BoolNet Inference (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] - GPL8227 Agilent-019118 Human miRNA Microarray 2.0 G4470B - Samples (11) - 822 miRNA

GSE47533 MCF-7 cells under hypoxia [mRNA] - GPL6884 Illumina HumanWG-6 v3.0 expression beadchip - Samples (12)

GSE47602 MCF-7 cells under hypoxia (miRNA-Seq) - GPL11154 Illumina HiSeq 2000 (Homo sapiens) - Samples (8) - Don't exist

```
packages_cran = c("igraph", "BoolNet", "BiocManager", "tidyverse", "fs", "ff")


# 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", "oligo", "ArrayExpress", "hgu133plus2.db", "preprocess

# 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)
```

```
download_dir <- fs::path(".data_tmp")
if (!dir_exists(download_dir)) { dir_create(download_dir) }
```

```
GSE47533 <-getGEO("GSE47533", destdir = download_dir, GSEMatrix = T)
```

```
## Found 1 file(s)

## GSE47533_series_matrix.txt.gz

## Using locally cached version: .data_tmp/GSE47533_series_matrix.txt.gz

##
## -- Column specification --------------------------------------------------------
## cols(
##   ID_REF = col_character(),
##   GSM1151682 = col_double(),
##   GSM1151683 = col_double(),
##   GSM1151684 = col_double(),
##   GSM1151685 = col_double(),
##   GSM1151686 = col_double(),
##   GSM1151687 = col_double(),
##   GSM1151688 = col_double(),
##   GSM1151689 = col_double(),
##   GSM1151690 = col_double(),
##   GSM1151691 = col_double(),
##   GSM1151692 = col_double(),
##   GSM1151693 = col_double()
## )

## Using locally cached version of GPL6884 found here:
## .data_tmp/GPL6884.soft
```

```
expr.GSE47533 <- exprs(GSE47533[[1]])
prob.GSE47533 <- unique(rownames(expr.GSE47533))
data.GSE47533 <- pData(GSE47533[[1]])

data.GSE47533 <- data.frame(
                  codes = as.character(data.GSE47533$geo_accession),
                  cell_line = "MCF7",
                  time = data.GSE47533$`time of exposure:ch1`,
                  condition = substr(as.character(data.GSE47533$description), 1, 4),
                  rep = data.GSE47533$description.1)

data.GSE47533 <- data.GSE47533 %>%
  mutate(rep = recode(rep, "replicate 1" = 1,
                           "replicate 2" = 2,
                           "replicate 3" = 3))

data.GSE47533$time <- as.character(data.GSE47533$time)
data.GSE47533$time[data.GSE47533$condition == "Norm"] <- ''


# Convert the probes to Symbol names

#  load/install the package
if(!require("illuminaHumanv3.db")) BiocManager::install("illuminaHumanv3.db")
```

```
## Loading required package: illuminaHumanv3.db
```

```
##
```

```
# The below function call will return a datafram with probe_id, gene symbol
# and refgene_id for your data

anno.GSE47533 <- AnnotationDbi::select(illuminaHumanv3.db,
       keys = prob.GSE47533,
       columns=c("ENSEMBL", "SYMBOL", "GENENAME"),
       keytype="PROBEID")
```

```
## 'select()' returned 1:many mapping between keys and columns
```

```
colnames(anno.GSE47533) <- c("probes", "ensgene", "symbol", "description")

rm(download_dir, GSE47533,  prob.GSE47533)
```

Selecting the HIF Genes

```
# Genes from Boolean Network:
# HIF1a, HIF2a, p53, BNIP3, VEGF, cMyc, Oct4, cdc20, cycA, cycB, cycE, cycD, p27, Rb, E2F, cdh1, mdm2,

# hif.symbols <- c("HIF1A", "HIF1", "PASD8", "MOP1", "EPAS1", "HIF2A", "HLF", "PASD2", "MOP2", "VEGFA",

# 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)


# Function to binarize according an consensus mean of probes, add the O2 state and rename columns
binNet <- function(b){
  binarizeTimeSeries(b[,-5], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  aggregate(., list(symbol = b$symbol), mean) %>%
  mutate_at(vars(-symbol), funs(ifelse(. > 0.4, 1, 0))) %>%
  rbind(., c("O2", 1,0,0,0)) %>%
    rename_at(vars(data.GSE47533$codes[data.GSE47533$codes %in% names(b)]),
            ~paste0(data.GSE47533$condition[data.GSE47533$codes %in% names(b)],".",
                    data.GSE47533$time[data.GSE47533$codes %in% names(b)],".",
```

```
                    data.GSE47533$rep[data.GSE47533$codes %in% names(b)])) %>%
  column_to_rownames("symbol")
}
```

```
breast1_MCF7 <-
expr.GSE47533.hif %>%
  dplyr::select(c(data.GSE47533$codes[data.GSE47533$rep == 1], "symbol")) %>% arrange(symbol)

names(breast1_MCF7) <- c("Norm..1","Hypo.16h.1","Hypo.32h.1","Hypo.48h.1", "symbol")

knitr::kable(breast1_MCF7[, c("symbol", "Norm..1","Hypo.16h.1","Hypo.32h.1","Hypo.48h.1")])
```

symbol	Norm..1	Hypo.16h.1	Hypo.32h.1	Hypo.48h.1
EP300	9.038936	9.183945	8.945772	8.979497
HIF1A	8.583756	7.783518	8.148891	8.482742
HIF1A	9.643793	9.077734	9.313412	9.673450
HIF1A	8.535129	7.744851	8.328545	8.302191
MDM2	7.601032	7.904100	7.560669	8.099927
MDM2	6.331443	6.243023	6.335099	6.310119
MDM2	6.100215	6.011316	6.099801	6.151320
TP53	9.443995	9.725640	9.315033	9.458588
VHL	8.048573	7.725032	8.081774	8.949112
VHL	11.624437	11.475865	11.251867	11.560166
VHL	9.742655	9.596538	9.390603	9.157433
VHL	9.501160	8.869732	9.394971	9.211784

```
binarizeTimeSeries(breast1_MCF7[,-5], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  add_column(symbol = breast1_MCF7$symbol) %>%   dplyr::select(c("symbol", "Norm..1","Hypo.16h.1","Hypo
  knitr::kable(.)
```

symbol	Norm..1	Hypo.16h.1	Hypo.32h.1	Hypo.48h.1
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[,-5], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  aggregate(., list(symbol = breast1_MCF7$symbol), mean) %>%
  mutate_at(vars(-symbol), funs(ifelse(. > 0.4, 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	Norm..1	Hypo.16h.1	Hypo.32h.1	Hypo.48h.1
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
O2	1	0	0	0

MDA-MB231 breast cancer

```
breast1_MCF7 <-
expr.GSE47533.hif %>%
  dplyr::select(c(data.GSE47533$codes[data.GSE47533$rep == 1], "symbol"))  %>%
  binNet(.)
knitr::kable(breast1_MCF7)
```

	Norm..1	Hypo.16h.1	Hypo.32h.1	Hypo.48h.1
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
O2	1	0	0	0

```
breast2_MCF7 <-
expr.GSE47533.hif %>%
  dplyr::select(c(data.GSE47533$codes[data.GSE47533$rep == 2], "symbol"))  %>%
  binNet(.)
knitr::kable(breast2_MCF7)
```

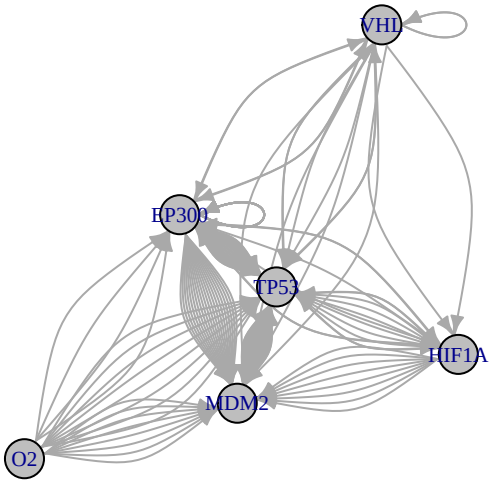
	Norm..2	Hypo.16h.2	Hypo.32h.2	Hypo.48h.2
EP300	0	0	1	0
HIF1A	1	0	0	1
MDM2	0	1	0	0
TP53	1	1	0	0
VHL	1	0	0	1
O2	1	0	0	0

```
breast3_MCF7 <-
expr.GSE47533.hif %>%
  dplyr::select(c(data.GSE47533$codes[data.GSE47533$rep == 3], "symbol"))  %>%
  binNet(.)
knitr::kable(breast3_MCF7)
```

	Norm..3	Hypo.16h.3	Hypo.32h.3	Hypo.48h.3
EP300	0	0	1	0
HIF1A	1	0	0	1
MDM2	0	0	0	1
TP53	1	1	1	0
VHL	1	0	0	1
O2	1	0	0	0

```
# All breast cancer nets merged:

net <- reconstructNetwork(list(breast1_MCF7, breast2_MCF7, breast3_MCF7), method="bestfit",returnPBN=TRU
plotNetworkWiring(net)
```



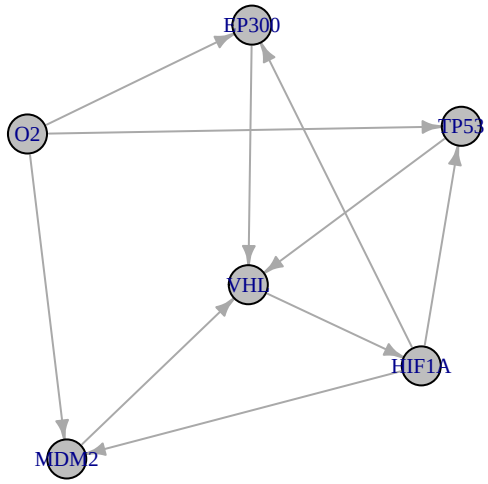

```
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (!EP300 & !TP53 & O2) | (!EP300 & TP53 & !O2) ( probability: 0.1, error: 0)
## EP300 = (!EP300 & !TP53 & O2) | (!EP300 & TP53 & !O2) | (EP300 & TP53 & O2) ( probability: 0.1, erro
## EP300 = (!TP53 & O2) | (!EP300 & TP53 & !O2) ( probability: 0.1, error: 0)
## EP300 = (!TP53 & O2) | (!EP300 & TP53 & !O2) | (EP300 & O2) ( probability: 0.1, error: 0)
## EP300 = (!EP300 & !TP53 & VHL) | (!EP300 & TP53 & !VHL) ( probability: 0.1, error: 0)
## EP300 = (!TP53 & VHL) | (!EP300 & TP53 & !VHL) ( probability: 0.1, error: 0)
## EP300 = (!EP300 & !HIF1A & TP53) | (!EP300 & HIF1A & !TP53) ( probability: 0.1, error: 0)
## EP300 = (!EP300 & !HIF1A & TP53) | (!EP300 & HIF1A & !TP53) | (EP300 & HIF1A & TP53) ( probability: 
## EP300 = (!EP300 & !HIF1A & TP53) | (HIF1A & !TP53) ( probability: 0.1, error: 0)
## EP300 = (!EP300 & !HIF1A & TP53) | (HIF1A & !TP53) | (EP300 & HIF1A) ( probability: 0.1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (!TP53 & !VHL) | (EP300 & !VHL) ( probability: 0.5, error: 0)
## HIF1A = (!TP53 & !VHL) | (EP300 & !TP53) | (EP300 & !VHL) ( probability: 0.5, error: 0)
##
```

```
## Alternative transition functions for gene MDM2:
## MDM2 = (!EP300 & !TP53 & !O2) | (EP300 & TP53 & !O2) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !TP53 & !O2) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !TP53 & !O2) | (EP300 & !TP53 & O2) | (EP300 & TP53 & !O2) ( probability: 0.05, erro
## MDM2 = (!EP300 & !TP53 & !O2) | (EP300 & O2) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !TP53 & !O2) | (!EP300 & TP53 & O2) | (EP300 & TP53 & !O2) ( probability: 0.05, erro
## MDM2 = (!EP300 & !TP53 & !O2) | (TP53 & O2) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !TP53 & !O2) | (!EP300 & TP53 & O2) | (EP300 & !TP53 & O2) | (EP300 & TP53 & !O2) (
## MDM2 = (!EP300 & !TP53 & !O2) | (TP53 & O2) | (EP300 & O2) | (EP300 & TP53) ( probability: 0.05, erro
## MDM2 = (!EP300 & !TP53 & !VHL) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !TP53 & !VHL) | (EP300 & VHL) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !TP53 & !VHL) | (TP53 & VHL) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !TP53 & !VHL) | (TP53 & VHL) | (EP300 & VHL) | (EP300 & TP53) ( probability: 0.05, e
## MDM2 = (!EP300 & !HIF1A & !TP53) | (EP300 & !HIF1A & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !HIF1A & !TP53) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !HIF1A & !TP53) | (EP300 & !HIF1A & TP53) | (EP300 & HIF1A & !TP53) ( probability: 0
## MDM2 = (!EP300 & !HIF1A & !TP53) | (EP300 & TP53) | (EP300 & HIF1A) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !HIF1A & !TP53) | (!EP300 & HIF1A & TP53) | (EP300 & !HIF1A & TP53) ( probability: 0
## MDM2 = (!EP300 & !HIF1A & !TP53) | (HIF1A & TP53) | (EP300 & TP53) ( probability: 0.05, error: 1)
## MDM2 = (!EP300 & !HIF1A & !TP53) | (!EP300 & HIF1A & TP53) | (EP300 & !HIF1A & TP53) | (EP300 & HIF1
## MDM2 = (!EP300 & !HIF1A & !TP53) | (HIF1A & TP53) | (EP300 & TP53) | (EP300 & HIF1A) ( probability: 0
##
## Alternative transition functions for gene TP53:
## TP53 = (!EP300 & !MDM2) | (!EP300 & O2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (MDM2 & O2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & O2) | (EP300 & MDM2 & !O2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (MDM2 & O2) | (EP300 & MDM2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & O2) | (!MDM2 & O2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (O2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & O2) | (!MDM2 & O2) | (EP300 & MDM2 & !O2) ( probability: 0.05, e
## TP53 = (!EP300 & !MDM2) | (O2) | (EP300 & MDM2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & VHL) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (MDM2 & VHL) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & VHL) | (EP300 & MDM2 & !VHL) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (MDM2 & VHL) | (EP300 & MDM2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & HIF1A) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (HIF1A & MDM2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & HIF1A) | (HIF1A & !MDM2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (HIF1A) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & HIF1A) | (EP300 & !HIF1A & MDM2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (HIF1A & MDM2) | (EP300 & MDM2) ( probability: 0.05, error: 0)
## TP53 = (!EP300 & !MDM2) | (!EP300 & HIF1A) | (EP300 & !HIF1A & MDM2) | (HIF1A & !MDM2) ( probability
## TP53 = (!EP300 & !MDM2) | (HIF1A) | (EP300 & MDM2) ( probability: 0.05, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!TP53 & !VHL) | (!EP300 & !TP53) | (EP300 & !VHL) ( probability: 0.5, error: 0)
## VHL = (!TP53) | (EP300 & !VHL) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## O2 = 0
```

```r
# Individual nets of each replica:

net <- reconstructNetwork(breast1_MCF7, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
plotNetworkWiring(net)
```

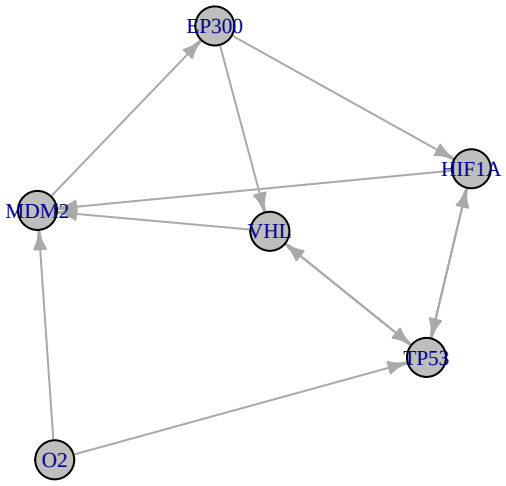



```r
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (O2) ( probability: 0.5, error: 0)
## EP300 = (HIF1A) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = (!O2) ( probability: 0.5, error: 0)
## MDM2 = (!HIF1A) ( probability: 0.5, error: 0)
##
```

```
## Alternative transition functions for gene TP53:
## TP53 = (O2) ( probability: 0.5, error: 0)
## TP53 = (HIF1A) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!TP53) ( probability: 0.3333333, error: 0)
## VHL = (MDM2) ( probability: 0.3333333, error: 0)
## VHL = (!EP300) ( probability: 0.3333333, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## O2 = 0
```

```
net <- reconstructNetwork(breast2_MCF7, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
plotNetworkWiring(net)
```

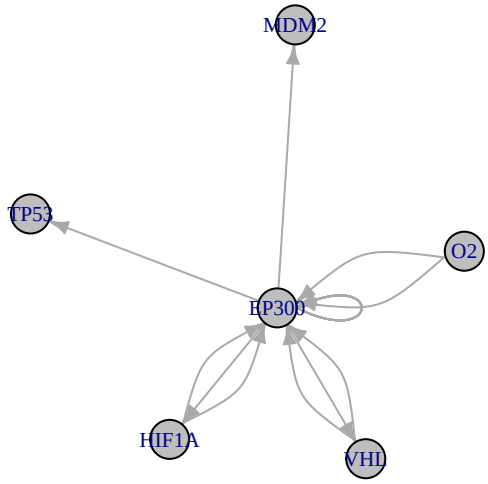



```
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
```

```
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (MDM2) ( 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 = (O2) ( probability: 0.3333333, error: 0)
## MDM2 = (VHL) ( probability: 0.3333333, error: 0)
## MDM2 = (HIF1A) ( probability: 0.3333333, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (O2) ( probability: 0.3333333, error: 0)
## TP53 = (VHL) ( probability: 0.3333333, error: 0)
## TP53 = (HIF1A) ( probability: 0.3333333, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!TP53) ( probability: 0.5, error: 0)
## VHL = (EP300) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## O2 = 0
```

```
net <- reconstructNetwork(breast3_MCF7, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
plotNetworkWiring(net)
```




```
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (!EP300 & !O2) ( probability: 0.1666667, error: 0)
## EP300 = (!EP300 & !O2) | (EP300 & O2) ( probability: 0.1666667, error: 0)
## EP300 = (!EP300 & !VHL) ( probability: 0.1666667, error: 0)
## EP300 = (!EP300 & !VHL) | (EP300 & VHL) ( probability: 0.1666667, error: 0)
## EP300 = (!EP300 & !HIF1A) ( probability: 0.1666667, error: 0)
## EP300 = (!EP300 & !HIF1A) | (EP300 & HIF1A) ( probability: 0.1666667, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (EP300) ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = (EP300) ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (!EP300) ( probability: 1, error: 0)
```

```
##
## Alternative transition functions for gene VHL:
## VHL = (EP300) ( probability: 1, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## O2 = 0
```