BoolNet Inference MCF-7 breast (GSE47533)

Integrated analysis of microRNA and mRNA expression and association with HIF binding in MCF-7 cells under hypoxia (GSE47533)

Camps C, Saini HK, Mole DR, Choudhry H et al. Integrated analysis of microRNA and mRNA expression and association with HIF binding reveals the complexity of microRNA expression regulation under hypoxia. Mol Cancer 2014 Feb 11;13:28. PMID: 24517586

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE47533

This SuperSeries is composed of the following SubSeries:

GSE47532 MCF-7 cells under hypoxia [miRNA] - Samples (11) - 822 miRNA

GSE47533 MCF-7 cells under hypoxia [mRNA] - GPL6884 - Samples (12)

GSE47602 MCF-7 cells under hypoxia (miRNA-Seq) - Samples (8) - missing

```
packages_cran = c("igraph", "BoolNet", "BiocManager", "tidyverse", "fs", "ff", "effectsize")
# 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)
  }
})
# For oligo and ArrayExpress First install:
#install.packages('https://cran.r-project.org/src/contrib/Archive/ff/ff_2.2-14.tar.gz',repos=NULL)
# packages_bioconductor = c("Biobase", "GEOquery", "affyPLM", "ArrayExpress", "illuminaHumanv3.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_cran)
```

Load the pre-processed data

```
load("../data/data.GSE47533.Rdata")
cols <- colnames(expr.GSE47533)
rows <- rownames(expr.GSE47533)
expr.GSE47533 <- as.data.frame(matrix(effectsize::normalize(as.matrix(expr.GSE47533)), ncol = length(co
colnames(expr.GSE47533) <- cols
rownames(expr.GSE47533) <- rows
```

Selecting the HIF Genes

```
# Selected genes from HIF Axis
hif.symbols <- c("TP53", "HIF1A", "EP300", "MDM2", "VHL")
hif.probes <- anno.GSE47533$probes[anno.GSE47533$symbol %in% hif.symbols]

# Select the probes and genes
expr.GSE47533.hif <- 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") %>%
  #distinct(symbol, .keep_all = TRUE) %>% # Take the first one
  dplyr::select(!(probes)) %>%
  arrange(symbol)
```

Example of Binarizing

```
cols <- (data.GSE47533$rep == 1)
breast1_MCF7 <-
expr.GSE47533.hif %>%
  dplyr::select(c("symbol", data.GSE47533$codes[cols])) %>% arrange(symbol) %>%
  arrange(symbol) %>%
  rename_at(vars(data.GSE47533$codes[cols]),
            ~paste0(substr(data.GSE47533$condition[cols],1,2),".",
                    data.GSE47533$time[cols],".",
                    substr(data.GSE47533$cell_line[cols],1,2)))

knitr::kable(breast1_MCF7)
```

symbol	No.0.MC	Hy.16h.MC	Hy.32h.MC	Hy.48h.MC
EP300	0.3905888	0.4059010	0.3807511	0.3843123
HIF1A	0.3425241	0.2580229	0.2966044	0.3318575
HIF1A	0.4544587	0.3946856	0.4195721	0.4575903
HIF1A	0.3373892	0.2539398	0.3155750	0.3127921
MDM2	0.2387532	0.2707557	0.2344911	0.2914341
MDM2	0.1046909	0.0953542	0.1050768	0.1024391
MDM2	0.0802744	0.0708871	0.0802306	0.0856708
TP53	0.4333610	0.4631014	0.4197433	0.4349020
VHL	0.2860114	0.2518470	0.2895173	0.3811038
VHL	0.6636049	0.6479165	0.6242634	0.6568183
VHL	0.4648981	0.4494688	0.4277231	0.4031015
VHL	0.4393973	0.3727216	0.4281843	0.4088407

```
binarizeTimeSeries(breast1_MCF7[,-1], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  add_column(symbol = breast1_MCF7$symbol, .before=0) %>%
  knitr::kable(.)
```

symbol	No.0.MC	Hy.16h.MC	Hy.32h.MC	Hy.48h.MC
EP300	0	1	0	0
HIF1A	1	0	0	1
HIF1A	1	0	0	1
HIF1A	1	0	1	1
MDM2	0	1	0	1
MDM2	1	0	1	1
MDM2	1	0	1	1
TP53	0	1	0	0
VHL	0	0	0	1
VHL	1	1	0	1
VHL	1	1	0	0
VHL	1	0	1	1

```
binarizeTimeSeries(breast1_MCF7[,-1], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  aggregate(., list(symbol = breast1_MCF7$symbol), mean) %>%
  mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%
  #rbind(., c("O2", 1,0,0,0)) %>%  # removing O2
  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.0.MC	Hy.16h.MC	Hy.32h.MC	Hy.48h.MC
EP300	0	1	0	0
HIF1A	1	0	0	1
MDM2	1	0	1	1
TP53	0	1	0	0
VHL	1	1	0	1

```
# Function to binarize according an consensus mean of probes, add the O2 state and rename columns
binNet <- function(b){

  cols <- data.GSE47533$codes %in% names(b)

  binarizeTimeSeries(b[,-1], method="kmeans")$binarizedMeasurements  %>%
  as.data.frame(.)  %>%
  aggregate(., list(symbol = b$symbol), mean) %>%  # mean of binarized probes
```

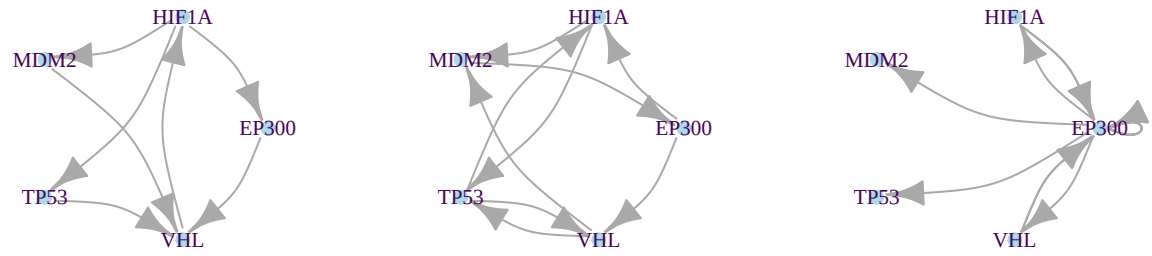
```
  mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%  # consensus with a bies to 1 (>= 0.5)
  #rbind(., c("O2", 1,0,0,0)) %>%  # removing O2
    rename_at(vars(data.GSE47533$codes[cols] ),
              ~paste0(substr(data.GSE47533$condition[cols],1,2),".",
                      data.GSE47533$time[cols],".",
                      substr(data.GSE47533$cell_line[cols],1,2), ".",
                      data.GSE47533$rep[cols])) %>%
  column_to_rownames("symbol")

}

breast_MCF7.1 <-
expr.GSE47533.hif %>%
  dplyr::select(c("symbol", data.GSE47533$codes[data.GSE47533$rep == 1]))  %>%
  binNet(.)

breast_MCF7.2 <-
expr.GSE47533.hif %>%
  dplyr::select(c("symbol", data.GSE47533$codes[data.GSE47533$rep == 2]))  %>%
  binNet(.)

breast_MCF7.3 <-
expr.GSE47533.hif %>%
  dplyr::select(c("symbol", data.GSE47533$codes[data.GSE47533$rep == 3]))  %>%
  binNet(.)

breast_MCF7.mean <-
cbind(breast_MCF7.1, breast_MCF7.2,breast_MCF7.3) %>%
  tibble::rownames_to_column('gene') %>%
  mutate_at(vars(-gene), as.numeric) %>%
  mutate(No.0.MC = rowMeans(select(.,starts_with("No.0.MC")), na.rm = TRUE)) %>%
  mutate(Hy.16h.MC = rowMeans(select(.,starts_with("Hy.16h.MC")), na.rm = TRUE)) %>%
  mutate(Hy.32h.MC = rowMeans(select(.,starts_with("Hy.32h.MC")), na.rm = TRUE)) %>%
  mutate(Hy.48h.MC = rowMeans(select(.,starts_with("Hy.48h.MC")), na.rm = TRUE)) %>%
  dplyr::select(c("No.0.MC", "Hy.16h.MC", "Hy.32h.MC", "Hy.48h.MC", "gene")) %>%
  mutate_at(c("No.0.MC", "Hy.16h.MC", "Hy.32h.MC", "Hy.48h.MC"), funs(ifelse(. >= 0.5, 1, 0)))  %>%  #
  tibble::column_to_rownames('gene')



# All breast cancer nets merged:
all.nets <- reconstructNetwork(list(breast_MCF7.1, breast_MCF7.2, breast_MCF7.3),
                               method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)

all.p <- plotNetworkWiring(all.nets, plotIt=F)



# Mean of replicate breast cancer net :
mean.net <- reconstructNetwork(breast_MCF7.mean,
                               method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
```

```r
mean.p <- plotNetworkWiring(mean.net, plotIt=F)

# # Mean of replicate breast cancer net without O2 :
# mean.nox.net <- reconstructNetwork(breast_MCF7.mean[c(1:5),],
#                                    method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
#
# mean.nox.p <- plotNetworkWiring(mean.nox.net, plotIt=F)

# Mean of replicate breast cancer net with only Hypoxia state:
#
# mean.hyp.net <- reconstructNetwork(breast_MCF7.mean[,c("Hy.16h.MC", "Hy.32h.MC", "Hy.48h.MC")],
#                                    method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
#
# mean.hyp.p <- plotNetworkWiring(mean.hyp.net, plotIt=F)
```

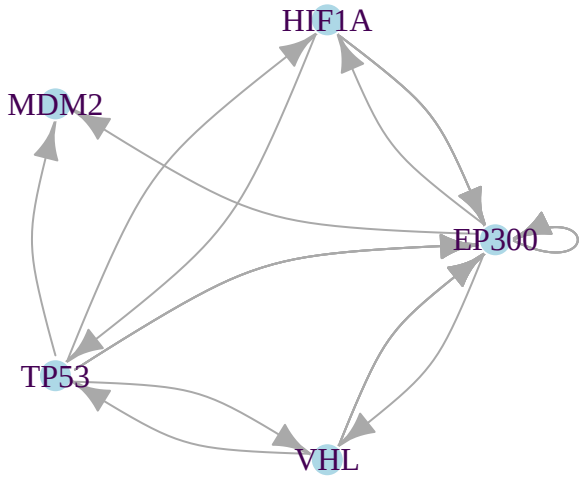
MCF7 breast cancer

```r
# MCF7 breast cancer - 4 time-points
breast_MCF7.1.net <- reconstructNetwork(breast_MCF7.1, method="bestfit",returnPBN=TRUE,readableFunctions
breast_MCF7.2.net <- reconstructNetwork(breast_MCF7.2, method="bestfit",returnPBN=TRUE,readableFunctions
breast_MCF7.3.net <- reconstructNetwork(breast_MCF7.3, method="bestfit",returnPBN=TRUE,readableFunctions

breast_MCF7.1.p <- plotNetworkWiring(breast_MCF7.1.net, plotIt=F)
breast_MCF7.2.p <- plotNetworkWiring(breast_MCF7.2.net, plotIt=F)
breast_MCF7.3.p <- plotNetworkWiring(breast_MCF7.3.net, plotIt=F)
```

```r
# MCF7 breast cancer - 4 time-points
par(mfrow = c(1,3))
plot(breast_MCF7.1.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="whi
     main="MCF7 breast\n 4 time-points, replicate 1")
plot(breast_MCF7.2.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="whi
     main="MCF7 breast\n 4 time-points, replicate 2")
plot(breast_MCF7.3.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="whi
     main="MCF7 breast\n 4 time-points, replicate 3")
```




```
par(mfrow = c(1,1))
plot(mean.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", layou
     main="MCF7 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 = (TP53 & !VHL) ( probability: 0.125, error: 0)
## EP300 = (!TP53 & VHL) | (TP53 & !VHL) ( probability: 0.125, error: 0)
## EP300 = (!HIF1A & TP53) ( probability: 0.125, error: 0)
## EP300 = (!HIF1A & TP53) | (HIF1A & !TP53) ( probability: 0.125, error: 0)
## EP300 = (!EP300 & !VHL) ( probability: 0.125, error: 0)
## EP300 = (!EP300 & !VHL) | (EP300 & VHL) ( probability: 0.125, error: 0)
## EP300 = (!EP300 & !HIF1A) ( probability: 0.125, error: 0)
## EP300 = (!EP300 & !HIF1A) | (EP300 & HIF1A) ( probability: 0.125, 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)
```

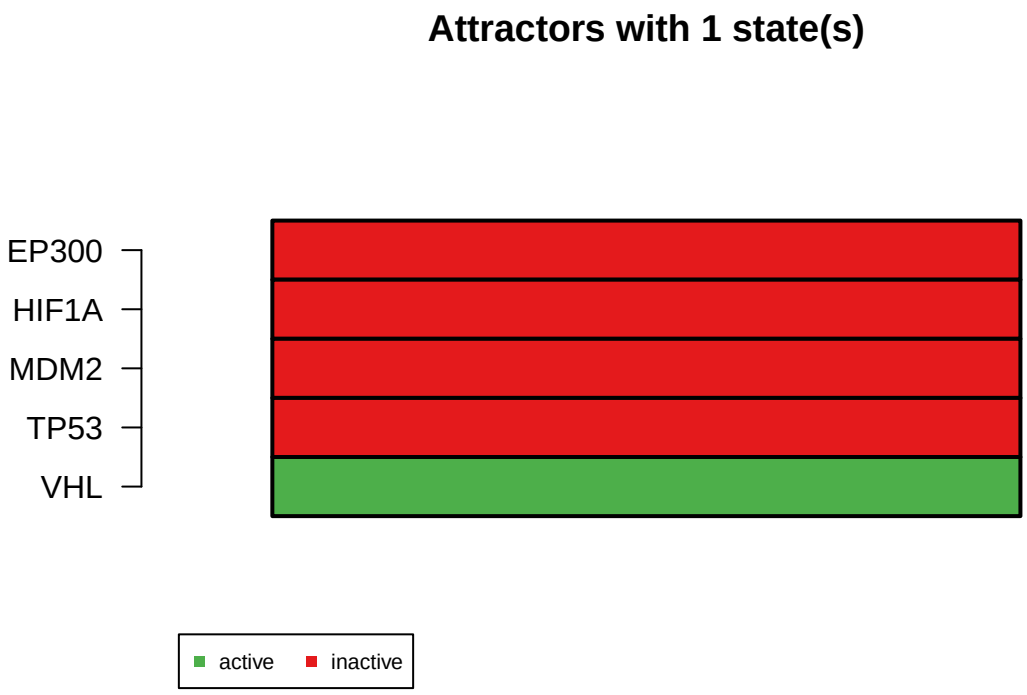
```
## MDM2 = (EP300) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (VHL) ( probability: 0.5, error: 0)
## TP53 = (HIF1A) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!TP53) ( probability: 0.5, error: 0)
## VHL = (EP300) ( probability: 0.5, error: 0)
```

```
# sink("ATOTS_inferred.bn")
# cat("targets, factors\n")
# cat("EP300, (TP53 & !VHL) | (!TP53 & VHL) | (TP53 & !VHL) | (!HIF1A & TP53) |  (!HIF1A & TP53) | (HIF
# cat("HIF1A, !TP53 | EP300\n")
# cat("MDM2, TP53 & !VHL\n")
# cat("TP53, VHL | HIF1A\n")
# cat("VHL, !TP53 | !EP300\n")
# sink()
try({
sink("ATOTS_inferred.bn")
cat("targets, factors\n")
cat("EP300, (!HIF1A & TP53) | (HIF1A & !TP53)\n")
cat("HIF1A, EP300\n")
cat("MDM2, EP300\n")
cat("TP53, HIF1A\n")
cat("VHL, !TP53\n")
sink()}, silent = T)
```

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

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

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


Attractors with 1 state(s)
EP300
HIF1A
MDM2
TP53
VHL
active
inactive


```
## $`1`
##       Attr1.1
## EP300       0
## HIF1A       0
## MDM2        0
## TP53        0
## VHL         1
##
## $`7`
##       Attr2.1 Attr2.2 Attr2.3 Attr2.4 Attr2.5 Attr2.6 Attr2.7
## EP300       1       0       1       1       1       0       0
## HIF1A       0       1       0       1       1       1       0
## MDM2        0       1       0       1       1       1       0
## TP53        0       0       1       0       1       1       1
## VHL         0       1       1       0       1       0       0
```