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")

}
```

```
# Function to calculate the mean and binarize after, according an consensus mean of probes

meanBinNet <- function(b){

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

  b <-b %>%
  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])) %>%
  mutate(no.ctrl = rowMeans(dplyr::select(.,starts_with("no.control")), na.rm = TRUE)) %>%
  mutate(hy.4h = rowMeans(dplyr::select(.,starts_with("hy.4h")), na.rm = TRUE)) %>%
  mutate(hy.8h = rowMeans(dplyr::select(.,starts_with("hy.8h")), na.rm = TRUE)) %>%
  mutate(hy.12h = rowMeans(dplyr::select(.,starts_with("hy.12h")), na.rm = TRUE)) %>%
  dplyr::select(c("symbol", "no.ctrl", "hy.4h", "hy.8h", "hy.12h"))
```

```
  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)) %>%
  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	2.546113	2.626751	2.654011	2.547178
EP300	2.617732	2.641452	2.655325	2.613432
HIF1A	3.452495	3.377380	3.200654	3.152428
MDM2	2.084020	2.013627	2.026330	2.060950
MDM2	1.461779	1.357165	1.484469	1.571494
MDM2	1.923286	1.865037	1.907608	1.889048
MDM2	2.343501	2.337237	2.354995	2.471546
MDM2	1.583780	1.794800	1.761824	1.668576
MDM2	2.784604	2.789847	2.797232	2.773494
MDM2	2.587246	2.569956	2.548837	2.510208
MDM2	1.220965	1.343511	1.305911	1.492542
MDM2	1.466768	1.505834	1.478688	1.571045
MDM2	2.793894	2.703201	2.674944	2.710847
TP53	2.930941	2.909605	2.970412	2.981704
TP53	2.873767	2.802698	2.882359	2.891784
VHL	2.683352	2.688519	2.603420	2.546111
VHL	1.290983	1.297170	1.309405	1.230831

```
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("02", 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

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

```
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

```
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

```
breast.mean <-
cbind(breast1x, breast2x,breast3x) %>%
  tibble::rownames_to_column('gene') %>%
  mutate_at(vars(-gene), as.numeric) %>%
  mutate(no.ctrl = rowMeans(dplyr::select(.,starts_with("no.control")), na.rm = TRUE)) %>%
  mutate(hy.4h = rowMeans(dplyr::select(.,starts_with("hy.4h")), na.rm = TRUE)) %>%
  mutate(hy.8h = rowMeans(dplyr::select(.,starts_with("hy.8h")), na.rm = TRUE)) %>%
  mutate(hy.12h = rowMeans(dplyr::select(.,starts_with("hy.12h")), na.rm = TRUE)) %>%
  dplyr::select(c("no.ctrl", "hy.4h", "hy.8h", "hy.12h", "gene")) %>%
  mutate_at(c("no.ctrl", "hy.4h", "hy.8h", "hy.12h"), funs(ifelse(. >= 0.5, 1, 0)))  %>%  # consensus w
  tibble::column_to_rownames('gene')

breast.mean %>%
  knitr::kable(.)
```

	no.ctrl	hy.4h	hy.8h	hy.12h
EP300	0	1	1	1
HIF1A	1	1	0	0
MDM2	1	0	1	1
TP53	0	1	1	1
VHL	1	1	1	0

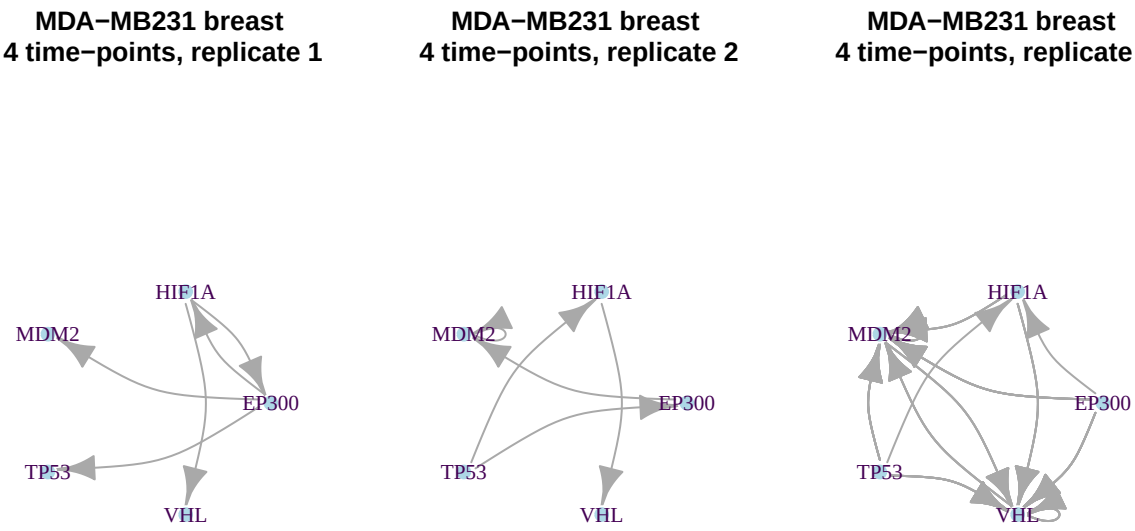
```
cellline.breast <- (data.EGEOD18494$cell_line == "MDA-MB231 breast cancer")

breast.meanBin <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.breast])) %>% meanBinNet(.)

breast.meanBin %>%
  knitr::kable(.)
```

	no.ctrl	hy.4h	hy.8h	hy.12h
EP300	0	1	1	0
HIF1A	1	1	0	0
MDM2	0	0	0	1
TP53	0	0	1	1
VHL	1	1	1	0

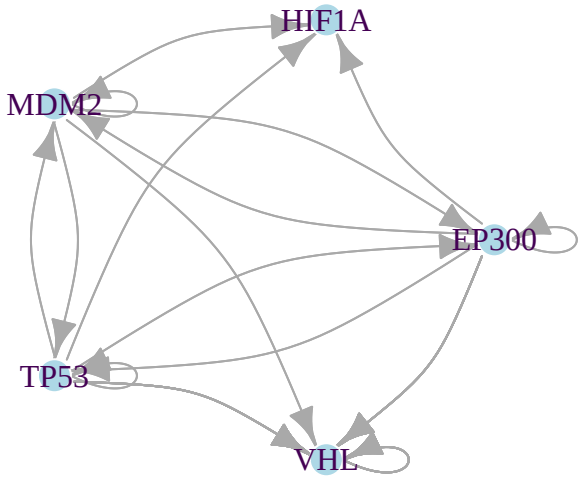
Network inference:

```
# 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", l
     main="MDA-MB231 breast\n 4 time-points, replicate 1")
plot(breast2x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", l
     main="MDA-MB231 breast\n 4 time-points, replicate 2")
plot(breast3x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", l
```

```
     main="MDA-MB231 breast\n 4 time-points, replicate 3")
```



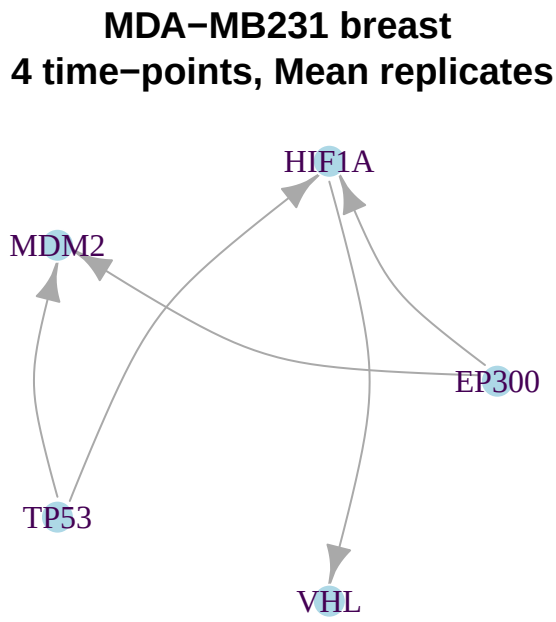

```
par(mfrow = c(1,1))
plot(breast.all.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
     main="MDA-MB231 breast\n 4 time-points, all replicate")
```




```
#
# # HepG2 hepatoma
# par(mfrow = c(1,3))
# plot(hepatoma1x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="whit
#      main="HepG2 hepatoma\n 4 steps, replicate 1")
# plot(hepatoma2x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="whit
#      main="HepG2 hepatoma\n 4 steps, replicate 2")
# plot(hepatoma3x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="whit
#      main="HepG2 hepatoma\n 4 steps, replicate 3")
#
# par(mfrow = c(1,1))
# plot(hepatoma.all.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="wh
#      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"
#      main="U87 glioma\n 4 steps, replicate 1")
# plot(glioma2x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
#      main="U87 glioma\n 4 steps, replicate 2")
# plot(glioma3x.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white"
#      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="whit
#      main="U87 glioma\n 4 steps, replicate 3")
```

Mean AFTER binarize the replicates of breast cancer net :

```
par(mfrow = c(1,1))
plot(mean.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", layou
     main="MDA-MB231 breast\n 4 time-points, Mean replicates")
```




```
print(mean.net)
```

```
## Probabilistic Boolean network with 5 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = 1 ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (!TP53) ( probability: 0.5, error: 0)
## HIF1A = (!EP300) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = (TP53) ( probability: 0.5, error: 0)
```

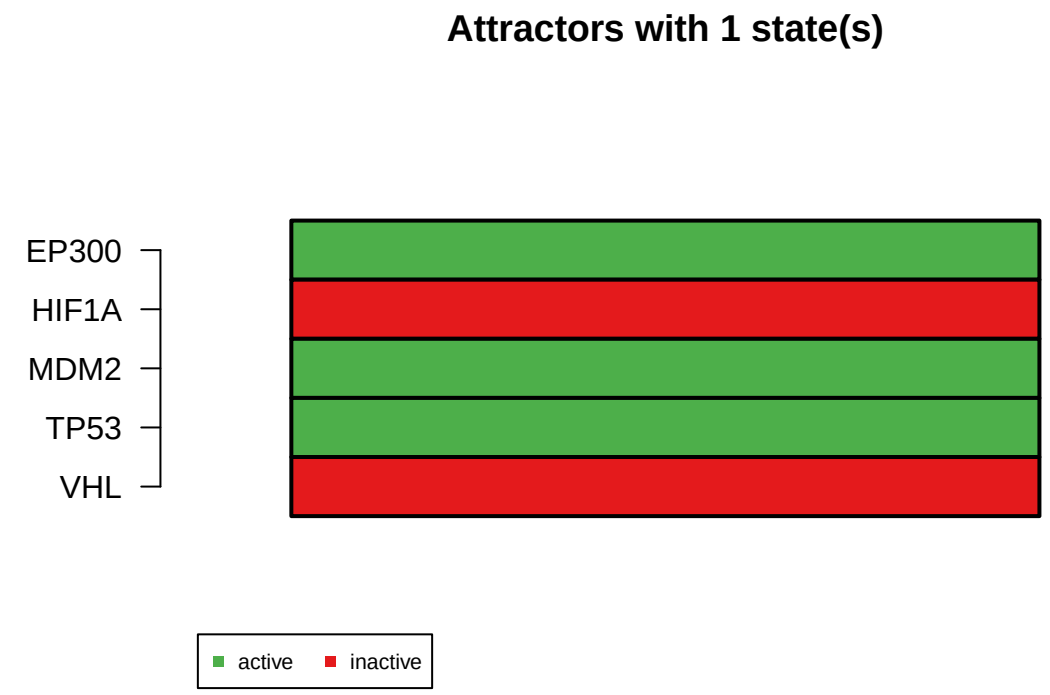
```
## MDM2 = (EP300) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = 1 ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (HIF1A) ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## EP300 = 1
## TP53 = 1
```

```
try({
sink("../data/ATOTS_inferred_EGEOD18494.bn")
cat("targets, factors\n")
cat("EP300, 1\n")
cat("HIF1A, (!TP53 | !EP300 )\n")
cat("MDM2, (TP53 | EP300)\n")
cat("TP53, 1\n")
cat("VHL, HIF1A\n")
sink()}, silent = T)
```

```
net <- loadNetwork("../data/ATOTS_inferred_EGEOD18494.bn")
print(net)
```

```
## Boolean network with 5 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL
##
## Transition functions:
## EP300 = 1
## HIF1A = (!TP53 | !EP300 )
## MDM2 = (TP53 | EP300)
## TP53 = 1
## VHL = HIF1A
##
## Knocked-out and over-expressed genes:
## EP300 = 1
## TP53 = 1
```

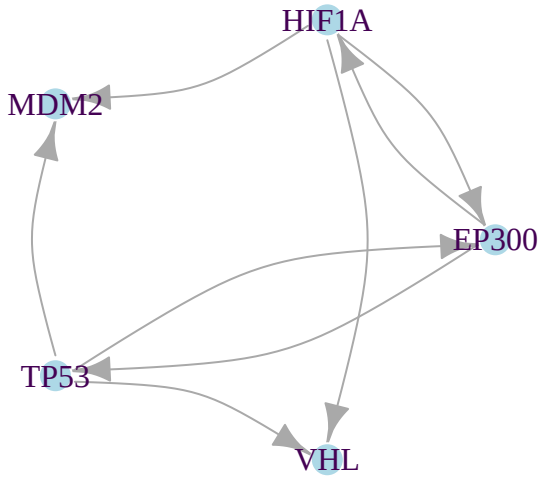
```
attr.syn <- getAttractors(net, type = "synchronous")
plotAttractors(attr.syn)
```




```
## $`1`
##       Attr1.1
## EP300       1
## HIF1A       0
## MDM2        1
## TP53        1
## VHL         0
```

Mean BEFORE binarize the replicates of breast cancer net :

```
par(mfrow = c(1,1))
plot(meanBin.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", la
     main="MDA-MB231 breast\n 4 time-points, Mean BEFORE binarize replicates")
```




```
print(meanBin.net)
```

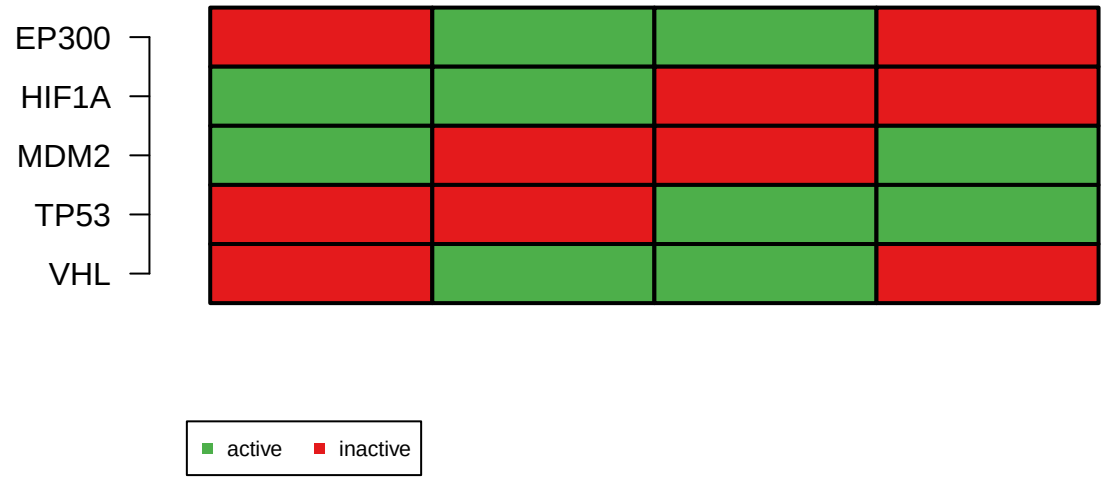
```
## Probabilistic Boolean network with 5 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (!TP53) ( probability: 0.5, error: 0)
## EP300 = (HIF1A) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (!EP300) ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = (TP53) ( probability: 0.5, error: 0)
## MDM2 = (!HIF1A) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (EP300) ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!TP53) ( probability: 0.5, error: 0)
## VHL = (HIF1A) ( probability: 0.5, error: 0)
```

```
try({
sink("../data/ATOTS_inferred_EGEOD18494_meanBin.bn")
cat("targets, factors\n")
cat("EP300, (!TP53 | HIF1A) \n")
cat("HIF1A,  !EP300 \n")
cat("MDM2, (TP53 | !HIF1A)\n")
cat("TP53, EP300\n")
cat("VHL, (!TP53 | HIF1A)\n")
sink()}, silent = T)
```

```
net <- loadNetwork("../data/ATOTS_inferred_EGEOD18494_meanBin.bn")
print(net)
```

```
## Boolean network with 5 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL
##
## Transition functions:
## EP300 = (!TP53 | HIF1A)
## HIF1A = !EP300
## MDM2 = (TP53 | !HIF1A)
## TP53 = EP300
## VHL = (!TP53 | HIF1A)
```

```
attr.syn <- getAttractors(net, type = "synchronous")
plotAttractors(attr.syn)
```




```
## $`4`
##       Attr1.1 Attr1.2 Attr1.3 Attr1.4
## EP300       0       1       1       0
## HIF1A       1       1       0       0
## MDM2        1       0       0       1
## TP53        0       0       1       1
## VHL         0       1       1       0
```