BoolNet Inference HepG2 hepatoma, U87 glioma, and MDA-MB231 breast cancer (E-GEOD-18494)

Expression profiling of hypoxic HepG2 hepatoma, U87 glioma, and MDA-MB231 breast cancer cells: time course (E-GEOD-18494)

Analysis of expression changes of cultured HepG2 hepatoma, U87 glioma, and MDA-MB231 breast cancer cells subjected to hypoxia (0.5% O2) for 0, 4, 8, 12 hours . Results provide insight to cell type-specific response to hypoxia. HepG2 hepatoma, U87 glioma, and MDA-MB231 breast cancer cells were collected under normoxic conditions (~19% O2, 0 hours) and after 4, 8 and 12 hours of hypoxia treatment (0.5% O2). For each cell line, three replicates of total RNA at each time point were prepared using Trizol and submitted to the DFCI Microarray Core for labeling, hybridization to Affymetrix HG-U133Plus2 oligonucleotide arrays and image scanning.

https://www.ebi.ac.uk/arrayexpress/experiments/E-GEOD-18494/

```
packages_cran = c("igraph", "BoolNet", "BiocManager", "tidyverse", "fs")
# Install and load packages
package.check <- lapply(packages_cran, FUN = function(x) {
  if (!require(x, character.only = TRUE)) {
    install.packages(x, dependencies = TRUE)
    library(x, character.only = TRUE)
  }
})
packages_bioconductor = c("Biobase", "GEOquery", "vsn", "hgu133plus2.db")
# Install and load packages
package.check <- lapply(packages_bioconductor, FUN = function(x) {
  if (!require(x, character.only = TRUE)) {
    BiocManager::install(x, dependencies = TRUE)
    library(x, character.only = TRUE)
  }
})

rm(package.check, packages_bioconductor, packages_cran)
```

Load the pre-processed

```
load("../data/data.EGEOD18494.Rdata")
eset <- ExpressionSet(assayData = as.matrix(expr.EGEOD18494),
                      probeNames = row.names(expr.EGEOD18494))
expr.EGEOD18494 <- exprs(justvsn(eset))
```

Selecting the HIF Genes

```
# Selecting genes from HIF Axis
hif.symbols <- c("TP53", "HIF1A", "EP300", "MDM2", "VHL")

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

# Select the probes and genes
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") %
  #distinct(symbol, .keep_all = TRUE) %>% # Take the first one
  dplyr::select(!(probes))
```

```
# Function to binarize according an consensus mean of probes, add the O2 state and rename columns

binNet <- function(b){

  cols <- data.EGEOD18494$codes %in% names(b)

  binarizeTimeSeries(b[,-1], method="kmeans")$binarizedMeasurements  %>%
  as.data.frame(.)  %>%
  aggregate(., list(symbol = b$symbol), mean) %>%  # mean of binarized probes
  mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%  # consensus with a bies to 1 (>= 0.5)
  rbind(., c("O2", 1,0,0,0)) %>%
    rename_at(vars(data.EGEOD18494$codes[cols] ),
              ~paste0(substr(data.EGEOD18494$condition[cols],1,2),".",
                      data.EGEOD18494$time[cols],".",
                      substr(data.EGEOD18494$cell_line[cols],1,2), ".",
                      data.EGEOD18494$rep[cols])) %>%
  column_to_rownames("symbol")

}
```

Exemplifying the Binarization

```
cols <- (data.EGEOD18494$cell_line == "MDA-MB231 breast cancer" & data.EGEOD18494$rep == 1)

breast1x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cols])) %>% arrange(symbol) %>%
  arrange(symbol) %>%
  rename_at(vars(data.EGEOD18494$codes[cols]),
            ~paste0(substr(data.EGEOD18494$condition[cols],1,2),".",
                    data.EGEOD18494$time[cols],".",
                    substr(data.EGEOD18494$cell_line[cols],1,2)))

breast1x %>%
  knitr::kable(.)
```

symbol	no.control.MD	hy.4h.MD	hy.8h.MD	hy.12h.MD
EP300	2.549070	2.629721	2.656562	2.550405
EP300	2.620624	2.644411	2.657875	2.616628
HIF1A	3.454857	3.379930	3.202643	3.155433
MDM2	2.087500	2.017202	2.029907	2.064475
MDM2	1.466399	1.361895	1.489469	1.575513
MDM2	1.927003	1.868816	1.911444	1.892719
MDM2	2.346662	2.340452	2.357972	2.474810
MDM2	1.588123	1.798685	1.766015	1.672476
MDM2	2.787360	2.792702	2.799612	2.776623
MDM2	2.590166	2.572969	2.551526	2.513453
MDM2	1.226232	1.348273	1.311544	1.496668
MDM2	1.471376	1.510237	1.483707	1.575065
MDM2	2.796643	2.706115	2.677469	2.714001
TP53	2.933592	2.912386	2.972610	2.984760
TP53	2.876457	2.805545	2.884647	2.894870
VHL	2.686189	2.691444	2.606037	2.549339
VHL	1.296047	1.302045	1.315025	1.235383

```
binarizeTimeSeries(breast1x[,-1], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  add_column(symbol = breast1x$symbol, .before=0) %>%
  knitr::kable(.)
```

symbol	no.control.MD	hy.4h.MD	hy.8h.MD	hy.12h.MD
EP300	0	1	1	0
EP300	0	1	1	0
HIF1A	1	1	0	0
MDM2	1	0	0	1
MDM2	1	0	1	1
MDM2	1	0	1	0
MDM2	0	0	0	1
MDM2	0	1	1	0
MDM2	1	1	1	0
MDM2	1	1	1	0
MDM2	0	0	0	1
MDM2	0	0	0	1
MDM2	1	0	0	0
TP53	0	0	1	1
TP53	1	0	1	1
VHL	1	1	0	0
VHL	1	1	1	0

```
binarizeTimeSeries(breast1x[,-1], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  aggregate(., list(symbol = breast1x$symbol), mean) %>%
  mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%
  rbind(., c("O2", 1,0,0,0)) %>%
  knitr::kable(.)
```

```
## 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.
```

symbol	no.control.MD	hy.4h.MD	hy.8h.MD	hy.12h.MD
EP300	0	1	1	0
HIF1A	1	1	0	0
MDM2	1	0	1	1
TP53	1	0	1	1
VHL	1	1	1	0
O2	1	0	0	0

MDA-MB231 breast cancer

```
cellline.rep1 <- (data.EGEOD18494$cell_line == "MDA-MB231 breast cancer" &  data.EGEOD18494$rep == 1)
cellline.rep2 <- (data.EGEOD18494$cell_line == "MDA-MB231 breast cancer" &  data.EGEOD18494$rep == 2)
cellline.rep3 <- (data.EGEOD18494$cell_line == "MDA-MB231 breast cancer" &  data.EGEOD18494$rep == 3)

breast1x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep1])) %>% binNet(.)

breast1x %>% knitr::kable(.)
```

	no.control.MD.1	hy.4h.MD.1	hy.8h.MD.1	hy.12h.MD.1
EP300	0	1	1	0
HIF1A	1	1	0	0
MDM2	1	0	1	1
TP53	1	0	1	1
VHL	1	1	1	0
O2	1	0	0	0

```
breast2x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep2])) %>% binNet(.)

breast2x  %>% knitr::kable(.)
```

	no.control.MD.2	hy.4h.MD.2	hy.8h.MD.2	hy.12h.MD.2
EP300	1	0	1	1
HIF1A	1	1	0	0
MDM2	1	0	1	0
TP53	0	1	1	1
VHL	1	1	1	0
O2	1	0	0	0

```
breast3x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep3])) %>% binNet(.)

breast3x %>% knitr::kable(.)
```

	no.control.MD.3	hy.4h.MD.3	hy.8h.MD.3	hy.12h.MD.3
EP300	0	1	1	1
HIF1A	1	1	0	0
MDM2	1	1	0	1
TP53	0	1	1	1
VHL	1	1	0	1
O2	1	0	0	0

HepG2 hepatoma

```
cellline.rep1 <- (data.EGEOD18494$cell_line == "HepG2 hepatoma" &  data.EGEOD18494$rep == 1)
cellline.rep2 <- (data.EGEOD18494$cell_line == "HepG2 hepatoma" &  data.EGEOD18494$rep == 2)
cellline.rep3 <- (data.EGEOD18494$cell_line == "HepG2 hepatoma" &  data.EGEOD18494$rep == 3)

hepatoma1x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep1]))  %>%
  binNet(.)

hepatoma1x %>%
  knitr::kable(.)
```

	no.control.He.1	hy.4h.He.1	hy.8h.He.1	hy.12h.He.1
EP300	1	1	0	0
HIF1A	0	0	1	0
MDM2	0	1	0	1
TP53	1	1	0	1
VHL	1	0	1	0
O2	1	0	0	0

```
hepatoma2x <-
expr.EGEOD18494.hif %>%
```

```
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep2]))  %>%
  binNet(.)

hepatoma2x %>%
  knitr::kable(.)
```

	no.control.He.2	hy.4h.He.2	hy.8h.He.2	hy.12h.He.2
EP300	0	1	1	1
HIF1A	0	0	1	0
MDM2	0	1	1	1
TP53	0	1	1	0
VHL	1	0	1	1
O2	1	0	0	0

```
hepatoma3x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep3]))  %>%
  binNet(.)

hepatoma3x %>%
  knitr::kable(.)
```

	no.control.He.3	hy.4h.He.3	hy.8h.He.3	hy.12h.He.3
EP300	0	1	0	1
HIF1A	0	1	1	0
MDM2	0	1	0	1
TP53	1	1	1	1
VHL	1	1	0	0
O2	1	0	0	0

U87 glioma

```
cellline.rep1 <- (data.EGEOD18494$cell_line == "U87 glioma" &  data.EGEOD18494$rep == 1)
cellline.rep2 <- (data.EGEOD18494$cell_line == "U87 glioma" &  data.EGEOD18494$rep == 2)
cellline.rep3 <- (data.EGEOD18494$cell_line == "U87 glioma" &  data.EGEOD18494$rep == 3)

glioma1x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep1]))  %>%
  binNet(.)

glioma1x %>%
  knitr::kable(.)
```

	no.control.U8.1	hy.4h.U8.1	hy.8h.U8.1	hy.12h.U8.1
EP300	1	0	1	1
HIF1A	1	0	0	0
MDM2	1	0	0	0
TP53	1	0	1	1
VHL	1	1	0	1
O2	1	0	0	0

```
glioma2x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep2]))  %>%
  binNet(.)

glioma2x %>%
  knitr::kable(.)
```

	no.control.U8.2	hy.4h.U8.2	hy.8h.U8.2	hy.12h.U8.2
EP300	1	0	1	0
HIF1A	1	1	0	0
MDM2	1	0	0	1
TP53	1	0	1	0
VHL	0	1	1	0
O2	1	0	0	0

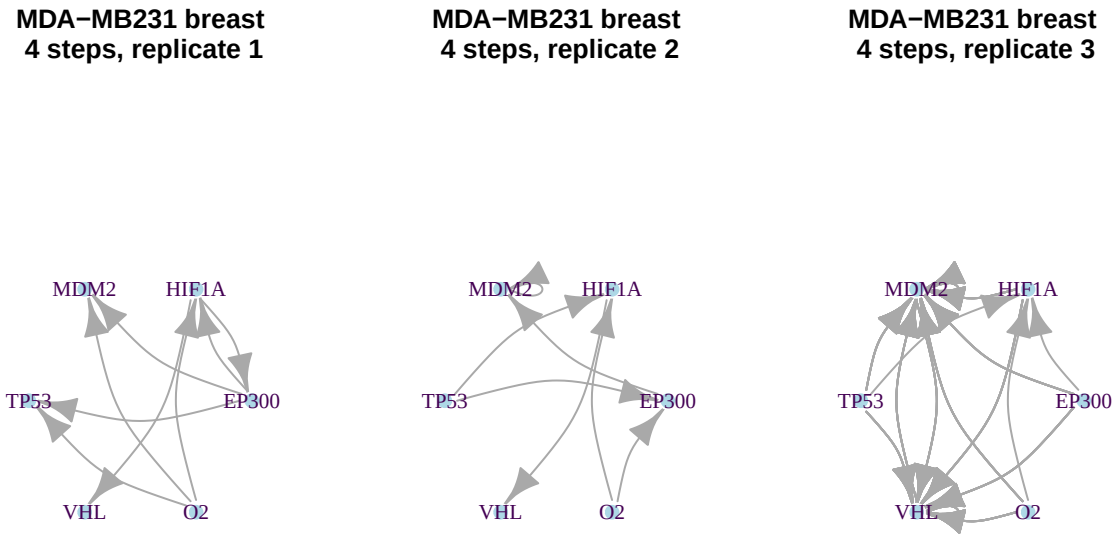
```
glioma3x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep3]))  %>%
  binNet(.)

glioma3x %>%
  knitr::kable(.)
```

	no.control.U8.3	hy.4h.U8.3	hy.8h.U8.3	hy.12h.U8.3
EP300	1	1	1	0
HIF1A	1	1	0	0
MDM2	1	1	1	0
TP53	1	1	1	1
VHL	1	1	1	1
O2	1	0	0	0

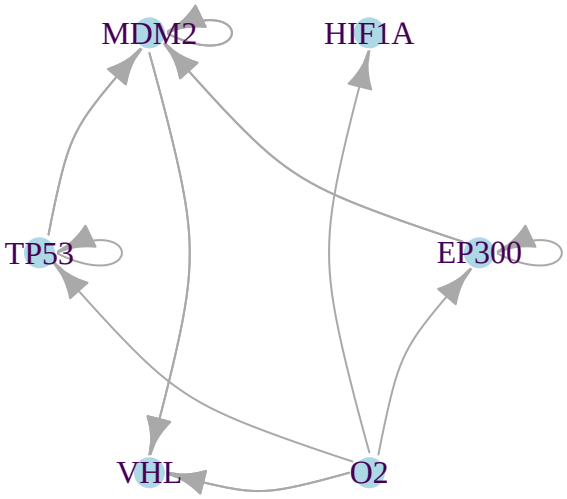
Network inference:

```
# MDA-MB231 breast cancer - 4 time-points
par(mfrow = c(1,3))
plot(breast1x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white",
     main="MDA-MB231 breast\n 4 steps, replicate 1")
```

```
plot(breast2x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", l
     main="MDA-MB231 breast\n 4 steps, replicate 2")
plot(breast3x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", l
     main="MDA-MB231 breast\n 4 steps, replicate 3")
```

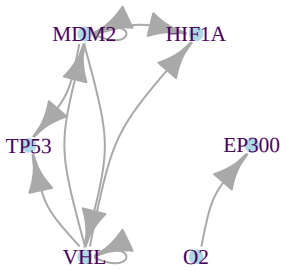



```
par(mfrow = c(1,1))
plot(breast.all.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
     main="MDA-MB231 breast\n 4 steps, replicate 3")
```

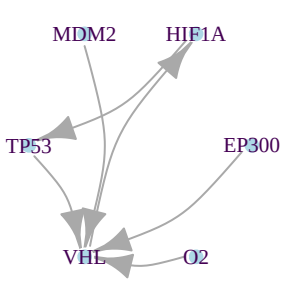



```
# HepG2 hepatoma
par(mfrow = c(1,3))
plot(hepatoma1x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
     main="HepG2 hepatoma\n 4 steps, replicate 1")
plot(hepatoma2x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
     main="HepG2 hepatoma\n 4 steps, replicate 2")
plot(hepatoma3x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
     main="HepG2 hepatoma\n 4 steps, replicate 3")
```

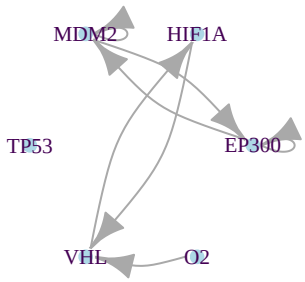
HepG2 hepatoma
4 steps, replicate 1



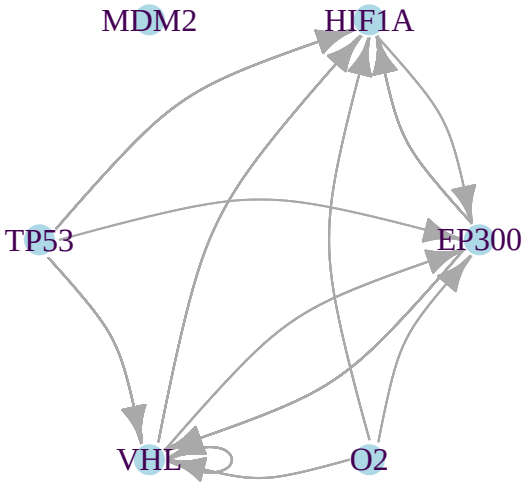
HepG2 hepatoma
4 steps, replicate 2



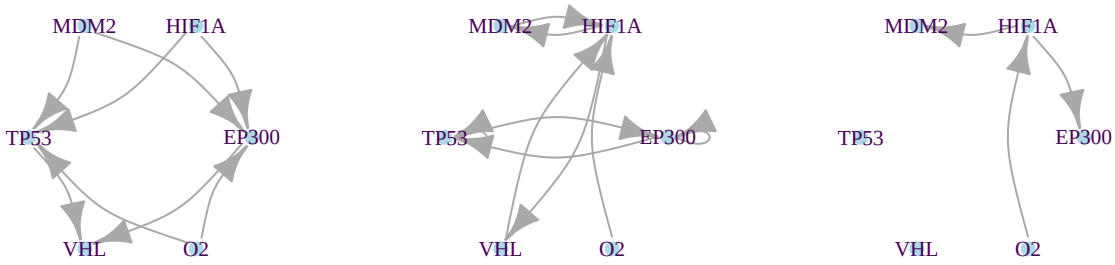
HepG2 hepatoma
4 steps, replicate 3




```
par(mfrow = c(1,1))
plot(hepatoma.all.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white
     main="HepG2 hepatoma\n 4 steps, replicate 3")
```




```
# U87 glioma
par(mfrow = c(1,3))
plot(glioma1x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", l
     main="U87 glioma\n 4 steps, replicate 1")
plot(glioma2x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", l
     main="U87 glioma\n 4 steps, replicate 2")
plot(glioma3x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", l
     main="U87 glioma\n 4 steps, replicate 3")
```




```
par(mfrow = c(1,1))
plot(glioma.all.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
     main="U87 glioma\n 4 steps, replicate 3")
```

U87 glioma
4 steps, replicate 3