BoolNet Inference (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", "ff", "RSQLite")


# 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 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", "ArrayExpress", "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)
```

```
download_dir <- fs::path(".data_tmp")
if (!dir_exists(download_dir)) {
    dir_create(download_dir)
    EGEOD18494 <- ArrayExpress( "E-GEOD-18494", save=TRUE, path=download_dir)
} else {
    EGEOD18494 <- ArrayExpress( "E-GEOD-18494", save=TRUE, path=download_dir)
}
```

```
data.EGEOD18494 <- Biobase::pData(EGEOD18494)

data.EGEOD18494 <- data.frame(
                  codes = substr(data.EGEOD18494$Source.Name,1,9),
                  cell_line = data.EGEOD18494$Characteristics..cell.line.,
                  time = data.EGEOD18494$Characteristics..time,
                  condition = data.EGEOD18494$Characteristics..stress.
                  )
data.EGEOD18494 <- data.EGEOD18494[order(data.EGEOD18494$codes),]
data.EGEOD18494$rep <- rep(1:3, n= length(data.EGEOD18494$codes))

# Normalisation
eset.EGEOD18494 <- oligo::rma(EGEOD18494,  normalize = TRUE)

expr.EGEOD18494 <- exprs(eset.EGEOD18494)

# Convert to a data.frame
expr.EGEOD18494 <- as.data.frame(as.ffdf(expr.EGEOD18494))

colnames(expr.EGEOD18494) <- substr(colnames(expr.EGEOD18494),1,9)

rm(download_dir, EGEOD18494, eset.EGEOD18494)
```

Convert the probes to Symbol names

```
anno.EGEOD18494 <- AnnotationDbi::select(hgu133plus2.db, keys=rownames(expr.EGEOD18494), columns=c("ENS
```

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

```
colnames(anno.EGEOD18494) <- c("probes", "ensgene", "symbol", "description")
```

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) %>%
  mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%
  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	7.117723	7.444650	7.564863	7.102371
EP300	7.413672	7.507501	7.570583	7.374402
HIF1A	12.201881	11.633014	10.456373	10.119609
MDM2	5.524042	5.320023	5.350573	5.446186
MDM2	4.045154	3.853332	4.078569	4.257243
MDM2	5.078994	4.927372	5.029658	4.981994
MDM2	6.355831	6.328876	6.389927	6.806724
MDM2	4.287158	4.755383	4.670058	4.462138
MDM2	8.162994	8.179121	8.219938	8.085525
MDM2	7.285900	7.207761	7.123573	6.955918
MDM2	3.623543	3.829355	3.753720	4.100483
MDM2	4.054654	4.129631	4.067410	4.256327
MDM2	8.207312	7.778604	7.656600	7.797764

symbol	no.control.MD	hy.4h.MD	hy.8h.MD	hy.12h.MD
TP53	8.895355	8.773830	9.104009	9.136858
TP53	8.600345	8.240599	8.641253	8.664151
VHL	7.698038	7.713089	7.348580	7.098092
VHL	3.738962	3.749649	3.759698	3.638137

```
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

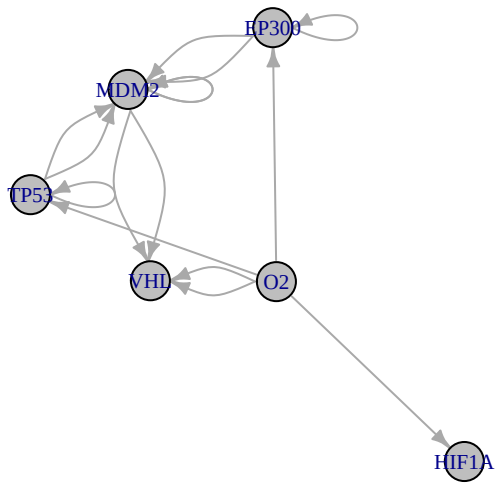
```
breast3x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep3])) %>%
  binNet(.)

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

```
# All breast cancer nets merged:

net <- reconstructNetwork(list(breast1x, breast2x, breast3x), method="bestfit",returnPBN=TRUE,readableF
plotNetworkWiring(net)
```

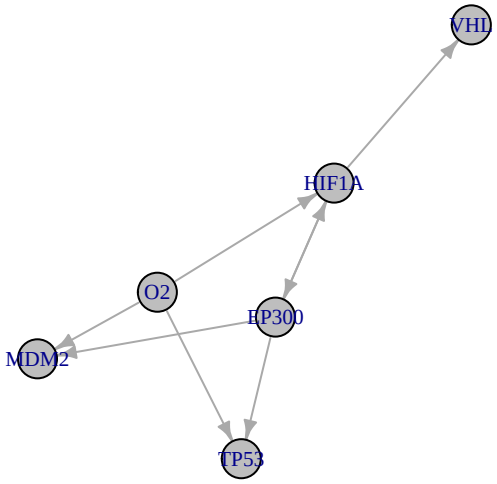



```
print(net)
```

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

```
# Individual nets of each replica:

net <- reconstructNetwork(breast1x, method="bestfit", returnPBN=TRUE, readableFunctions=TRUE)
plotNetworkWiring(net)
```

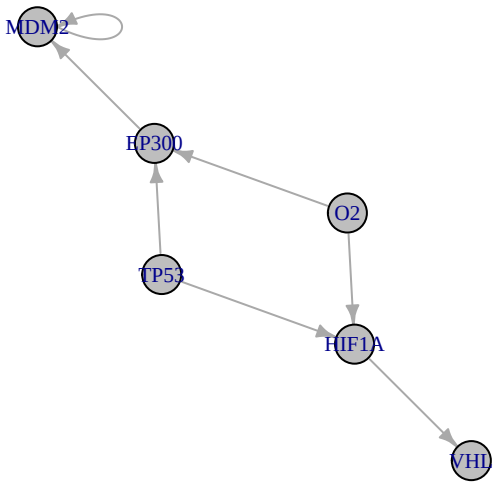



```
print(net)
```

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

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

```
net <- reconstructNetwork(breast2x, method="bestfit", returnPBN=TRUE, readableFunctions=TRUE)
plotNetworkWiring(net)
```

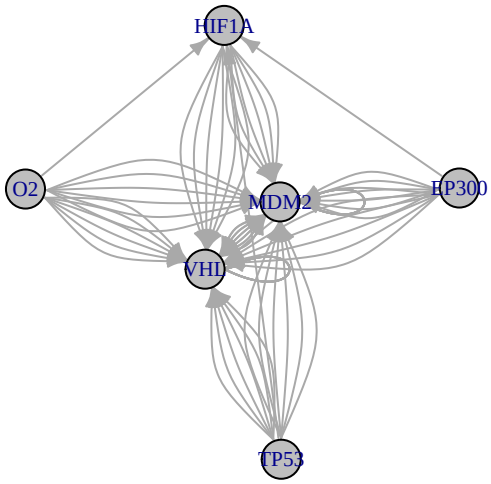



```
print(net)
```

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

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

```
net <- reconstructNetwork(breast3x, 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 = 1 ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (O2) ( probability: 0.3333333, error: 0)
## HIF1A = (!TP53) ( probability: 0.3333333, error: 0)
## HIF1A = (!EP300) ( probability: 0.3333333, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = (!VHL & !O2) | (VHL & O2) ( probability: 0.05555556, error: 0)
## MDM2 = (!VHL) | (O2) ( probability: 0.05555556, error: 0)
## MDM2 = (!TP53 & VHL) | (TP53 & !VHL) ( probability: 0.05555556, error: 0)
## MDM2 = (!VHL) | (!TP53) ( probability: 0.05555556, error: 0)
## MDM2 = (!MDM2 & !O2) | (MDM2 & O2) ( probability: 0.05555556, error: 0)
## MDM2 = (!MDM2) | (O2) ( probability: 0.05555556, error: 0)
## MDM2 = (!MDM2 & TP53) | (MDM2 & !TP53) ( probability: 0.05555556, error: 0)
## MDM2 = (!TP53) | (!MDM2) ( probability: 0.05555556, error: 0)
## MDM2 = (!HIF1A & !O2) | (HIF1A & O2) ( probability: 0.05555556, error: 0)
## MDM2 = (!HIF1A) | (O2) ( probability: 0.05555556, error: 0)
## MDM2 = (!HIF1A & TP53) | (HIF1A & !TP53) ( probability: 0.05555556, error: 0)
## MDM2 = (!TP53) | (!HIF1A) ( probability: 0.05555556, error: 0)
## MDM2 = (!EP300 & VHL) | (EP300 & !VHL) ( probability: 0.05555556, error: 0)
## MDM2 = (!VHL) | (!EP300) ( probability: 0.05555556, error: 0)
## MDM2 = (!EP300 & MDM2) | (EP300 & !MDM2) ( probability: 0.05555556, error: 0)
## MDM2 = (!MDM2) | (!EP300) ( probability: 0.05555556, error: 0)
## MDM2 = (!EP300 & HIF1A) | (EP300 & !HIF1A) ( probability: 0.05555556, error: 0)
## MDM2 = (!HIF1A) | (!EP300) ( probability: 0.05555556, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = 1 ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!VHL & !O2) | (VHL & O2) ( probability: 0.05555556, error: 0)
## VHL = (!VHL) | (O2) ( probability: 0.05555556, error: 0)
## VHL = (!TP53 & VHL) | (TP53 & !VHL) ( probability: 0.05555556, error: 0)
## VHL = (!VHL) | (!TP53) ( probability: 0.05555556, error: 0)
## VHL = (!MDM2 & !O2) | (MDM2 & O2) ( probability: 0.05555556, error: 0)
## VHL = (!MDM2) | (O2) ( probability: 0.05555556, error: 0)
## VHL = (!MDM2 & TP53) | (MDM2 & !TP53) ( probability: 0.05555556, error: 0)
## VHL = (!TP53) | (!MDM2) ( probability: 0.05555556, error: 0)
## VHL = (!HIF1A & !O2) | (HIF1A & O2) ( probability: 0.05555556, error: 0)
## VHL = (!HIF1A) | (O2) ( probability: 0.05555556, error: 0)
## VHL = (!HIF1A & TP53) | (HIF1A & !TP53) ( probability: 0.05555556, error: 0)
## VHL = (!TP53) | (!HIF1A) ( probability: 0.05555556, error: 0)
## VHL = (!EP300 & VHL) | (EP300 & !VHL) ( probability: 0.05555556, error: 0)
## VHL = (!VHL) | (!EP300) ( probability: 0.05555556, error: 0)
## VHL = (!EP300 & MDM2) | (EP300 & !MDM2) ( probability: 0.05555556, error: 0)
## VHL = (!MDM2) | (!EP300) ( probability: 0.05555556, error: 0)
```

```
## VHL = (!EP300 & HIF1A) | (EP300 & !HIF1A) ( probability: 0.05555556, error: 0)
## VHL = (!HIF1A) | (!EP300) ( probability: 0.05555556, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## EP300 = 1
## TP53 = 1
## O2 = 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	1	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

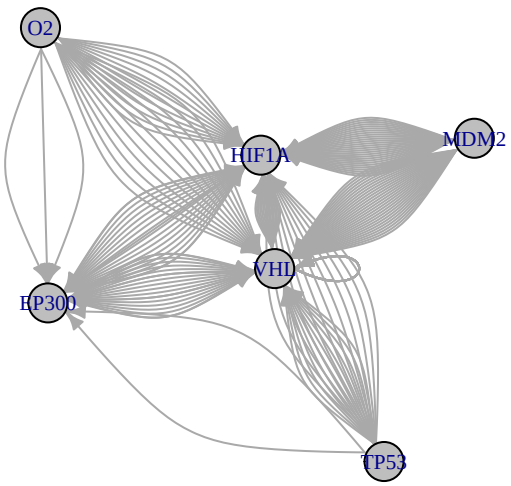
```
hepatoma3x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep3]))  %>%
  binNet(.)

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

	no.control.He.3	hy.4h.He.3	hy.8h.He.3	hy.12h.He.3
EP300	1	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

```
# All nets hepatoma merged:

net <- reconstructNetwork(list(hepatoma1x, hepatoma2x, hepatoma3x), method="bestfit",returnPBN=TRUE,rea
plotNetworkWiring(net)
```



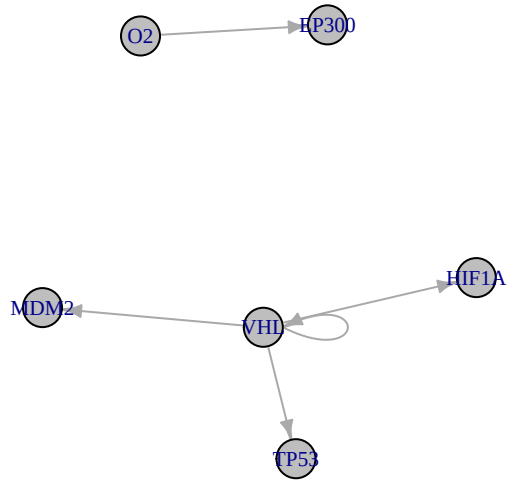

```
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (!VHL & !O2) | (VHL & O2) ( probability: 0.1666667, error: 2)
## EP300 = (!VHL) | (O2) ( probability: 0.1666667, error: 2)
## EP300 = (O2) | (TP53) ( probability: 0.1666667, error: 2)
## EP300 = (!HIF1A & VHL) | (HIF1A & !VHL) ( probability: 0.1666667, error: 2)
## EP300 = (!VHL) | (!HIF1A) ( probability: 0.1666667, error: 2)
## EP300 = (!HIF1A) | (TP53) ( probability: 0.1666667, error: 2)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (!MDM2 & TP53 & O2) | (MDM2 & TP53 & !O2) ( probability: 0.03571429, error: 1)
## HIF1A = (!MDM2 & TP53 & O2) | (MDM2 & !TP53 & O2) | (MDM2 & TP53 & !O2) ( probability: 0.03571429, e
## HIF1A = (!MDM2 & TP53 & O2) | (MDM2 & !O2) ( probability: 0.03571429, error: 1)
## HIF1A = (!MDM2 & TP53 & O2) | (MDM2 & !O2) | (MDM2 & !TP53) ( probability: 0.03571429, error: 1)
## HIF1A = (!MDM2 & TP53 & VHL) | (MDM2 & TP53 & !VHL) ( probability: 0.03571429, error: 1)
## HIF1A = (!MDM2 & TP53 & VHL) | (MDM2 & !TP53 & VHL) | (MDM2 & TP53 & !VHL) ( probability: 0.03571429
## HIF1A = (!MDM2 & TP53 & VHL) | (MDM2 & !VHL) ( probability: 0.03571429, error: 1)
## HIF1A = (!MDM2 & TP53 & VHL) | (MDM2 & !VHL) | (MDM2 & !TP53) ( probability: 0.03571429, error: 1)
## HIF1A = (!MDM2 & !TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & TP53 & !VHL) ( probability: 0.035714
## HIF1A = (!MDM2 & !TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & !TP53 & VHL) | (MDM2 & TP53 & !VHL)
## HIF1A = (!TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & !VHL) ( probability: 0.03571429, error: 1)
## HIF1A = (!TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & !TP53) | (MDM2 & !VHL) ( probability: 0.0357
## HIF1A = (EP300 & !MDM2 & O2) | (EP300 & MDM2 & !O2) ( probability: 0.03571429, error: 1)
## HIF1A = (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## HIF1A = (!EP300 & MDM2 & O2) | (EP300 & !MDM2 & O2) | (EP300 & MDM2 & !O2) ( probability: 0.03571429
## HIF1A = (!EP300 & MDM2 & O2) | (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## HIF1A = (MDM2 & !O2) | (EP300 & !MDM2 & O2) ( probability: 0.03571429, error: 1)
## HIF1A = (MDM2 & !O2) | (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## HIF1A = (MDM2 & !O2) | (!EP300 & MDM2) | (EP300 & !MDM2 & O2) ( probability: 0.03571429, error: 1)
## HIF1A = (MDM2 & !O2) | (!EP300 & MDM2) | (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, e
## HIF1A = (EP300 & !MDM2 & VHL) | (EP300 & MDM2 & !VHL) ( probability: 0.03571429, error: 1)
## HIF1A = (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## HIF1A = (!EP300 & MDM2 & VHL) | (EP300 & !MDM2 & VHL) | (EP300 & MDM2 & !VHL) ( probability: 0.035714
## HIF1A = (!EP300 & MDM2 & VHL) | (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1
## HIF1A = (MDM2 & !VHL) | (EP300 & !MDM2 & VHL) ( probability: 0.03571429, error: 1)
## HIF1A = (MDM2 & !VHL) | (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## HIF1A = (MDM2 & !VHL) | (!EP300 & MDM2) | (EP300 & !MDM2 & VHL) ( probability: 0.03571429, error: 1)
## HIF1A = (MDM2 & !VHL) | (!EP300 & MDM2) | (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429
##
## Alternative transition functions for gene MDM2:
## MDM2 = 1 ( probability: 1, error: 2)
##
## Alternative transition functions for gene TP53:
## TP53 = 1 ( probability: 1, error: 2)
##
## Alternative transition functions for gene VHL:
## VHL = (!MDM2 & TP53 & O2) | (MDM2 & TP53 & !O2) ( probability: 0.03571429, error: 1)
## VHL = (!MDM2 & TP53 & O2) | (MDM2 & !TP53 & O2) | (MDM2 & TP53 & !O2) ( probability: 0.03571429, erro
```

```
## VHL = (!MDM2 & TP53 & O2) | (MDM2 & !O2) ( probability: 0.03571429, error: 1)
## VHL = (!MDM2 & TP53 & O2) | (MDM2 & !O2) | (MDM2 & !TP53) ( probability: 0.03571429, error: 1)
## VHL = (!MDM2 & TP53 & VHL) | (MDM2 & TP53 & !VHL) ( probability: 0.03571429, error: 1)
## VHL = (!MDM2 & TP53 & VHL) | (MDM2 & !TP53 & VHL) | (MDM2 & TP53 & !VHL) ( probability: 0.03571429, e
## VHL = (!MDM2 & TP53 & VHL) | (MDM2 & !VHL) ( probability: 0.03571429, error: 1)
## VHL = (!MDM2 & TP53 & VHL) | (MDM2 & !VHL) | (MDM2 & !TP53) ( probability: 0.03571429, error: 1)
## VHL = (!MDM2 & !TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & TP53 & !VHL) ( probability: 0.03571429
## VHL = (!MDM2 & !TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & !TP53 & VHL) | (MDM2 & TP53 & !VHL) ( p
## VHL = (!TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & !VHL) ( probability: 0.03571429, error: 1)
## VHL = (!TP53 & !VHL) | (!MDM2 & TP53 & VHL) | (MDM2 & !TP53) | (MDM2 & !VHL) ( probability: 0.035714
## VHL = (EP300 & !MDM2 & O2) | (EP300 & MDM2 & !O2) ( probability: 0.03571429, error: 1)
## VHL = (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## VHL = (!EP300 & MDM2 & O2) | (EP300 & !MDM2 & O2) | (EP300 & MDM2 & !O2) ( probability: 0.03571429, e
## VHL = (!EP300 & MDM2 & O2) | (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !O2) | (EP300 & !MDM2 & O2) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !O2) | (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !O2) | (!EP300 & MDM2) | (EP300 & !MDM2 & O2) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !O2) | (!EP300 & MDM2) | (EP300 & !O2) | (EP300 & !MDM2) ( probability: 0.03571429, er
## VHL = (EP300 & !MDM2 & VHL) | (EP300 & MDM2 & !VHL) ( probability: 0.03571429, error: 1)
## VHL = (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## VHL = (!EP300 & MDM2 & VHL) | (EP300 & !MDM2 & VHL) | (EP300 & MDM2 & !VHL) ( probability: 0.0357142
## VHL = (!EP300 & MDM2 & VHL) | (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !VHL) | (EP300 & !MDM2 & VHL) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !VHL) | (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !VHL) | (!EP300 & MDM2) | (EP300 & !MDM2 & VHL) ( probability: 0.03571429, error: 1)
## VHL = (MDM2 & !VHL) | (!EP300 & MDM2) | (EP300 & !VHL) | (EP300 & !MDM2) ( probability: 0.03571429, e
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## MDM2 = 1
## TP53 = 1
## O2 = 0
```

```
# Individual nets of each replica:

net <- reconstructNetwork(hepatoma1x, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
plotNetworkWiring(net)
```

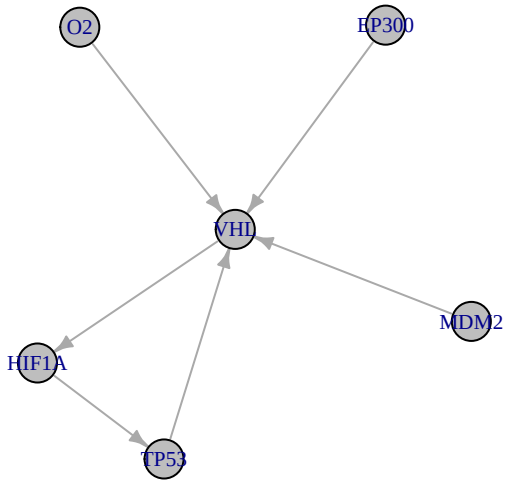



```
print(net)
```

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

```
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## O2 = 0
```

```
net <- reconstructNetwork(hepatoma2x, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
plotNetworkWiring(net)
```

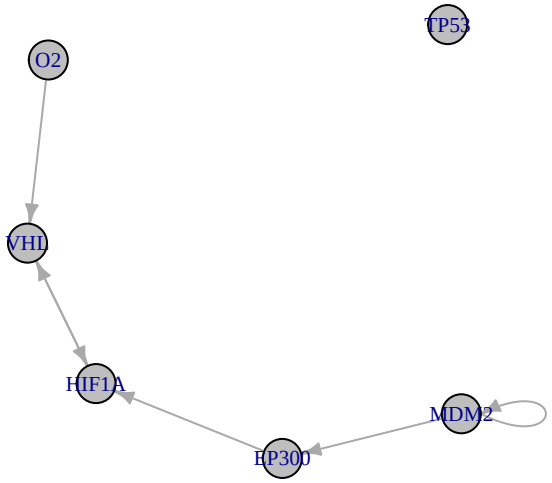



```
print(net)
```

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

```
##
## Alternative transition functions for gene TP53:
## TP53 = (!HIF1A) ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!O2) ( probability: 0.25, error: 0)
## VHL = (TP53) ( probability: 0.25, error: 0)
## VHL = (MDM2) ( probability: 0.25, error: 0)
## VHL = (EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## EP300 = 1
## MDM2 = 1
## O2 = 0
```

```
net <- reconstructNetwork(hepatoma3x, 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 = (VHL) ( probability: 0.5, error: 0)
## HIF1A = (EP300) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = (!MDM2) ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = 1 ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (O2) ( probability: 0.5, error: 0)
## VHL = (!HIF1A) ( probability: 0.5, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## TP53 = 1
## O2 = 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	0	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.rep1]))  %>%
  binNet(.)

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

	no.control.U8.1	hy.4h.U8.1	hy.8h.U8.1	hy.12h.U8.1
EP300	1	0	0	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

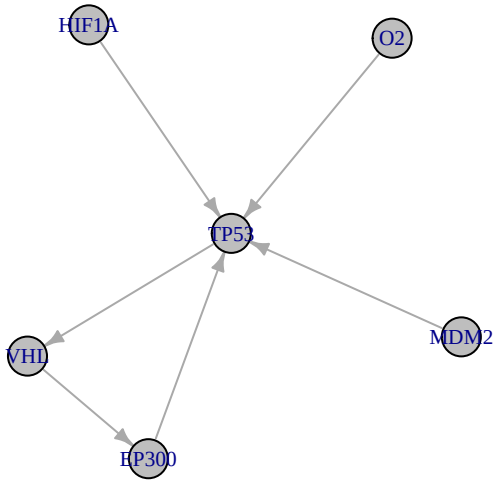
```
glioma3x <-
expr.EGEOD18494.hif %>%
  dplyr::select(c("symbol", data.EGEOD18494$codes[cellline.rep1]))  %>%
  binNet(.)

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

	no.control.U8.1	hy.4h.U8.1	hy.8h.U8.1	hy.12h.U8.1
EP300	1	0	0	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

```
# All glioma nets merged:

net <- reconstructNetwork(list(glioma1x, glioma2x, glioma3x), method="bestfit",returnPBN=TRUE,readableF
plotNetworkWiring(net)
```



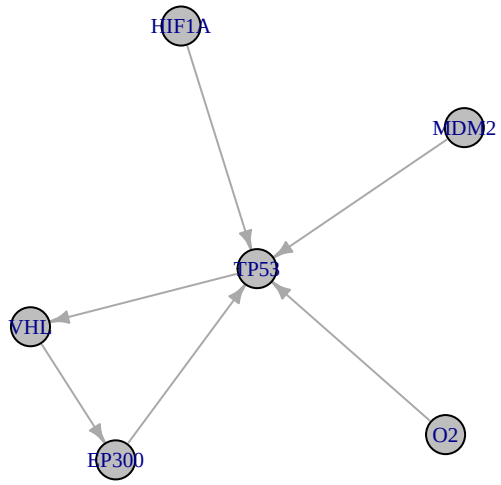

```
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (!O2) ( probability: 0.25, error: 0)
## TP53 = (!MDM2) ( probability: 0.25, error: 0)
## TP53 = (!HIF1A) ( probability: 0.25, error: 0)
## TP53 = (!EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene VHL:
```

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

```
# Individual nets of each replica:

net <- reconstructNetwork(glioma1x, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
plotNetworkWiring(net)
```

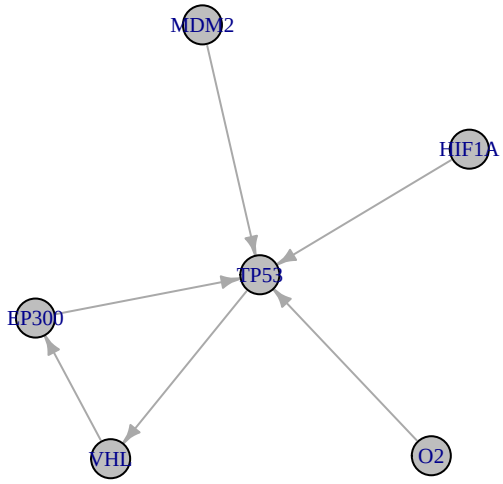



```
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
```

```
## EP300 = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (!O2) ( probability: 0.25, error: 0)
## TP53 = (!MDM2) ( probability: 0.25, error: 0)
## TP53 = (!HIF1A) ( probability: 0.25, error: 0)
## TP53 = (!EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (TP53) ( probability: 1, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## HIF1A = 0
## MDM2 = 0
## O2 = 0
```

```
net <- reconstructNetwork(glioma2x, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
plotNetworkWiring(net)
```

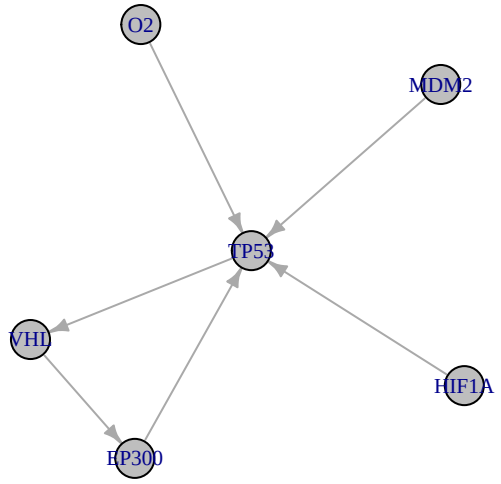



```
print(net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (!O2) ( probability: 0.25, error: 0)
## TP53 = (!MDM2) ( probability: 0.25, error: 0)
## TP53 = (!HIF1A) ( probability: 0.25, error: 0)
## TP53 = (!EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene VHL:
```

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

```
net <- reconstructNetwork(glioma3x, 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 = (!VHL) ( probability: 1, error: 0)
##
```

```
## Alternative transition functions for gene HIF1A:
## HIF1A = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (!O2) ( probability: 0.25, error: 0)
## TP53 = (!MDM2) ( probability: 0.25, error: 0)
## TP53 = (!HIF1A) ( probability: 0.25, error: 0)
## TP53 = (!EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (TP53) ( probability: 1, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## HIF1A = 0
## MDM2 = 0
## O2 = 0
```