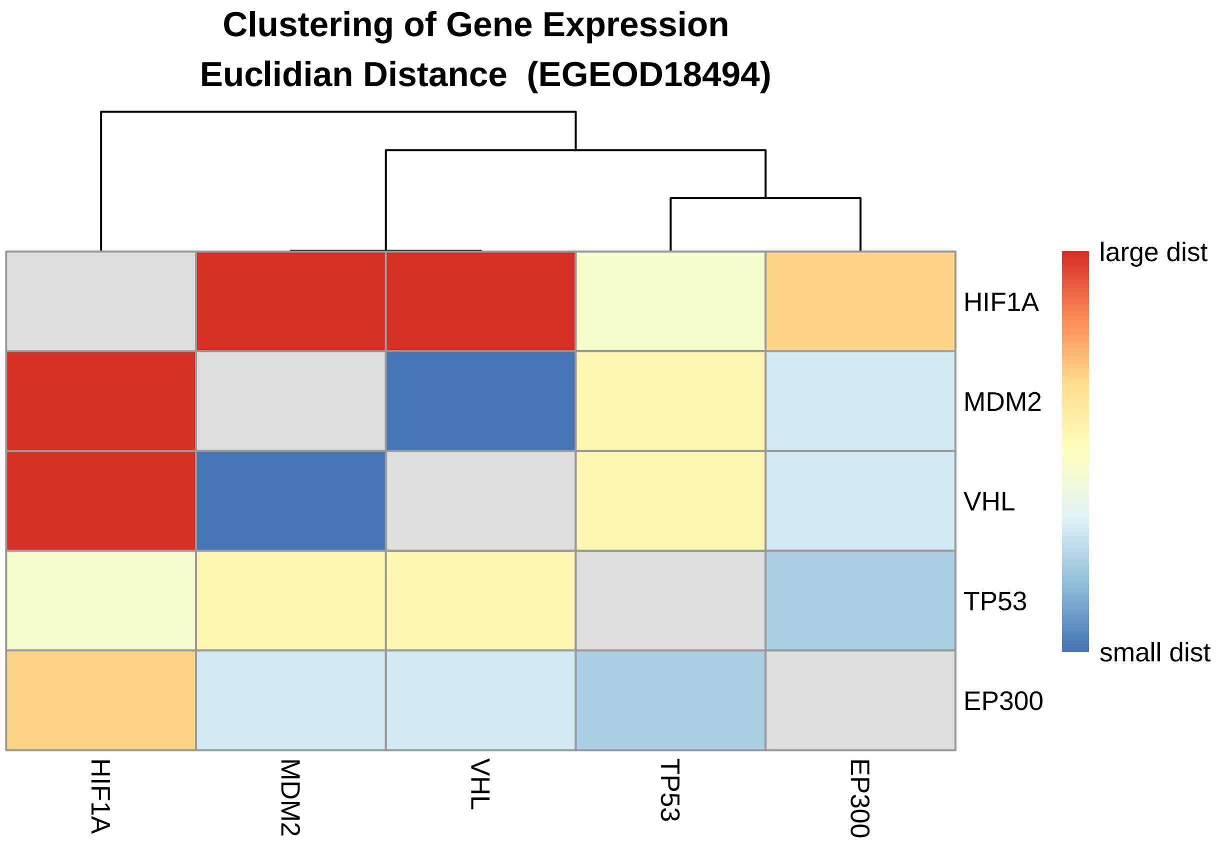
```r
data.EGEOD18494$time <- factor(data.EGEOD18494$time,  levels =  c("control", "4h" , "8h" , "12h"))

row <- data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"

annotation_for_heatmap <- droplevels(data.frame(time = data.EGEOD18494$time[row], condition = data.EGEO

row.names(annotation_for_heatmap) <- paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18

dists <- as.matrix(dist(t(expr.EGEOD18494.hif), method = "manhattan"))

rownames(dists) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18494$time[row],
colnames(dists) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18494$time[row],

hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))

diag(dists) <- NA

ann_colors <- list(
  time = RColorBrewer::brewer.pal(length(levels(data.EGEOD18494$time)), "Set2"),
  condition = c("red", "blue")
)

ann_colors
```

```
## $time
## [1] "#66C2A5" "#FC8D62" "#8DA0CB" "#E78AC3"
##
## $condition
## [1] "red"  "blue"
```

```r
names(ann_colors$time) <- levels(data.EGEOD18494$time)
names(ann_colors$condition) <- levels(data.EGEOD18494$condition)

pheatmap(dists, col = (hmcol),
         annotation_row = annotation_for_heatmap,
         annotation_colors = ann_colors,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Samples (EGEOD18494)")
```



```
dists <- as.matrix(dist(expr.EGEOD18494.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.EGEOD18494.hif)
colnames(dists) <- rownames(expr.EGEOD18494.hif)
diag(dists) <- NA

pheatmap(dists, #row = (hmcol),
         #annotation_col = annotation_for_heatmap,
         #annotation_colors = ann_colors,
         legend = TRUE,
         #display_numbers = T,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression \n Euclidian Distance  (EGEOD18494)")
```



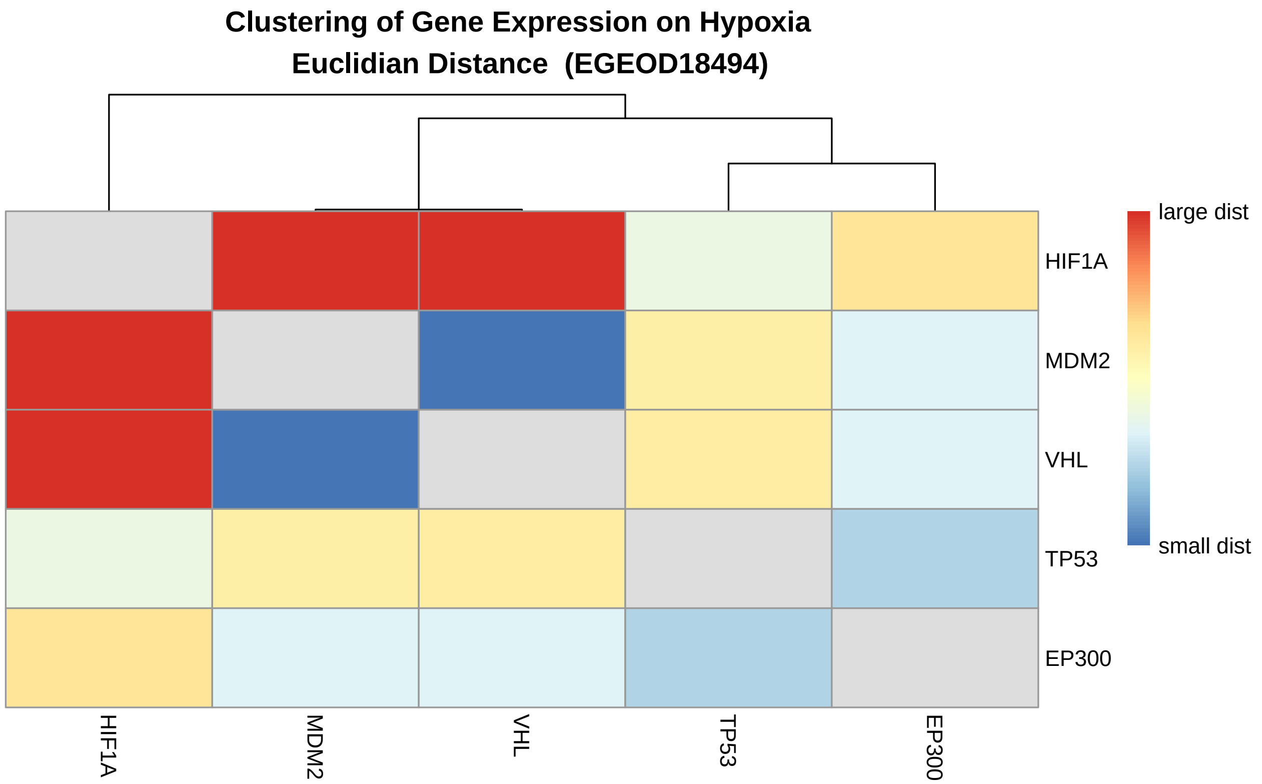
```
#---------------------------------------------------------------------------------

expr.row <- (colnames(expr.EGEOD18494.hif) %in% data.EGEOD18494$codes[data.EGEOD18494$cell_line == "MDA-
dists <- as.matrix(dist(expr.EGEOD18494.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])
colnames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])
diag(dists) <- NA

p1 <- pheatmap(dists,
         legend = TRUE,
         #display_numbers = T,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Hypoxia \n  Euclidian Distance  (EGEOD18494)",
         silent=T)

#---------------------------------------------------------------------------------

row <- data.EGEOD18494$cell_line == "MDA-MB231 breast cancer" &  data.EGEOD18494$condition == "normoxia

annotation_for_heatmap <- droplevels(data.frame(time = data.EGEOD18494$time[row], condition = data.EGEOD

expr.row <- (colnames(expr.EGEOD18494.hif) %in% data.EGEOD18494$codes[data.EGEOD18494$cell_line == "MDA-

row.names(annotation_for_heatmap) <- colnames(expr.EGEOD18494.hif[expr.row])
```

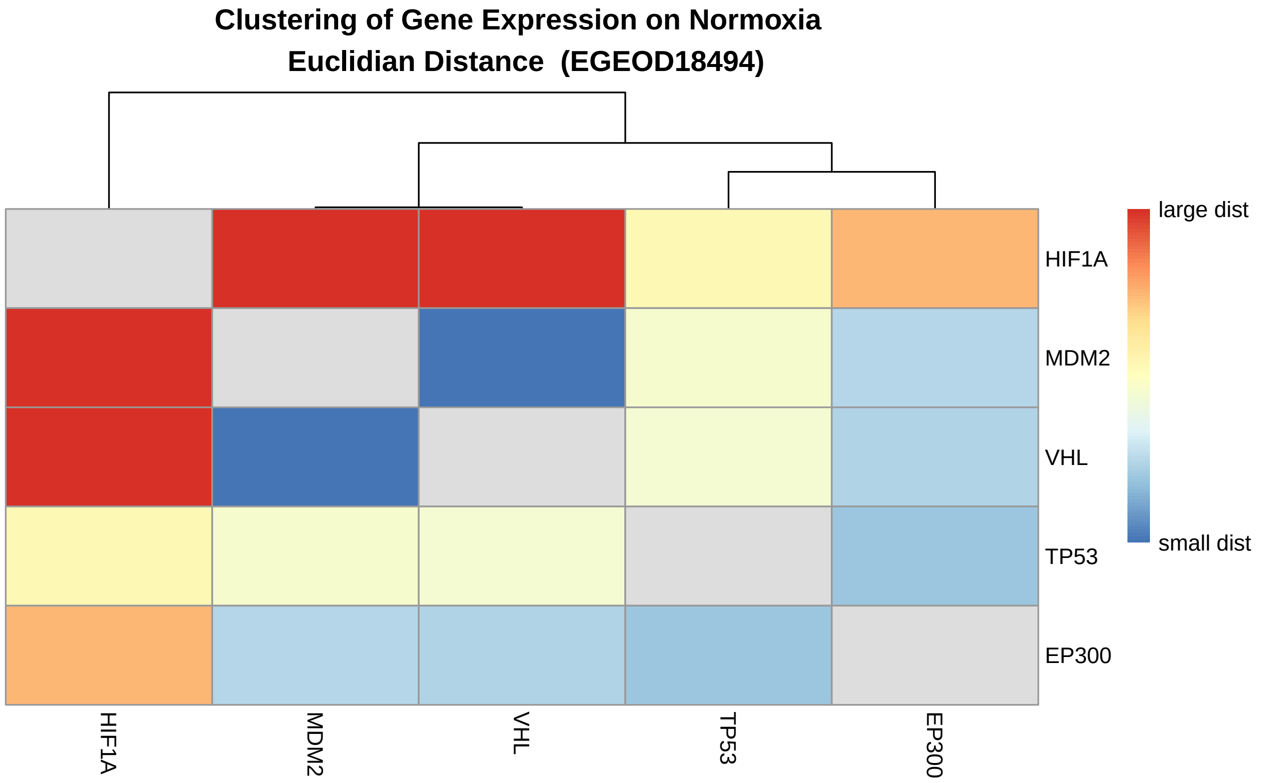
```
dists <- as.matrix(dist(expr.EGEOD18494.hif[expr.row], method = "euclidean"))

rownames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])

hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))
colnames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])
diag(dists) <- NA

ann_colors <- list(
  time = RColorBrewer::brewer.pal(length(levels(data.EGEOD18494$time)), "Set2"),
  condition = c("#EF8A62", "#67A9CF")
)

ann_colors
```

```
## $time
## [1] "#66C2A5" "#FC8D62" "#8DA0CB" "#E78AC3"
##
## $condition
## [1] "#EF8A62" "#67A9CF"
```

```
names(ann_colors$time) <- levels(data.EGEOD18494$time)
names(ann_colors$condition) <- levels(data.EGEOD18494$condition)

p2 <- pheatmap(dists, #row = (hmcol),
         #annotation_col = annotation_for_heatmap,
         #annotation_colors = ann_colors,
         legend = TRUE,
         #display_numbers = T,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance  (EGEOD18494)",
         silent=T)
```

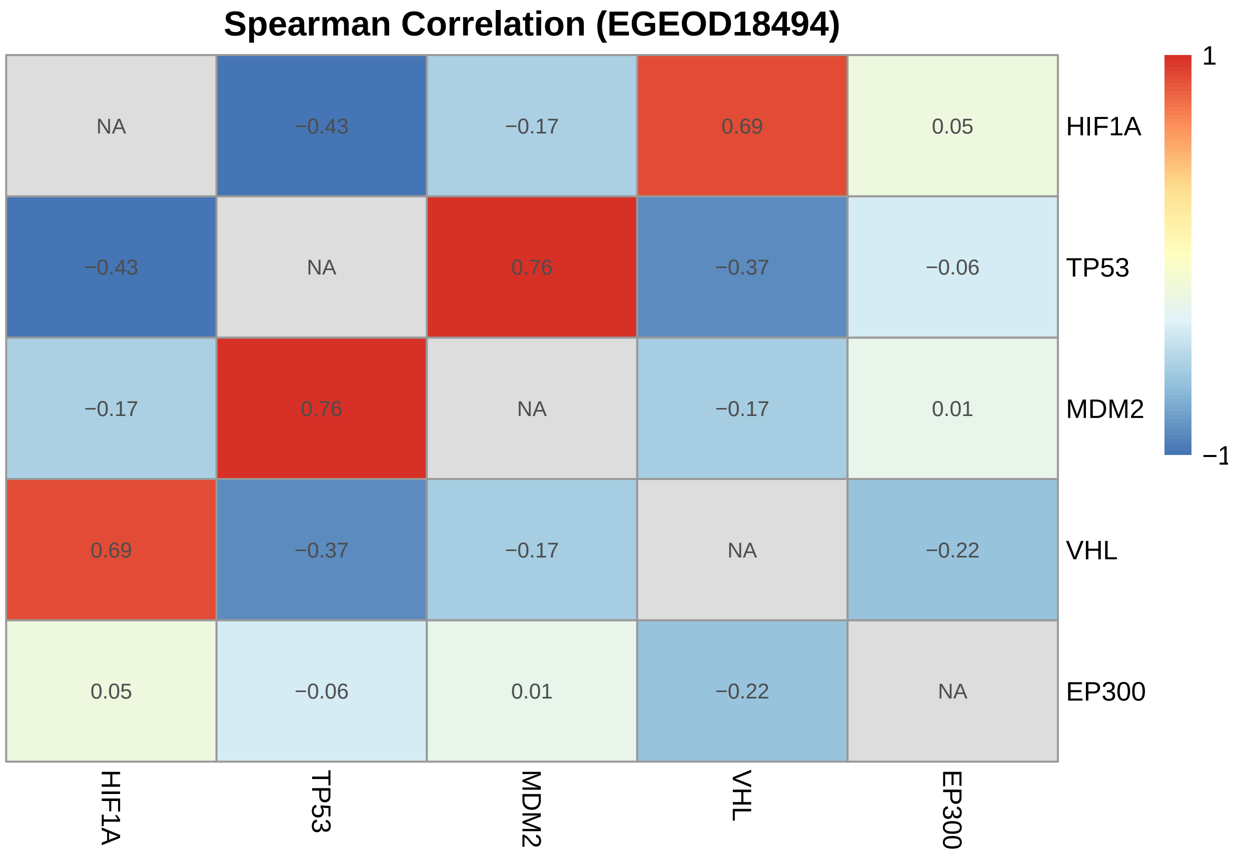
```
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
                        nrow = 2 , labels=c('A', 'B'))
```



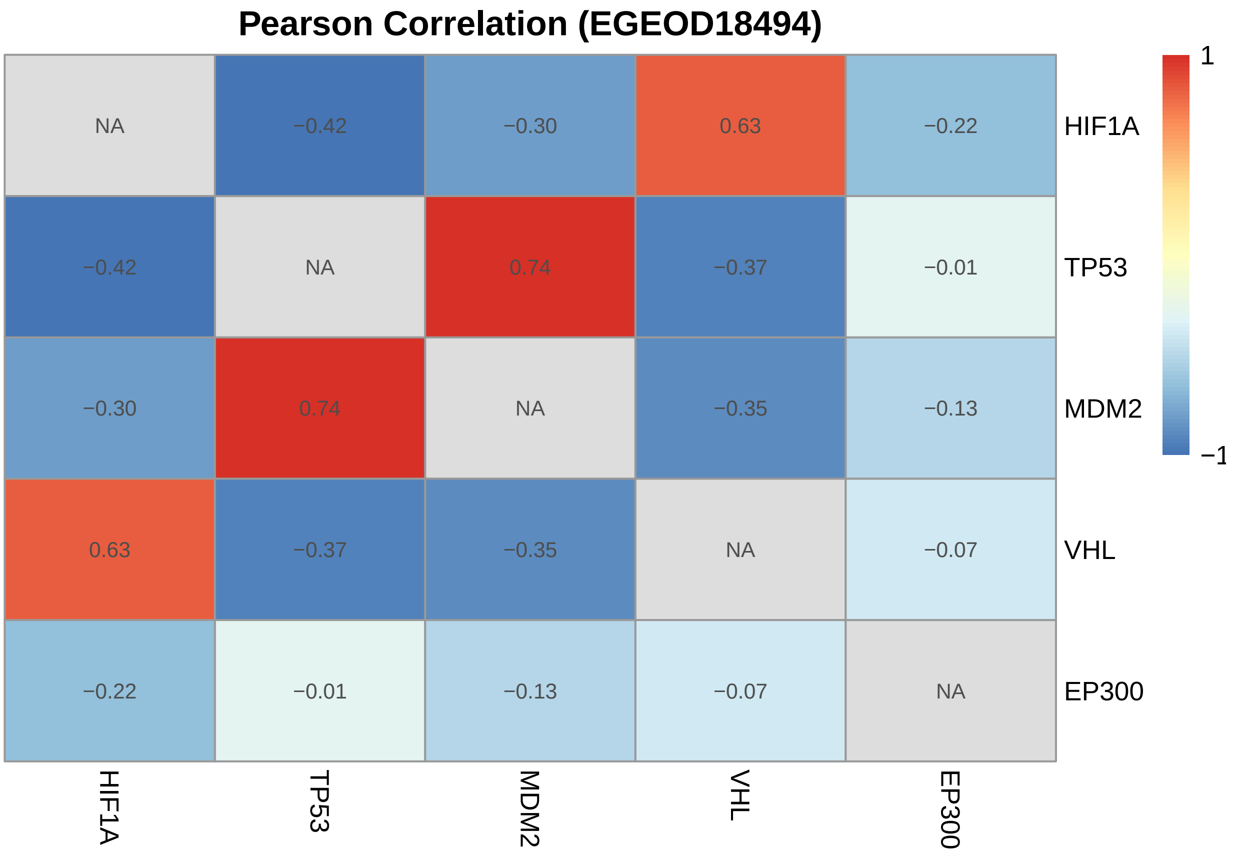


```
data.EGEOD18494$time <- factor(data.EGEOD18494$time,  levels =  c("control", "4h" , "8h" , "12h 4h"))

dists.EGEOD18494_spearman <- cor(t(expr.EGEOD18494.hif), use = "pairwise.complete.obs", method = "spear
rownames(dists.EGEOD18494_spearman) <- rownames(expr.EGEOD18494.hif)
```

```r
hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))
colnames(dists.EGEOD18494_spearman) <- rownames(expr.EGEOD18494.hif)
diag(dists.EGEOD18494_spearman) <- NA

ps.EGEOD18494_spearman <- pheatmap(dists.EGEOD18494_spearman, #row = (hmcol),
                               #annotation_col = annotation_for_heatmap,
                               #annotation_colors = ann_colors,
                               legend = TRUE,
                               display_numbers = T,
                               cluster_rows = F,
                               cluster_cols = F,
                               treeheight_row = 0,
                               legend_breaks = c(min(dists.EGEOD18494_spearman, na.rm = TRUE),
                                                 max(dists.EGEOD18494_spearman, na.rm = TRUE)),
                               legend_labels = (c("-1", "1")),
                               main = "Spearman Correlation (EGEOD18494)")
```



```r
dists.EGEOD18494_pearson <- cor(t(expr.EGEOD18494.hif), use = "pairwise.complete.obs", method = "pearso
rownames(dists.EGEOD18494_pearson) <- rownames(expr.EGEOD18494.hif)
hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))
colnames(dists.EGEOD18494_pearson) <- rownames(expr.EGEOD18494.hif)
diag(dists.EGEOD18494_pearson) <- NA

ps.EGEOD18494_pearson <- pheatmap(dists.EGEOD18494_pearson, #row = (hmcol),
         #annotation_col = annotation_for_heatmap,
         #annotation_colors = ann_colors,
         legend = TRUE,
         display_numbers = T,
         cluster_rows = F,
         cluster_cols = F,
         treeheight_row = 0,
         legend_breaks = c(min(dists.EGEOD18494_pearson, na.rm = TRUE),
                           max(dists.EGEOD18494_pearson, na.rm = TRUE)),
         legend_labels = (c("-1", "1")),
         main = "Pearson Correlation (EGEOD18494)")
```



Heatmaps - GSE47533

Multivariate Shapiro-Wilk normality test

From the output, the p-value > 0.05 implying that the distribution of the data are not significantly different from normal distribution. In other words, we can assume the normality.

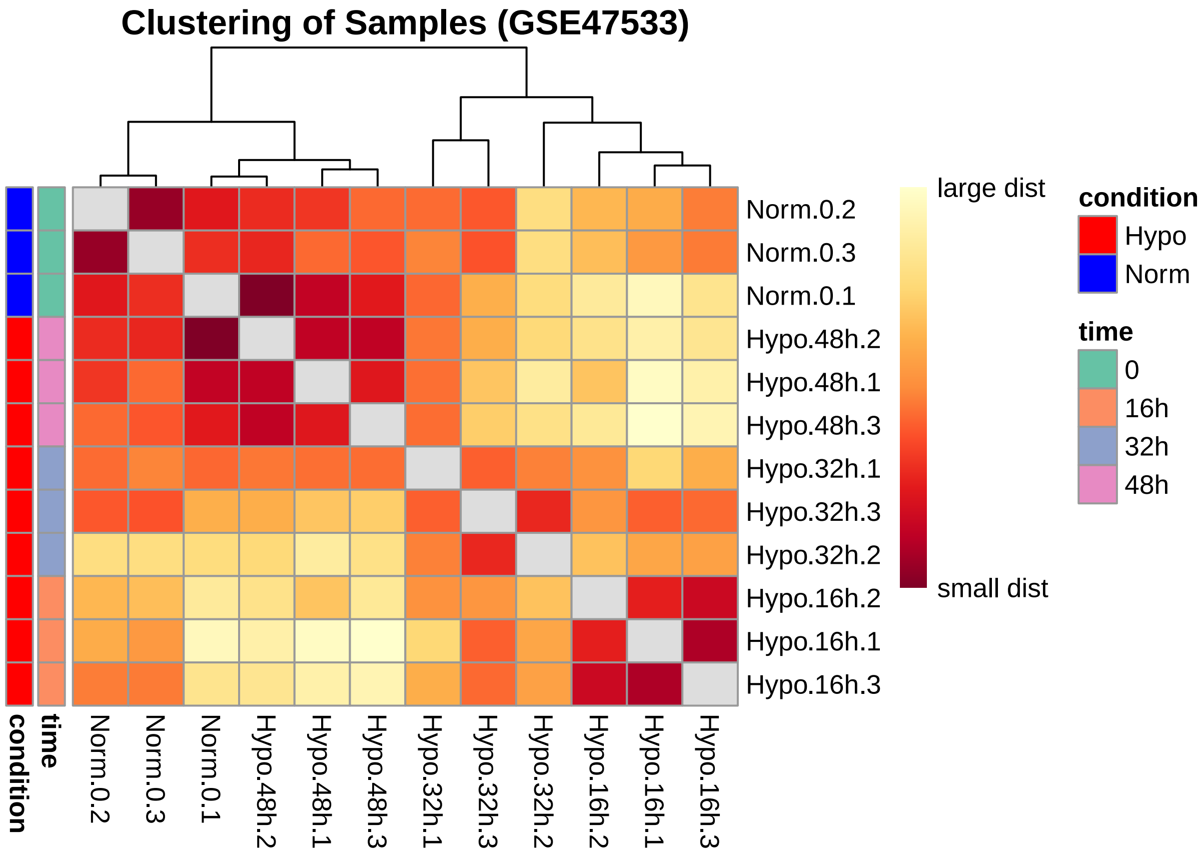
```
# library(rstatix)
#
# rstatix::mshapiro_test(expr.GSE47533.hif)  %>%
#   knitr::kable(.)
```

```
library("pheatmap")
library("ComplexHeatmap")

data.GSE47533$time <- factor(data.GSE47533$time,  levels =  c("0", "16h" , "32h" , "48h"))
```

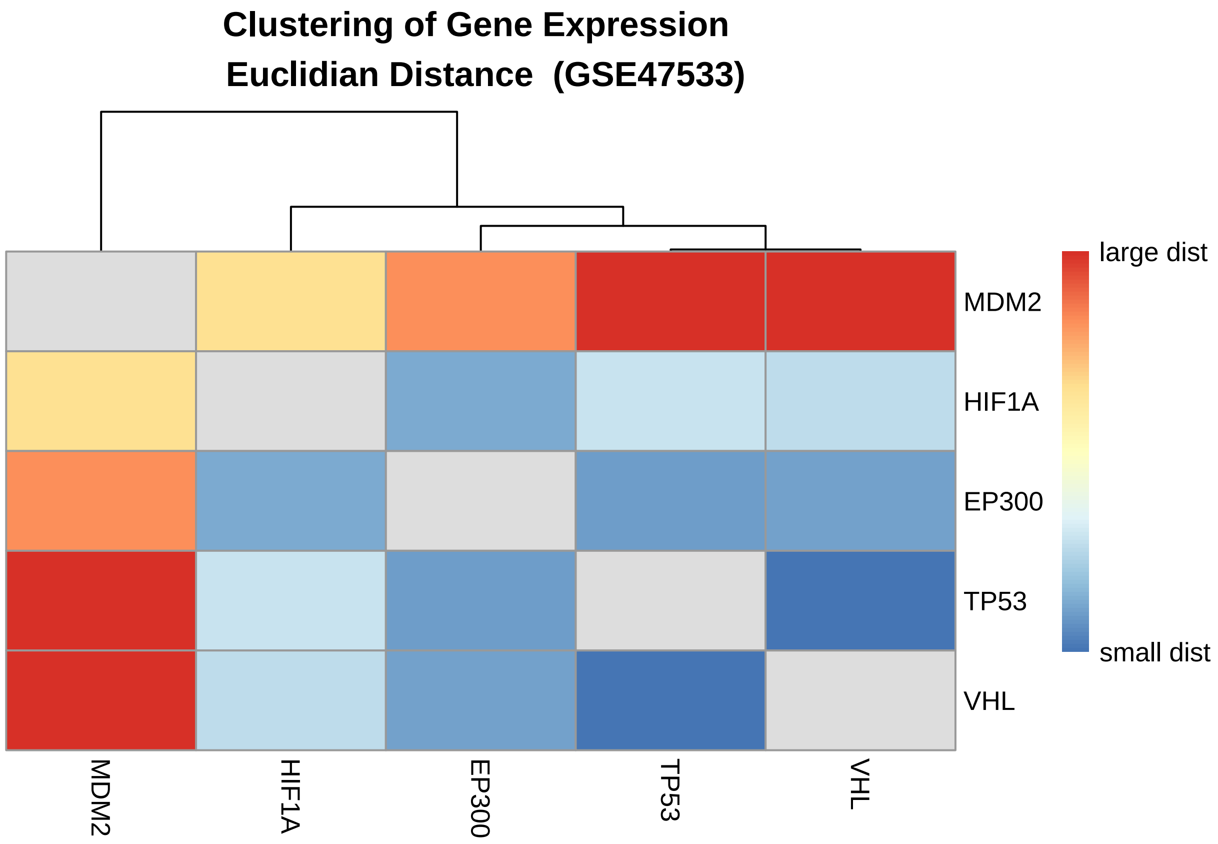
```r
annotation_for_heatmap <- droplevels(data.frame(time = data.GSE47533$time, condition = data.GSE47533$con

row.names(annotation_for_heatmap) <- paste0(substr(data.GSE47533$condition,1,4),".", data.GSE47533$time

dists <- as.matrix(dist(t(expr.GSE47533.hif), method = "manhattan"))

rownames(dists) <- c(paste0(substr(data.GSE47533$condition,1,4),".", data.GSE47533$time, ".", data.GSE47
colnames(dists) <- c(paste0(substr(data.GSE47533$condition,1,4),".", data.GSE47533$time, ".", data.GSE47

hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))

diag(dists) <- NA

ann_colors <- list(
  time = RColorBrewer::brewer.pal(length(levels(data.GSE47533$time)), "Set2"),
  condition = c("red", "blue")
)

ann_colors
```

```
## $time
## [1] "#66C2A5" "#FC8D62" "#8DA0CB" "#E78AC3"
##
## $condition
## [1] "red"  "blue"
```

```r
names(ann_colors$time) <- levels(data.GSE47533$time)
names(ann_colors$condition) <- levels(data.GSE47533$condition)

pheatmap(dists, col = (hmcol),
         annotation_row = annotation_for_heatmap,
         annotation_colors = ann_colors,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Samples (GSE47533)")
```



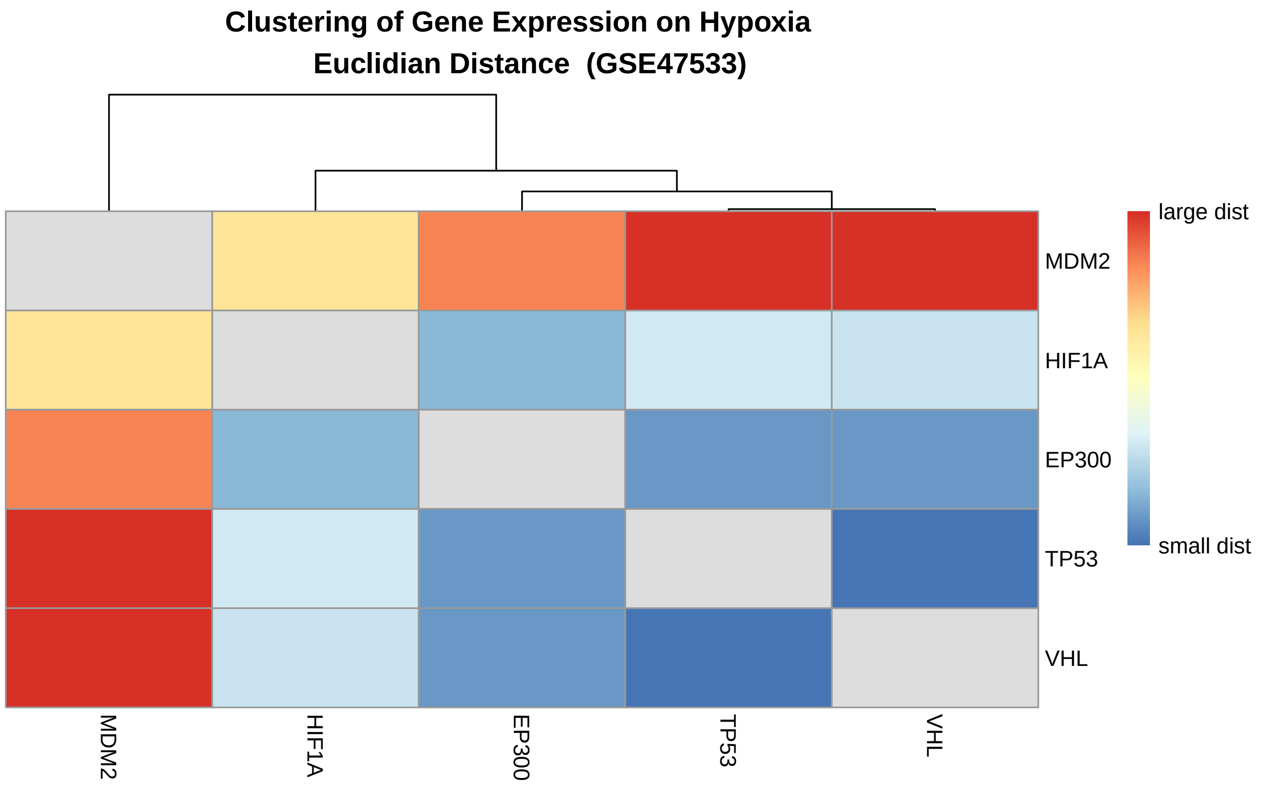
```
dists <- as.matrix(dist(expr.GSE47533.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.GSE47533.hif)
colnames(dists) <- rownames(expr.GSE47533.hif)
diag(dists) <- NA

pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression \n Euclidian Distance  (GSE47533)")
```



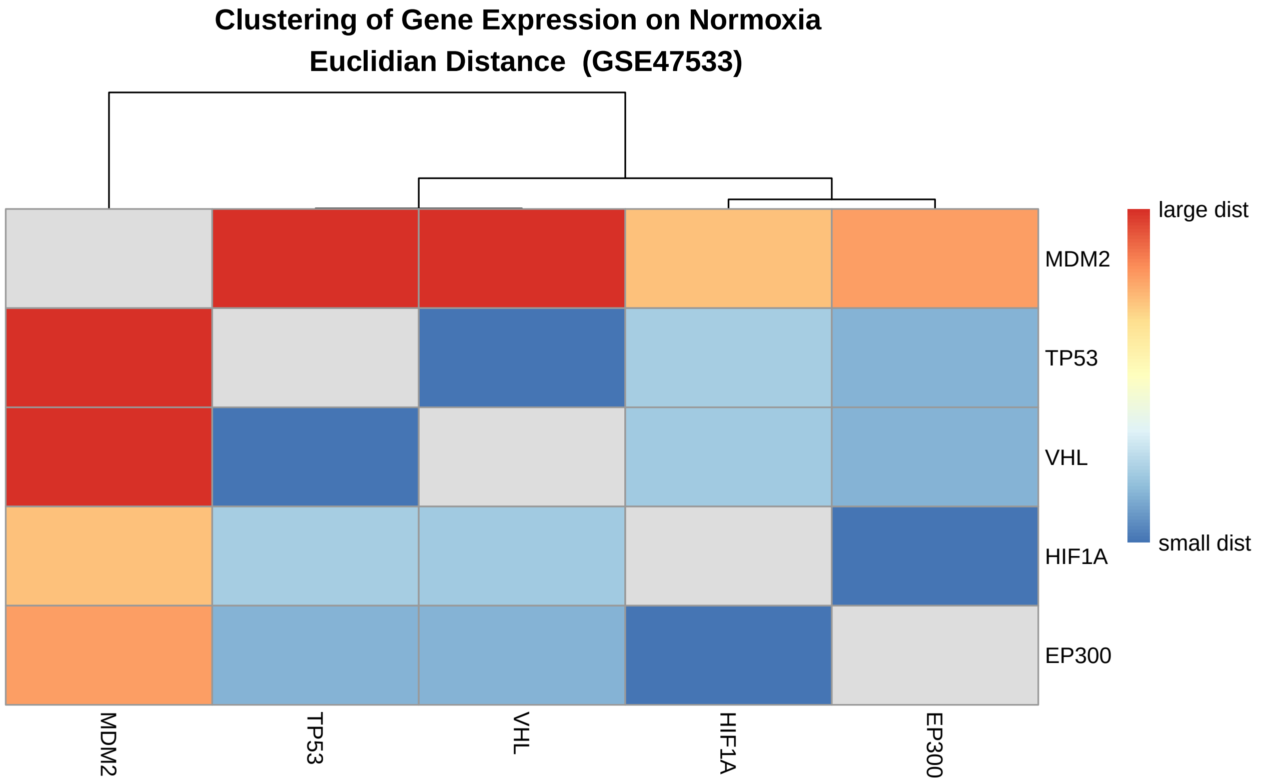
```
#---------------------------------------------------------------------------------

expr.row <- (colnames(expr.GSE47533.hif) %in% data.GSE47533$codes[data.GSE47533$condition == "Hypo"])
dists <- as.matrix(dist(expr.GSE47533.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.GSE47533.hif[expr.row])
colnames(dists) <- rownames(expr.GSE47533.hif[expr.row])
diag(dists) <- NA


p1 <- pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Hypoxia \n  Euclidian Distance  (GSE47533)",
         silent=T)

#---------------------------------------------------------------------------------

expr.row <- (colnames(expr.GSE47533.hif) %in% data.GSE47533$codes[data.GSE47533$condition == "Norm"])
dists <- as.matrix(dist(expr.GSE47533.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.GSE47533.hif[expr.row])
colnames(dists) <- rownames(expr.GSE47533.hif[expr.row])
diag(dists) <- NA
```

```
p2 <- pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance  (GSE47533)",
         silent=T)
```

```
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
                        nrow = 2 , labels=c('A', 'B'))
```



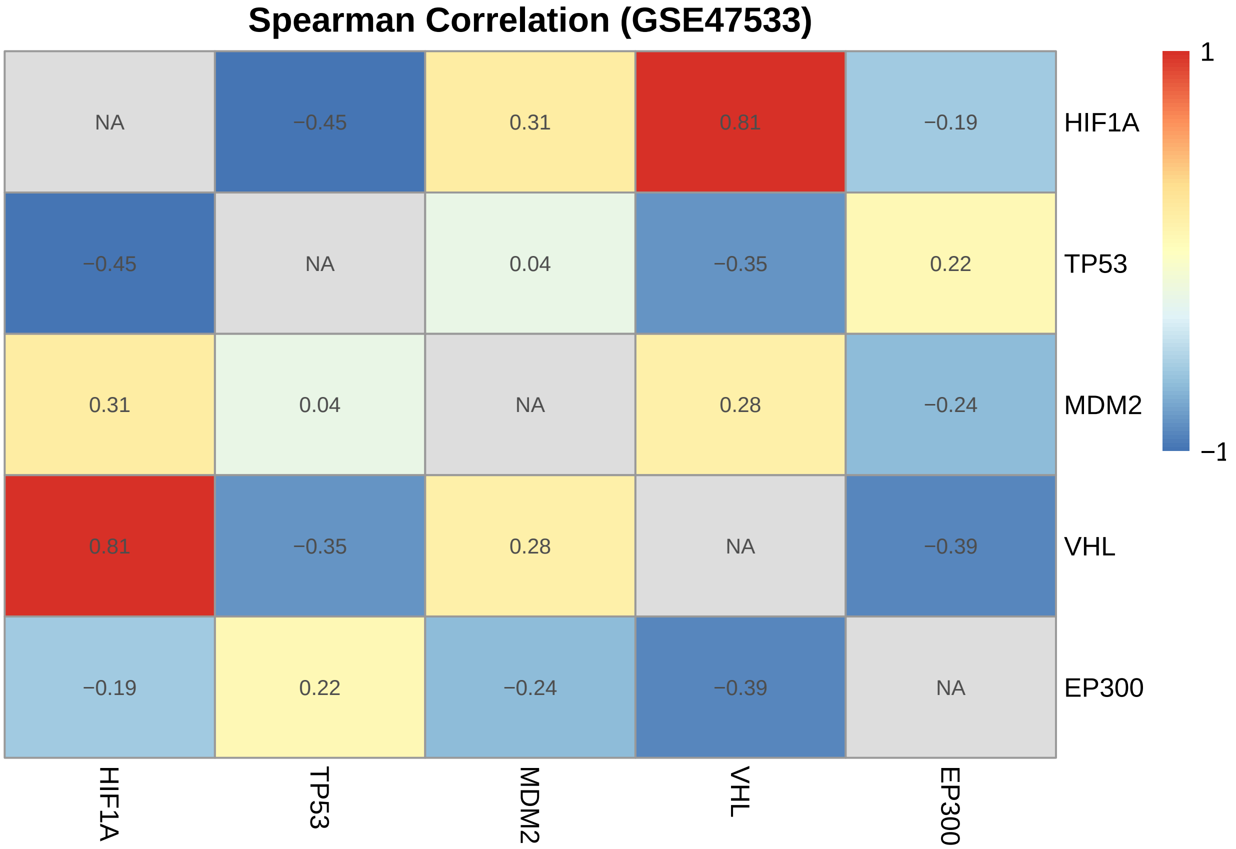
Clustering of Gene Expression on Hypoxia
Euclidian Distance (GSE47533)



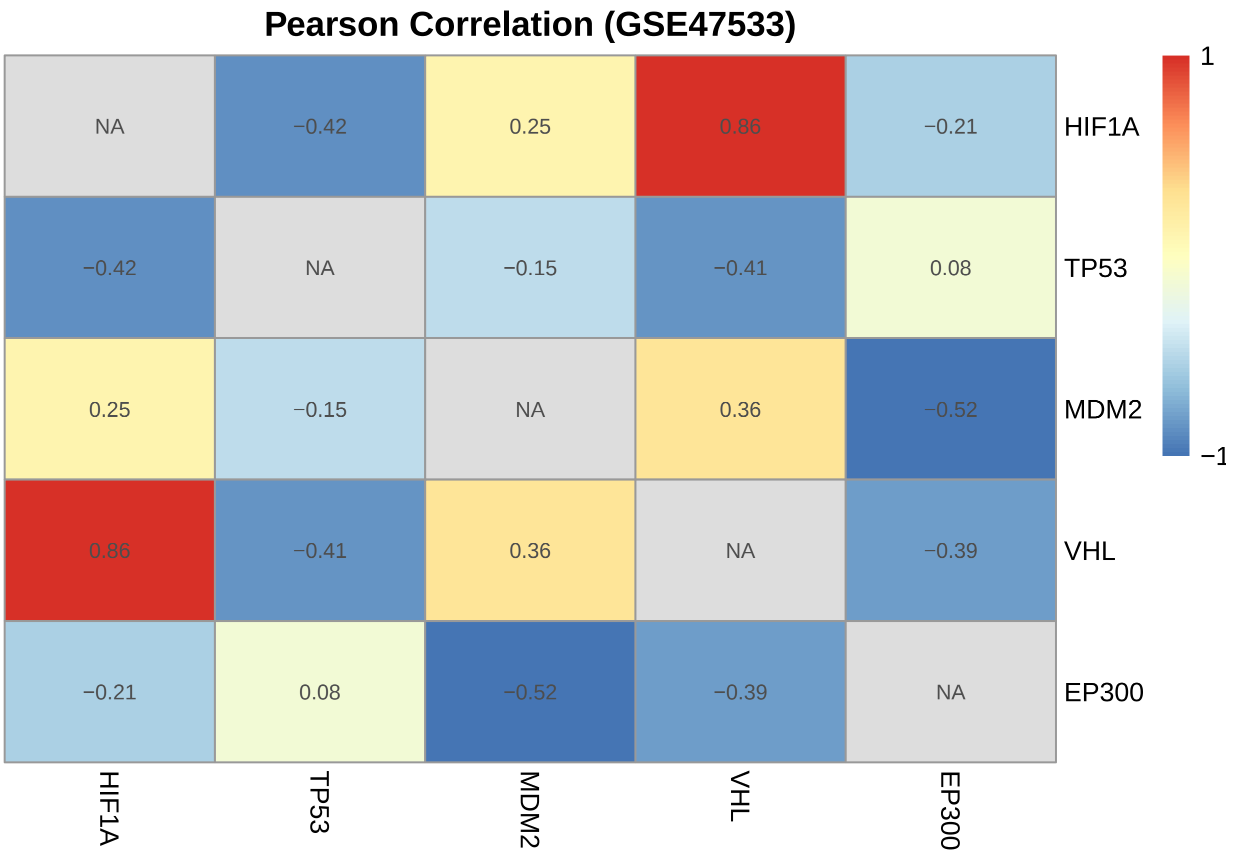
Clustering of Gene Expression on Normoxia
Euclidian Distance (GSE47533)

```r
dists.GSE47533_spearman <- cor(t(expr.GSE47533.hif), use = "pairwise.complete.obs", method = "spearman"
rownames(dists.GSE47533_spearman) <- rownames(expr.GSE47533.hif)
colnames(dists.GSE47533_spearman) <- rownames(expr.GSE47533.hif)
diag(dists.GSE47533_spearman) <- NA
```

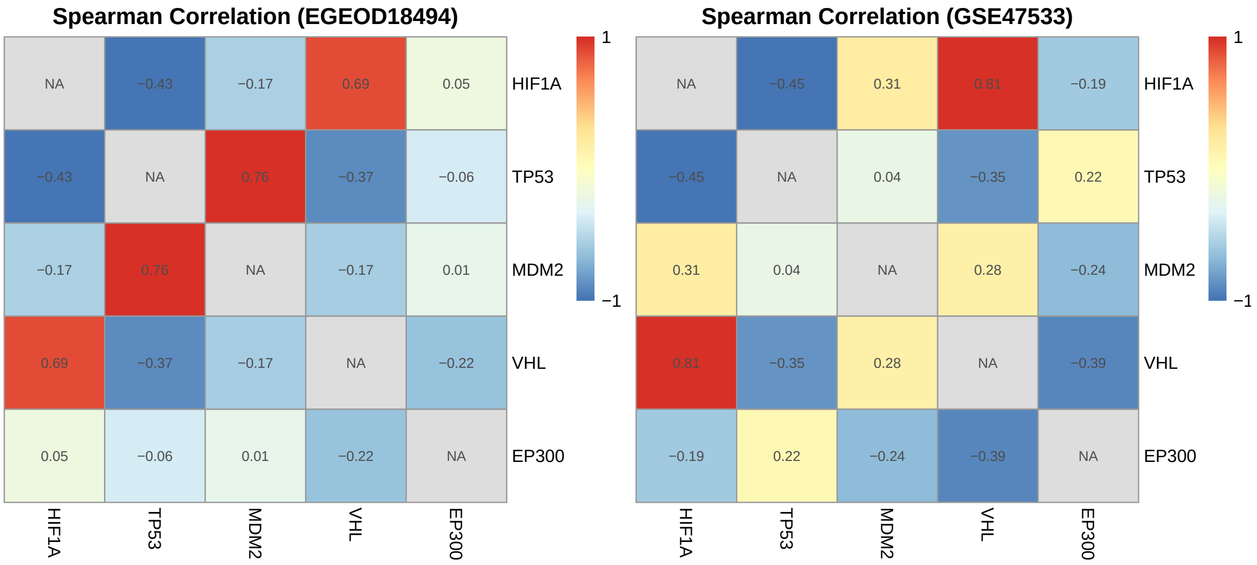
```
ps.GSE47533_spearman <- pheatmap(dists.GSE47533_spearman,
                           legend = TRUE,
                           display_numbers = T,
                           treeheight_row = 0,
                           cluster_rows = F,
                           cluster_cols = F,
                           legend_breaks = c(min(dists.GSE47533_spearman, na.rm = TRUE),
                                             max(dists.GSE47533_spearman, na.rm = TRUE)),
                           legend_labels = (c("-1", "1")),
                           main = "Spearman Correlation (GSE47533)")
```



```
dists.GSE47533_pearson <- cor(t(expr.GSE47533.hif), use = "pairwise.complete.obs", method = "pearson")
rownames(dists.GSE47533_pearson) <- rownames(expr.GSE47533.hif)
colnames(dists.GSE47533_pearson) <- rownames(expr.GSE47533.hif)
diag(dists.GSE47533_pearson) <- NA

ps.GSE47533_pearson <- pheatmap(dists.GSE47533_pearson,
         legend = TRUE,
         display_numbers = T,
         cluster_rows = F,
         cluster_cols = F,
         treeheight_row = 0,
         legend_breaks = c(min(dists.GSE47533_pearson, na.rm = TRUE),
                           max(dists.GSE47533_pearson, na.rm = TRUE)),
         legend_labels = (c("-1", "1")),
         main = "Pearson Correlation (GSE47533)")
```



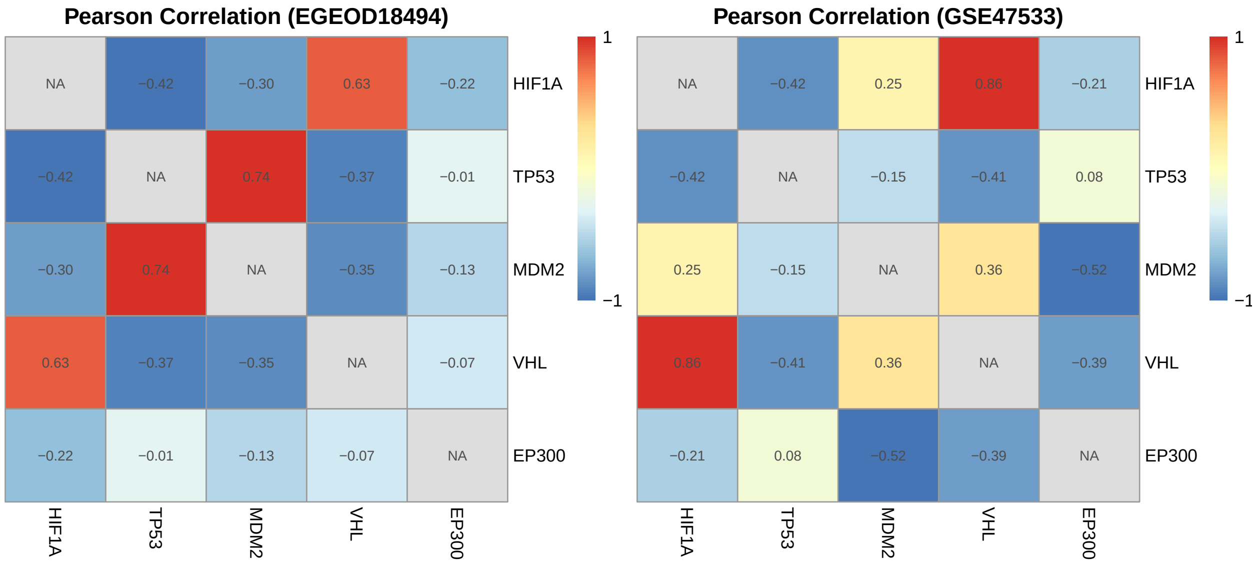
```
gridExtra::grid.arrange(grobs=list(ps.EGEOD18494_spearman$gtable, ps.GSE47533_spearman$gtable),
                      nrow = 1 , labels=c('A', 'B'))
```



```
cortest(dists.EGEOD18494_spearman, dists.GSE47533_spearman)
```

```
## Warning in cortest(dists.EGEOD18494_spearman, dists.GSE47533_spearman): n not
## specified, 100 used

## Tests of correlation matrices
## Call:cortest(R1 = dists.EGEOD18494_spearman, R2 = dists.GSE47533_spearman)
##  Chi Square value 78.83  with df =  10   with probability < 8.5e-13
## z of differences =  0.24
```

```
gridExtra::grid.arrange(grobs=list(ps.EGEOD18494_pearson$gtable, ps.GSE47533_pearson$gtable),
                        nrow = 1 , labels=c('A', 'B'))
```



```
cortest(dists.EGEOD18494_pearson, dists.GSE47533_pearson)
```

```
## Warning in cortest(dists.EGEOD18494_pearson, dists.GSE47533_pearson): n not
## specified, 100 used
```

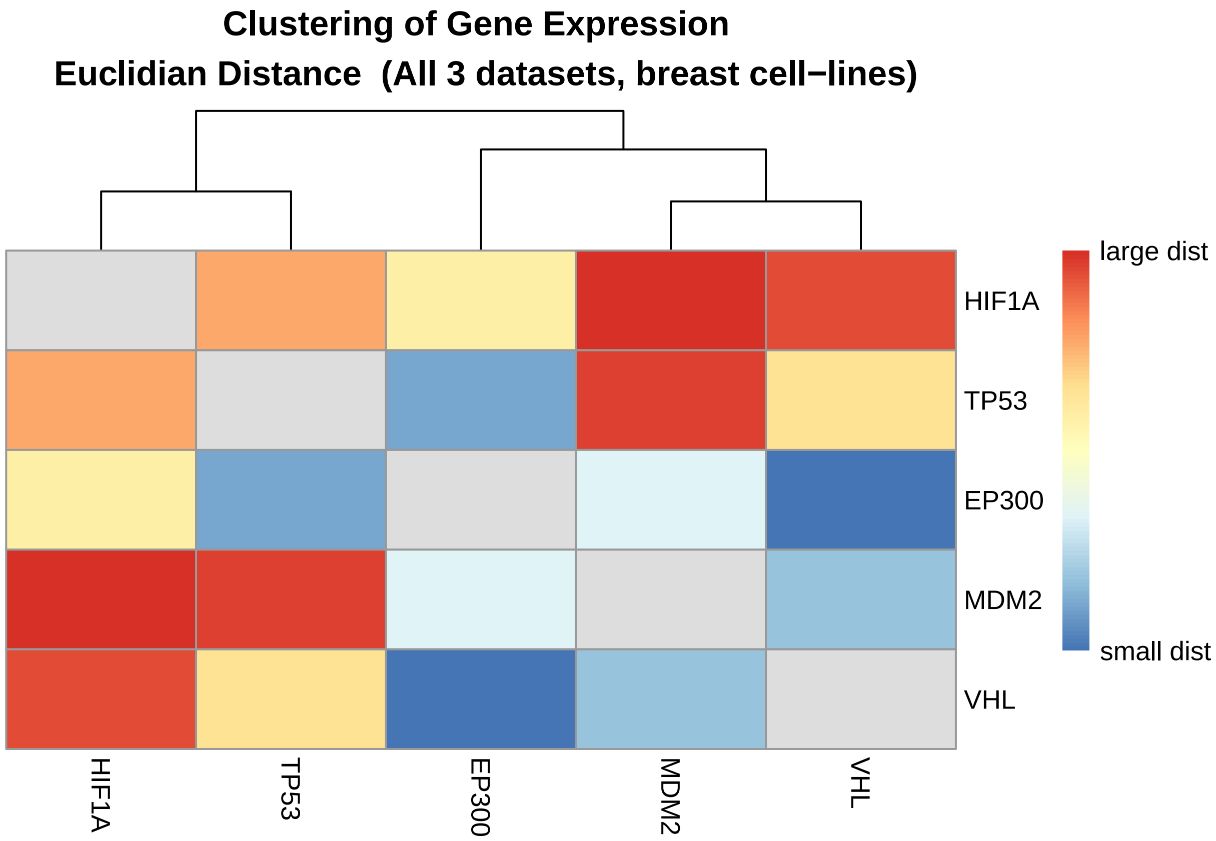
```
## Tests of correlation matrices
## Call:cortest(R1 = dists.EGEOD18494_pearson, R2 = dists.GSE47533_pearson)
##  Chi Square value 127.9  with df =  10   with probability < 1.3e-22
## z of differences =  0.4
```

Heatmaps - All datasets Breast Cell-lines (E-GEOD-18494, GSE47533, and GSE41491)

- E-GEOD-18494 2012 / MDA-MB231 / breast / 4h, 8h, 12h / microarray
- GSE41491 2012 / MCF7 / breast / 1h, 2h, 4h, 8h, 12h, 16h, 24h / microarray
- GSE47534 2014 / MCF7 / breast / normoxia, 16h, 32h, 48h / mRNA

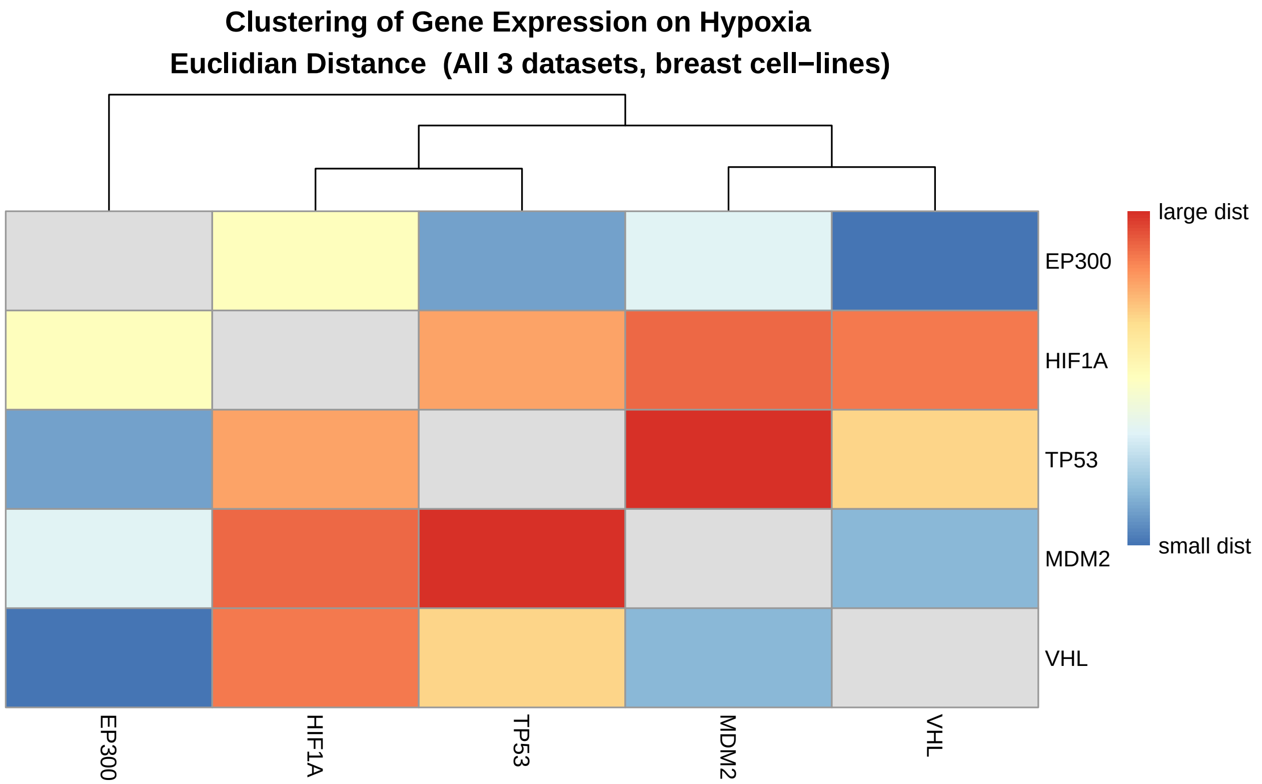
```
# Imput the mean of all VHL values
mean.vhl <- mean(unlist(expr.GSE47533.hif["VHL",], expr.EGEOD18494.hif["VHL",]))
expr.GSE41491.hif["VHL",] <- rep(mean.vhl, 24)

expr.all.hif <- cbind(expr.GSE47533.hif, expr.EGEOD18494.hif, expr.GSE41491.hif)

col_brca <- union(data.GSE47533$codes[data.GSE47533$cell_line == "MCF7"],
                  union(data.EGEOD18494$codes[data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"],
                        data.GSE41491$codes[data.GSE41491$cell_line == "MCF7"]))

expr.all.hif <- expr.all.hif[, (colnames(expr.all.hif) %in% col_brca)]
```

```
dists <- as.matrix(dist(expr.all.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif)
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA

pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression \n Euclidian Distance  (All 3 datasets, breast cell-line
```

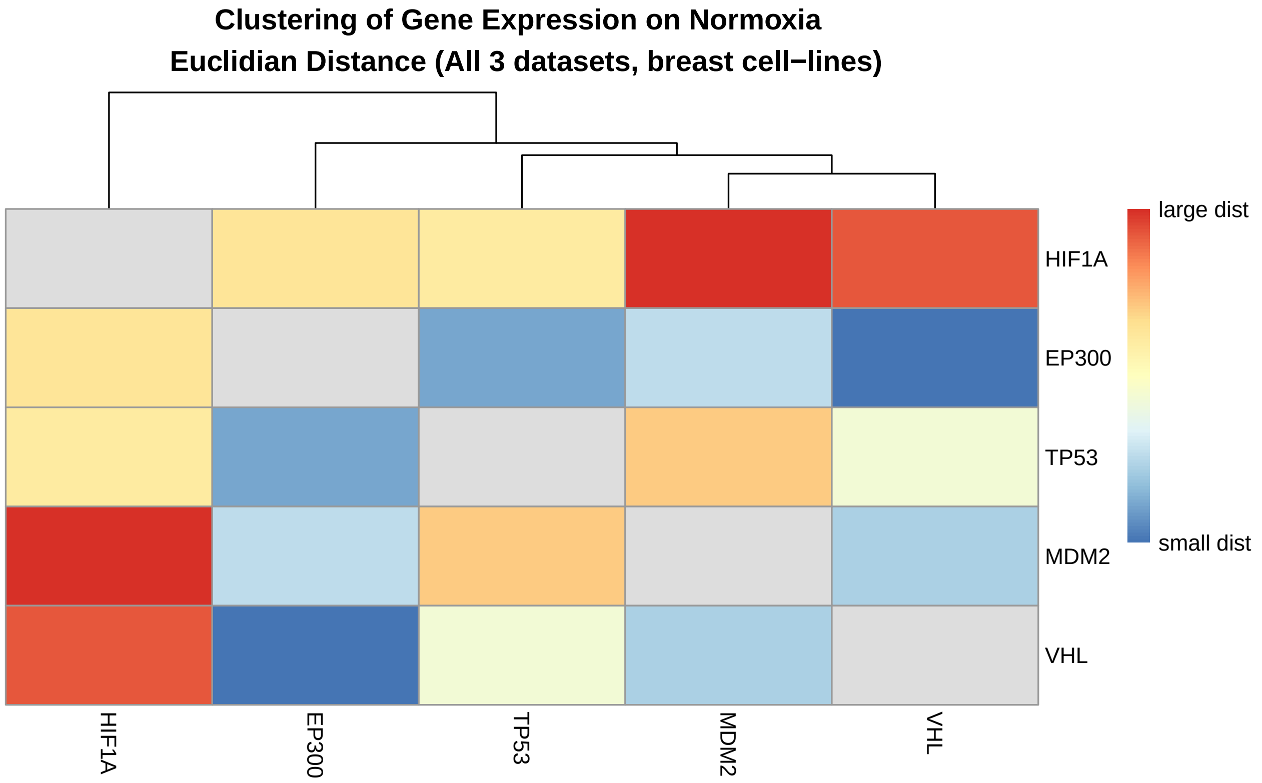


```
#--------------------------------------------------------------------------------

col_hypo <- union(data.GSE47533$codes[data.GSE47533$condition == "Hypo"],
                  union(data.EGEOD18494$codes[data.EGEOD18494$condition == "hypoxia"],
                        data.GSE41491$codes[data.GSE41491$condition == "hy"]))

expr.row <- (colnames(expr.all.hif) %in% col_hypo)
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA


p1 <- pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Hypoxia \n  Euclidian Distance  (All 3 datasets, breas
         silent=T)

#----------------------------------------------------------------------------------

col_norm <- union(data.GSE47533$codes[data.GSE47533$condition == "Norm"],
                  union(data.EGEOD18494$codes[data.EGEOD18494$condition == "normoxia"],
                        data.GSE41491$codes[data.GSE41491$condition == "no"]))

expr.row <- (colnames(expr.all.hif) %in% col_norm)
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA


p2 <- pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance (All 3 datasets, breast
         silent=T)
```

```
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
                        nrow = 2 , labels=c('A', 'B'))
```



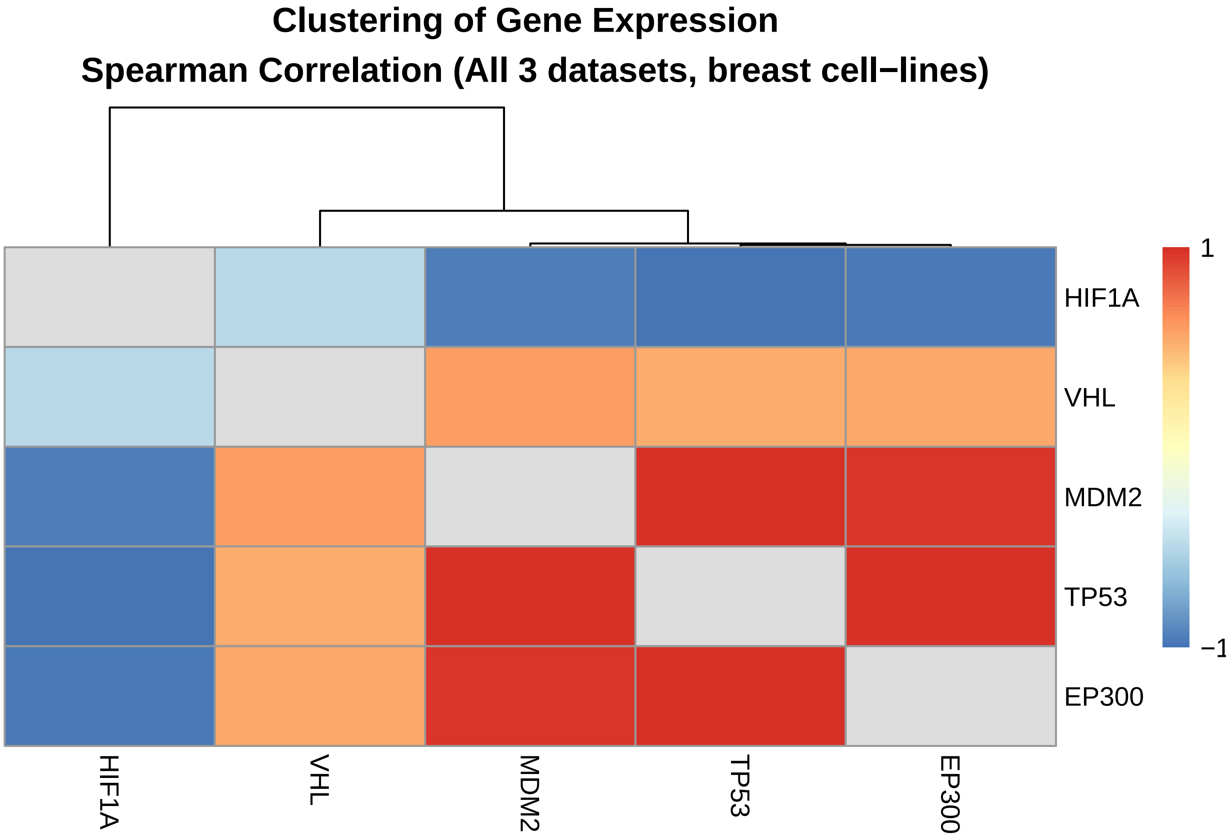
Clustering of Gene Expression on Hypoxia
Euclidian Distance (All 3 datasets, breast cell−lines)



Clustering of Gene Expression on Normoxia
Euclidian Distance (All 3 datasets, breast cell−lines)

```r
dists <- cor(t(expr.all.hif), use = "pairwise.complete.obs", method = "spearman")
rownames(dists) <- rownames(expr.all.hif)
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA
```

```
pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("-1", "1")),
         main = "Clustering of Gene Expression \n Spearman Correlation (All 3 datasets, breast cell-lin
```

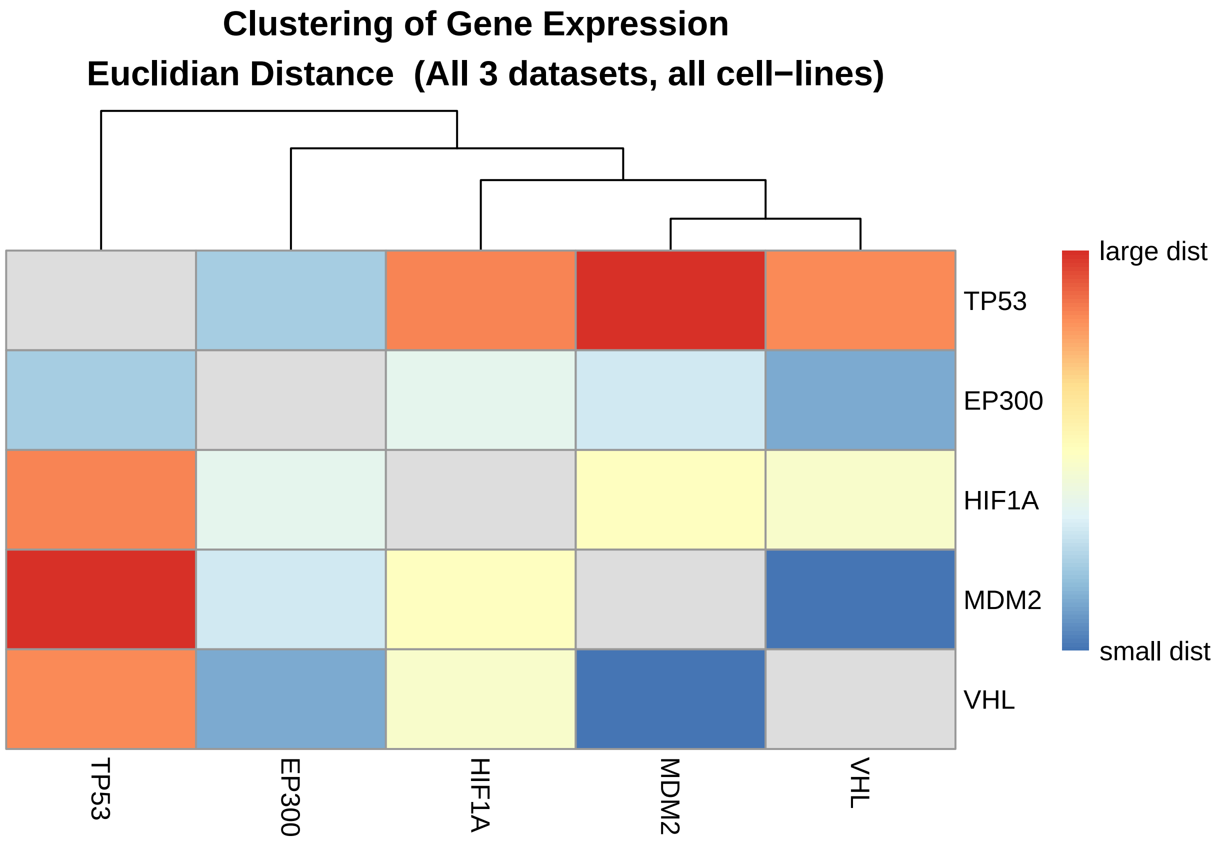


Heatmaps - All datasets All Cell-lines (E-GEOD-18494, GSE47533, and GSE41491)

- E-GEOD-18494 2012 / HepG2, U87, MDA-MB231 / hepatoma, glioma, breast / 4h, 8h, 12h / microarray
- GSE41491 2012 / DU145, HT29, MCF7 / prostate, colon, breast / 1h, 2h, 4h, 8h, 12h, 16h, 24h / microarray
- GSE47534 2014 / MCF7 / breast / normoxia, 16h, 32h, 48h / mRNA

```
# Imput the mean of all VHL values
mean.vhl <- mean(unlist(expr.GSE47533.hif["VHL",], expr.EGEOD18494.hif["VHL",]))
expr.GSE41491.hif["VHL",] <- rep(mean.vhl, 24)

expr.all.hif <- cbind(expr.GSE47533.hif, expr.EGEOD18494.hif, expr.GSE41491.hif)
```

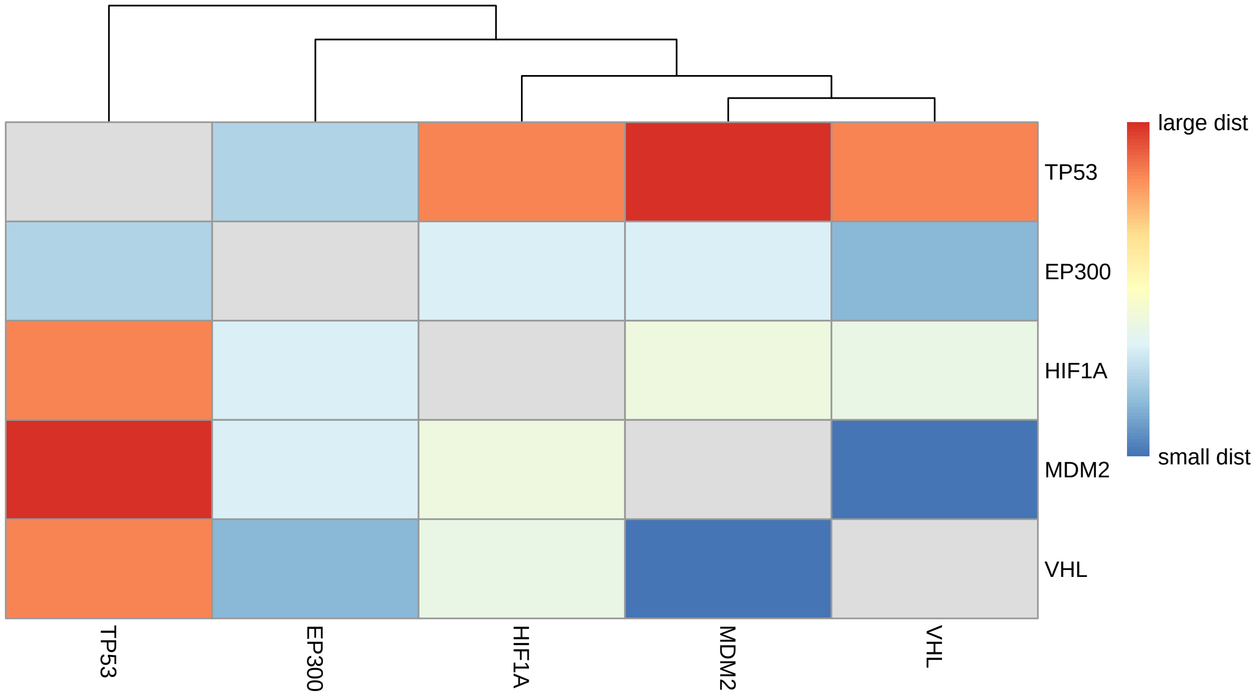
```r
dists <- as.matrix(dist(expr.all.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif)
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA

pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression \n Euclidian Distance  (All 3 datasets, all cell-lines)"
```



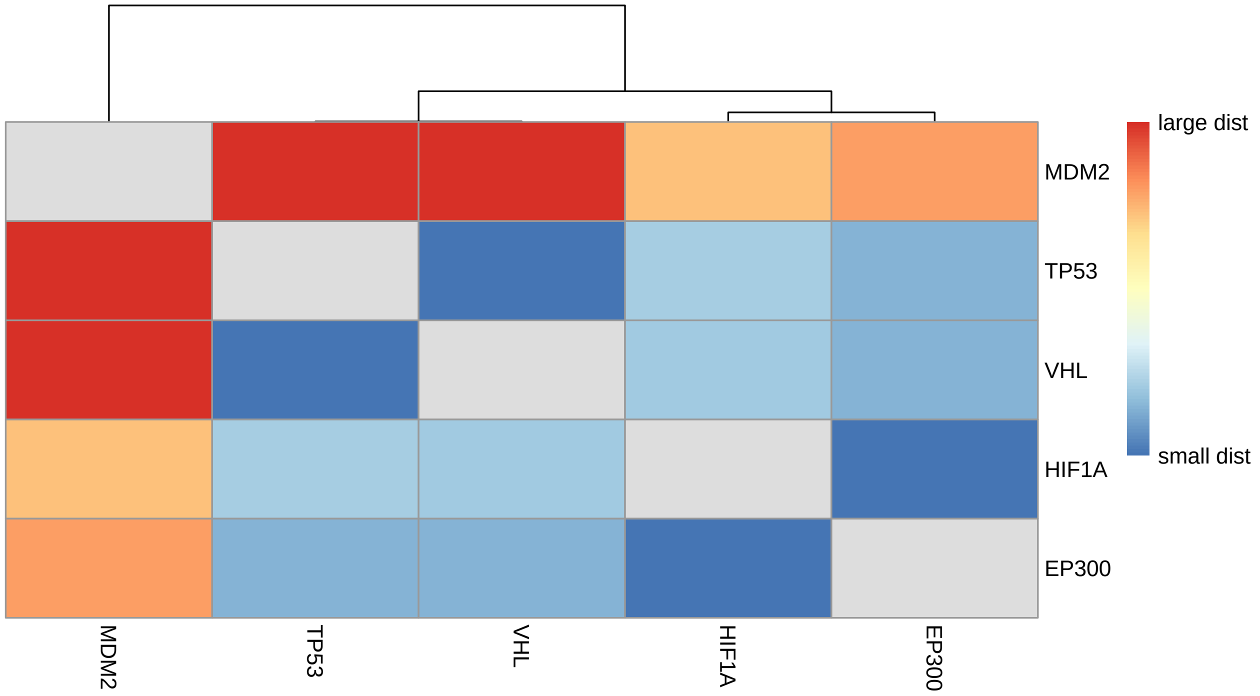
```r
#---------------------------------------------------------------------------------

col_hypo <- union(data.GSE47533$codes[data.GSE47533$condition == "Hypo"],
                  union(data.EGEOD18494$codes[data.EGEOD18494$condition == "hypoxia"],
                        data.GSE41491$codes[data.GSE41491$condition == "hy"]))

expr.row <- (colnames(expr.all.hif) %in% col_hypo)
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA


p1 <- pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Hypoxia \n  Euclidian Distance  (All 3 datasets, all
         silent=T)

#----------------------------------------------------------------------------------

expr.row <- (colnames(expr.all.hif) %in% data.GSE47533$codes[data.GSE47533$condition == "Norm"])
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA


p2 <- pheatmap(dists,
         legend = TRUE,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("small dist", "large dist")),
         main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance (All 3 datasets, all c
         silent=T)
```

```
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
                        nrow = 2 , labels=c('A', 'B'))
```

Clustering of Gene Expression on Hypoxia
Euclidian Distance (All 3 datasets, all cell−lines)



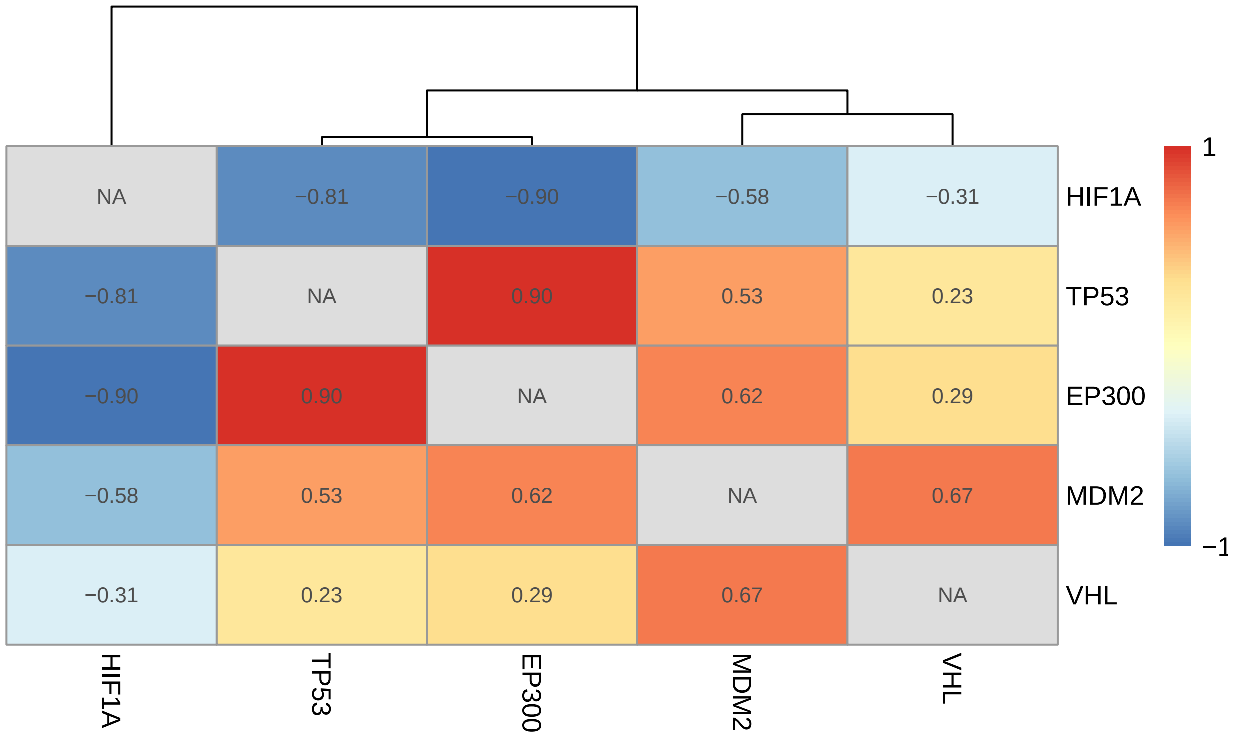
Clustering of Gene Expression on Normoxia
Euclidian Distance (All 3 datasets, all cell−lines)



```
dists <- cor(t(expr.all.hif), use = "pairwise.complete.obs", method = "spearman")
rownames(dists) <- rownames(expr.all.hif)
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA
```

```r
pheatmap(dists,
         legend = TRUE,
         display_numbers = T,
         treeheight_row = 0,
         legend_breaks = c(min(dists, na.rm = TRUE),
                           max(dists, na.rm = TRUE)),
         legend_labels = (c("-1", "1")),
         main = "Clustering of Gene Expression \n Spearman Correlation (All 3 datasets, all cell-lines)
```

Clustering of Gene Expression
Spearman Correlation (All 3 datasets, all cell−lines)



	HIF1A	TP53	EP300	MDM2	VHL
HIF1A	NA	−0.81	−0.90	−0.58	−0.31
TP53	−0.81	NA	0.90	0.53	0.23
EP300	−0.90	0.90	NA	0.62	0.29
MDM2	−0.58	0.53	0.62	NA	0.67
VHL	−0.31	0.23	0.29	0.67	NA