HTG-validation_RS-GGI-SET-Pen.R

t

2024-08-13

```
# HEADER ####
#
# Version: 2024-08-13
#
# Comparison of signature scores in TCGA-BRCA-RNA-Seq based on
# complete gene list and the subset available in the HTG-Panel
# for the following signature scores:
#
#
# -  onctotypeDX/Recurrence Score surrogate: adapted from genefu (PMID 26607490)
#                 based on Paik 2004 (PMID 15591335)
# -  GGI (Genomic Grade Index): genefu version with 112 genes (PMID 26607490)
#                 based on Sotiriou 2006 (PMID 16478745)
# -  SET-Index: Robust 18-gene predictor from Sinn 2019 (PMID 31231679 )
#                 based on Symmans 2010 (PMID 20697068)
# -  Pen355: n355 DEG from paired analysis of 1080 paired pre/post Penelope samples
#                 (Different scores for subclusters of the 355 genes)
#
#
#
#
# SETUP ####

Sys.setenv(lang = "en_US")
```

Install required packages if missing ——————————————–

```
# Package names from CRAN
packs <- c("dplyr")

# Install packages not yet installed
installed_packages <- packs %in% rownames(installed.packages())
if (any(installed_packages == FALSE)) {
  install.packages(packs[!installed_packages])
}



# Package names from Bioconductor
bcpacks <- c("genefu", "cBioPortalData")

# Install bc-packages if not yet installed from Bioconductor
installed_packages <- bcpacks %in% rownames(installed.packages())
if (any(installed_packages == FALSE)) {
  if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")
  BiocManager::install(bcpacks[!installed_packages])
}
```

Load required packages ——————————————————

```
invisible(lapply(packs, library, character.only = TRUE))
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
invisible(lapply(bcpacks, library, character.only = TRUE))
```

```
## Loading required package: survcomp
```

```
## Loading required package: survival
```

```
## Loading required package: prodlim
```

```
## Warning: package 'prodlim' was built under R version 4.3.1
```

```
## Loading required package: biomaRt
```

```
## Loading required package: iC10
```

```
## Warning: package 'iC10' was built under R version 4.3.1
```

```
## Loading required package: pamr
```

```
## Warning: package 'pamr' was built under R version 4.3.1
```

```
## Loading required package: cluster
```

```
## Loading required package: impute
```

```
## Loading required package: iC10TrainingData
```

```
## Loading required package: AIMS
```

```
## Loading required package: e1071
```

```
## Loading required package: Biobase
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:dplyr':
##
##     combine, intersect, setdiff, union
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which.max, which.min

## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.

## Loading required package: AnVIL

## Loading required package: MultiAssayExperiment

## Loading required package: SummarizedExperiment

## Loading required package: MatrixGenerics

## Warning: package 'MatrixGenerics' was built under R version 4.3.1

## Loading required package: matrixStats

## Warning: package 'matrixStats' was built under R version 4.3.1

##
## Attaching package: 'matrixStats'

## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians

## The following object is masked from 'package:dplyr':
##
##     count

##
## Attaching package: 'MatrixGenerics'

## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
```

```
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars

## The following object is masked from 'package:Biobase':
##
##     rowMedians

## Loading required package: GenomicRanges

## Loading required package: stats4

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following objects are masked from 'package:dplyr':
##
##     first, rename

## The following object is masked from 'package:utils':
##
##     findMatches

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: IRanges

## Warning: package 'IRanges' was built under R version 4.3.1

##
## Attaching package: 'IRanges'

## The following objects are masked from 'package:dplyr':
##
##     collapse, desc, slice

## The following object is masked from 'package:grDevices':
##
##     windows

## Loading required package: GenomeInfoDb

## Warning: package 'GenomeInfoDb' was built under R version 4.3.1
```

```r
# FUNCTION Definitions ####
# ***************************************************
#
# Function oncotypedxHTG   ####
#    with "CTSL2" updated to "CTSV"
#    excluding "GRB7"
#
#
# The function oncotypedxHTG is a modified version
# of the oncotypedx function from library genefu
#
# In function oncotypedxHTG the gene symbol "CTSL2" is replaced by the
# current HUGO symbol "CTSV".
#
# In addition the gene "GRB7" (which is not available in HTG-Panel) is
# left out of the algorithm in oncotypedxHTG.
#
# Moreover, the variable "sig.oncotypedx" is replaced by "sig.oncotypedx.htg"
#  --> within this new table "sig.oncotypedx.htg" the gene symbol "CTSL2" is
#   updated to the current HUGO symbol "CTSV" and the row with "GRB7" has
#   been removed.
#
# The scaling and clipping of rsu values in the original function
# from genefu package has been modified in order to adapt the
# distribution of Recurrence Score values from RNA-Seq
# to those from clinical RS data (PMID 32565552)


oncotypedxHTG <- function (data, annot, do.mapping = FALSE, mapping, verbose = FALSE)
{
  sig2 <- sig.oncotypedx.htg[sig.oncotypedx.htg[, "group"] != "reference",
                             , drop = FALSE]
  dimnames(sig2)[[1]] <- sig2[, "symbol"]
  gt <- nrow(sig2)
  if (do.mapping) {
    gid1 <- as.numeric(as.character(sig2[, "EntrezGene.ID"]))
    names(gid1) <- dimnames(sig2)[[1]]
    gid2 <- as.numeric(as.character(annot[, "EntrezGene.ID"]))
    names(gid2) <- dimnames(annot)[[1]]
    rm.ix <- is.na(gid1) | duplicated(gid1)
    gid1 <- gid1[!rm.ix]
    rr <- geneid.map(geneid1 = gid2, data1 = data, geneid2 = gid1,
                     verbose = FALSE)
    gm <- length(rr$geneid2)
    mymapping <- c(mapped = gm, total = gt)
    if (length(rr$geneid1) != gt) {
      res <- rep(NA, nrow(data))
      names(res) <- dimnames(data)[[1]]
      warning(sprintf("Probe candidates: %i/%i", gm, gt),
              "\nIncomplete overlap between the gene signature EntrezGene.IDs",
              " and the EntrezGene.ID column of annot... Returning all NAs.")
      return(list(score = res, risk = res, mapping = mymapping,
                  probe = NA))
    }
    gid1 <- rr$geneid2
```

```
  gid2 <- rr$geneid1
  data <- rr$data1
  myprobe <- cbind(probe = names(gid1), EntrezGene.ID = gid1,
                   new.probe = names(gid2))
  dimnames(data)[[2]] <- names(gid2) <- names(gid1)
}else {
  myprobe <- NA
  data <- data[, intersect(dimnames(sig2)[[1]], dimnames(data)[[2]])]
  #data <- data[, intersect(sig2$symbol, dimnames(data)[[2]])]
  gm <- ncol(data)
  mymapping <- c(mapped = gm, total = gt)
  if (nrow(sig2) != ncol(data)) {
    res <- rep(NA, nrow(data))
    names(res) <- dimnames(data)[[1]]
    warning(sprintf("Probe candidates: %i/%i", gm, gt),
            "\nIncomplete overlap between the gene signature EntrezGene.IDs",
            " and the colnames of data... Returning all NAs.")
    return(list(score = res, risk = res, mapping = mymapping,
                probe = myprobe))
  }
}
dimnames(data)[[2]] <- dimnames(sig2)[[1]] <- sig2[, "symbol"]
data <- apply(data, 2, function(x) {
  xx <- (x - min(x, na.rm = TRUE))/(max(x, na.rm = TRUE) -
                                      min(x, na.rm = TRUE))
  return(xx * 15)
})
cc.ix <- complete.cases(data)
rs <- rs.unscaled <- rsrisk <- NULL
for (i in 1:nrow(data)) {
  if (cc.ix[i]) {
    # grb7.gs <- 0.9 * data[i, "GRB7"] + 0.1 * data[i, "ERBB2"]
    grb7.gs <- 0.7 * data[i, "ERBB2"] # grb7.gs replaced by just ERBB2 value
    # 0.7 represents the median expression of GRB7 vs ERBB2 in TCGA-ERpos
    if (grb7.gs < 8) {
      grb7.gs <- 8
    }
    er.gs <- (0.8 * data[i, "ESR1"] + 1.2 * data[i, "PGR"] +
                data[i, "BCL2"] + data[i, "SCUBE2"])/4
    proliferation.gs <- (data[i, "BIRC5"] + data[i, "MKI67"] +
                           data[i, "MYBL2"] + data[i, "CCNB1"] +
                           data[i, "AURKA"])/5
    if (proliferation.gs < 6.5) {
      proliferation.gs <- 6.5
    }
    invasion.gs <- (data[i, "CTSV"] + data[i, "MMP11"])/2
    rsu <- 0.47 * (grb7.gs) - 0.34 * (er.gs) + 1.04 *
      (proliferation.gs) + 0.1 * (invasion.gs) + 0.05 *
      data[i, "CD68"] - 0.08 * data[i, "GSTM1"] - 0.07 *
      data[i, "BAG1"]
    rsu2 <- rsu
    rsu <- rsu * 4 - 18  # adapted from distribution from PMID 32565552
    if (rsu < 11) {
      rsr <- 0
    }
```

```
      if (rsu >= 11 & rsu < 26) {
        rsr <- 0.5
      }
      if (rsu >= 26) {
        rsr <- 1
      }
    }
    else {
      rsu <- rsr <- rsu2 <- NA
    }
    rs.unscaled <- c(rs.unscaled, rsu2)
    rs <- c(rs, rsu)
    rsrisk <- c(rsrisk, rsr)
  }
  names(rs) <- names(rs.unscaled) <- names(rsrisk) <- dimnames(data)[[1]]
  return(list(score = rs, risk = rsrisk, mapping = mymapping,
              probe = myprobe))
}
```

```r
# Function oncotypedxCTSV  with "CTSL2" updated to "CTSV" ####
#
#
# The function oncotypedxCTSV is a modified version
# of the oncotypedx function from library genefu
#
# In function oncotypedxCTSV the gene symbol "CTSL2" is replaced by the
# current HUGO symbol "CTSV". Moreover, the variable "sig.oncotypedx"
# is replaced by "sig.oncotypedx.new"
#  --> within this new table "sig.oncotypedx.new" the gene symbol "CTSL2" is
#   updated to the current HUGO symbol "CTSV".
#
# The scaling and clipping of rsu values in the original function
# from genefu package has been modified in order to adapt the
# distribution of Recurrence Score values from RNA-Seq
# to those from clinical RS data (PMID 32565552)

oncotypedxCTSV <- function (data, annot, do.mapping = FALSE, mapping, verbose = FALSE)
{
  sig2 <- sig.oncotypedx.new[sig.oncotypedx.new[, "group"] != "reference",
                             , drop = FALSE]
  dimnames(sig2)[[1]] <- sig2[, "symbol"]
  gt <- nrow(sig2)
  if (do.mapping) {
    gid1 <- as.numeric(as.character(sig2[, "EntrezGene.ID"]))
    names(gid1) <- dimnames(sig2)[[1]]
    gid2 <- as.numeric(as.character(annot[, "EntrezGene.ID"]))
    names(gid2) <- dimnames(annot)[[1]]
    rm.ix <- is.na(gid1) | duplicated(gid1)
    gid1 <- gid1[!rm.ix]
    rr <- geneid.map(geneid1 = gid2, data1 = data, geneid2 = gid1,
                     verbose = FALSE)
    gm <- length(rr$geneid2)
    mymapping <- c(mapped = gm, total = gt)
    if (length(rr$geneid1) != gt) {
      res <- rep(NA, nrow(data))
      names(res) <- dimnames(data)[[1]]
      warning(sprintf("Probe candidates: %i/%i", gm, gt),
              "\nIncomplete overlap between the gene signature EntrezGene.IDs",
              " and the EntrezGene.ID column of annot... Returning all NAs.")
      return(list(score = res, risk = res, mapping = mymapping,
                  probe = NA))
    }
    gid1 <- rr$geneid2
    gid2 <- rr$geneid1
    data <- rr$data1
    myprobe <- cbind(probe = names(gid1), EntrezGene.ID = gid1,
                     new.probe = names(gid2))
    dimnames(data)[[2]] <- names(gid2) <- names(gid1)
  }else {
    myprobe <- NA
    data <- data[, intersect(dimnames(sig2)[[1]], dimnames(data)[[2]])]
    #data <- data[, intersect(sig2$symbol, dimnames(data)[[2]])]
    gm <- ncol(data)
    mymapping <- c(mapped = gm, total = gt)
```

```
  if (nrow(sig2) != ncol(data)) {
    res <- rep(NA, nrow(data))
    names(res) <- dimnames(data)[[1]]
    warning(sprintf("Probe candidates: %i/%i", gm, gt),
            "\nIncomplete overlap between the gene signature EntrezGene.IDs",
            " and the colnames of data... Returning all NAs.")
    return(list(score = res, risk = res, mapping = mymapping,
                probe = myprobe))
  }
}
dimnames(data)[[2]] <- dimnames(sig2)[[1]] <- sig2[, "symbol"]
data <- apply(data, 2, function(x) {
  xx <- (x - min(x, na.rm = TRUE))/(max(x, na.rm = TRUE) -
                                      min(x, na.rm = TRUE))
  return(xx * 15)
})
cc.ix <- complete.cases(data)
rs <- rs.unscaled <- rsrisk <- NULL
for (i in 1:nrow(data)) {
  if (cc.ix[i]) {
    grb7.gs <- 0.9 * data[i, "GRB7"] + 0.1 * data[i, "ERBB2"]
    if (grb7.gs < 8) {
      grb7.gs <- 8
    }
    er.gs <- (0.8 * data[i, "ESR1"] + 1.2 * data[i, "PGR"] +
                data[i, "BCL2"] + data[i, "SCUBE2"])/4
    proliferation.gs <- (data[i, "BIRC5"] + data[i, "MKI67"] +
                           data[i, "MYBL2"] + data[i, "CCNB1"] +
                           data[i, "AURKA"])/5
    if (proliferation.gs < 6.5) {
      proliferation.gs <- 6.5
    }
    invasion.gs <- (data[i, "CTSV"] + data[i, "MMP11"])/2
    rsu <- 0.47 * (grb7.gs) - 0.34 * (er.gs) + 1.04 *
      (proliferation.gs) + 0.1 * (invasion.gs) + 0.05 *
      data[i, "CD68"] - 0.08 * data[i, "GSTM1"] - 0.07 *
      data[i, "BAG1"]
    rsu2 <- rsu
    rsu <- rsu * 4 - 18  # adapted from distribution from PMID 32565552
    if (rsu < 11) {
      rsr <- 0
    }
    if (rsu >= 11 & rsu < 26) {
      rsr <- 0.5
    }
    if (rsu >= 26) {
      rsr <- 1
    }
  }
  else {
    rsu <- rsr <- rsu2 <- NA
  }
  rs.unscaled <- c(rs.unscaled, rsu2)
  rs <- c(rs, rsu)
  rsrisk <- c(rsrisk, rsr)
```

```
  }
  names(rs) <- names(rs.unscaled) <- names(rsrisk) <- dimnames(data)[[1]]
  return(list(score = rs, risk = rsrisk, mapping = mymapping,
              probe = myprobe))
}

# ****************************************************
```

```r
# Function tcgaRseqGenelist  ####
#
#
# The function tcgaRseqGenelist obtains RNA-Seq data of a provided genelist
#  from TCGA using the cBioPortal access tools.
#
# We apply the cBioPortalData package to access data from the cBIO Portal
# at www.cbioportal.org
# This will allow to download RNA-Seq data from the TCGA-BRCA cohort.

library(cBioPortalData)
# First we setup some parameters for the cBioportal-access
# Define api
cbio <- cBioPortal()
```

```
## Warning in .service_validate_md5sum(api_reference_url, api_reference_md5sum, : service
version differs from validated version
##     service url: https://www.cbioportal.org/api/v2/api-docs
##     observed md5sum: 7314de5c5e8056e4e07b411b3e5a0cb9
##     expected md5sum: 07ceb76cc5afcf54a9cf2e1a689b18f7
```

```r
# Function definition:
#  (genelist is a vector of gene symbols)

tcgaRseqGenelist <- function (genelist) {
  # Download BRCA RNA-Seq data for this genelist from cBioPortal
  #  as a "MultiAssayExperiemnt" brca_rnaseq
  brca_rnaseq <- cBioPortalData(
    api = cbio,
    studyId = "brca_tcga",
    genes = genelist, by = "hugoGeneSymbol",
    molecularProfileIds = "brca_tcga_rna_seq_v2_mrna"
    )
  # Extract the RNA-Seq data from the MultiAssayExperiment
  tcgaRseqGenelist <- assay(brca_rnaseq[["brca_tcga_rna_seq_v2_mrna"]])

}
```

```r
# Function tcgaRseqGenelistERpos  ####
#
#
# The function tcgaRseqGenelistERpos obtains RNA-Seq data of a provided genelist
#  from TCGA using the cBioPortal access tools
#  and delivers only the data of ERpos BRCA samples.
#
# We apply the cBioPortalData package to access data from the cBIO Portal
# at www.cbioportal.org
# This will allow to download RNA-Seq data from the TCGA-BRCA cohort.

library(cBioPortalData)
# First we setup some parameters for the cBioportal-access
# Define api
cbio <- cBioPortal()

# Function definition:
#  (genelist is a vector of gene symbols)

tcgaRseqGenelistERpos <- function (genelist) {
  # Download BRCA RNA-Seq data for this genelist from cBioPortal
  #  as a "MultiAssayExperiemnt" brca_rnaseq
  brca_rnaseq <- cBioPortalData(
    api = cbio,
    studyId = "brca_tcga",
    genes = genelist, by = "hugoGeneSymbol",
    molecularProfileIds = "brca_tcga_rna_seq_v2_mrna"
  )
  # Extract the RNA-Seq data from the MultiAssayExperiment
  tcgaRseqGenelist <- assay(brca_rnaseq[["brca_tcga_rna_seq_v2_mrna"]])

  # Extract the phenotype data for TCGA-samples (by patientId)
  pheno <- colData(brca_rnaseq)

  # Extract the link-information between
  # the patientId ("primary") and  the RNA-seq-colnames ("colname")
  # from the MultiAssayExperiment
  sample_info <- unique(sampleMap(brca_rnaseq)[,2:3])


  # Now we use dplyr functions from tidyR to join
  #    the "ER_STATUS_BY_IHC" from pheno
  #    with the "colname" from sample_info
  # by linking the cases using the patientId == primary

  pdata <- as.data.frame(pheno) %>%
    dplyr::select(patientId, ER_STATUS_BY_IHC) %>%
    left_join(as.data.frame(sample_info), by = join_by(patientId == primary))

  # We can now use pdata to select only ER-positive samples

  pdata.erpos <- pdata %>% filter(ER_STATUS_BY_IHC == "Positive")

  tcgaRseqGenelistERpos <- tcgaRseqGenelist[, colnames(tcgaRseqGenelist)
```

```
                                        %in% pdata.erpos$colname]
}
```

```
# DATA IMPORT ####

library(dplyr)
```

Import list of genes from HTG-panel

```
htgprobes <- pull(read.table("HTG-OncBiomarkerPanel_n2559-Genelist.txt",
                        header=FALSE, sep=","))
```

Definition of genelist for Oncotype Recurrence Score

```
library(genefu)
# Load the Oncotype gene info table from the genefu package
data(sig.oncotypedx)
# Save original copy of this gene info table
sig.oncotypedx.ori <- sig.oncotypedx

# CTSL2 (EntrezGene.ID 1515) has been renamed to CTSV in current HUGO versions
# Thus we need to rename this gene in the new version "sig.oncotypedx.new":
sig.oncotypedx.new <- sig.oncotypedx
sig.oncotypedx.new[rownames(sig.oncotypedx.new)=="CTSL2", ]$symbol <- "CTSV"
rownames(sig.oncotypedx.new) <- sig.oncotypedx.new$symbol

# Remove the row with the gene "GRB7" unavailable in HTG-panel:
sig.oncotypedx.htg <- sig.oncotypedx.new[!(rownames(sig.oncotypedx.ori)
                                      %in% c("GRB7")), ]
```

Import GGI genelist from genefu package

```
library(genefu)
data(sig.ggi)
```

Import SET-ER/PR index genelist from PMID 31231679 (Sinn 2019) Suppl.Table 2

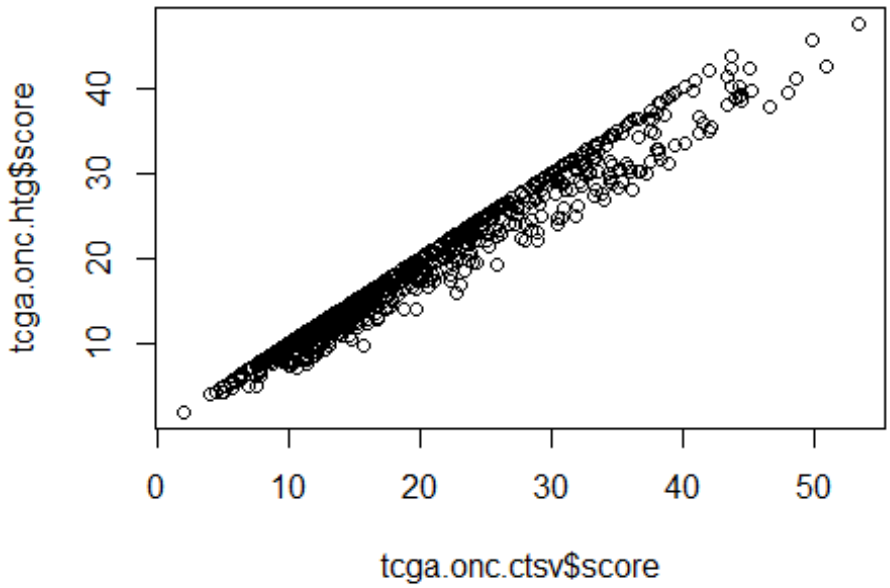
```
sig.set18 <-  pull(read.table("SET-ERPR-genes_PMID_31231679.txt",
                              header=FALSE, sep=","))
```

Import 355 Penelope signature infos

```
sig.pen355 <-  read.table("Penelope_n355genes_info.txt",
                          header=TRUE, sep="\t")
```

```r
# ANALYSIS ####

# Recurrence Score ####

# IMPORT TCGA-RNAseq data for oncotype genes

genelist <- sig.oncotypedx.new$symbol

# RNAseq for all TCGA-BRCA (including ERneg)
tcga.RS.Rseq <- tcgaRseqGenelist(genelist)

## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'

# RNAseq for ERpos TCGA-BRCA
tcga.RS.Rseq.ERpos <- tcgaRseqGenelistERpos(genelist)

## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'

# ************************************************************
#  Calculate Oncotype Recurrence Score for ERpos TCGA samples  *
# ************************************************************


# Format data for oncotype function from genefu package
#
#   Transpose the count matrix of the ERpos subset
tcga <- t(tcga.RS.Rseq.ERpos)

# log2 transform count data
log2tcga <- log2(tcga + 1)

# First we run the oncotypedxCTSV function for oncotype classification
# This includes the GRB7 gene and gene symbol "CTSL2" is updated to "CTSV".

tcga.onc.ctsv <- oncotypedxCTSV(data=log2tcga,
                          annot=sig.oncotypedx.new,
                          do.mapping = TRUE, verbose = TRUE)


# Next we run the oncotypedxHTG function for oncotype classification
# This excludes the GRB7 gene (but includes "CTSL2" updated to "CTSV").

tcga.onc.htg <- oncotypedxHTG(data=log2tcga,
                          annot=sig.oncotypedx.new,
                          do.mapping = TRUE, verbose = TRUE)



# ********************************************************************
```

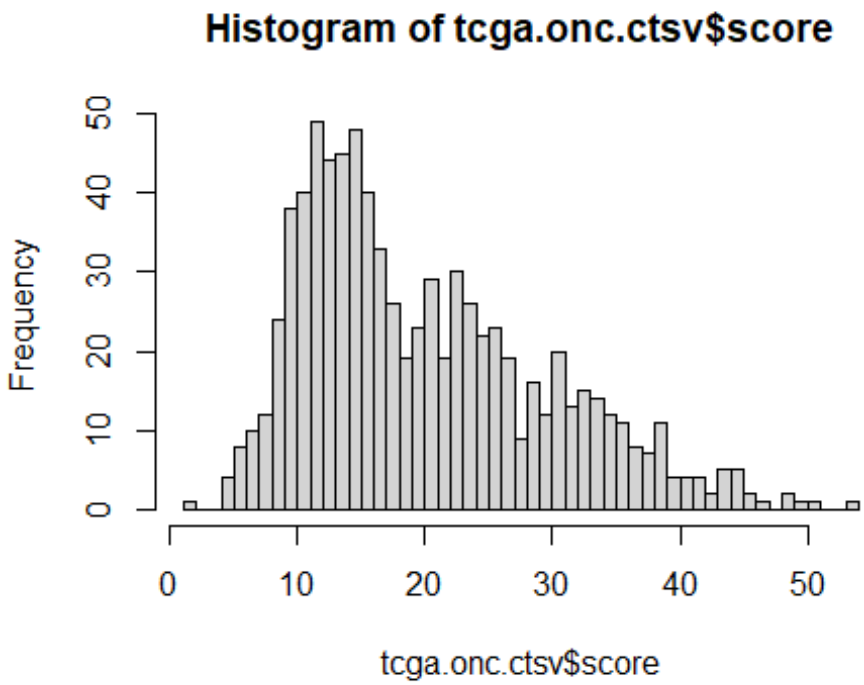
```r
# COMPARE OBTAINED RESULTS FOR RS ####

# Finally we compare the results obtained from the two different functions

plot(tcga.onc.ctsv$score, tcga.onc.htg$score)
```



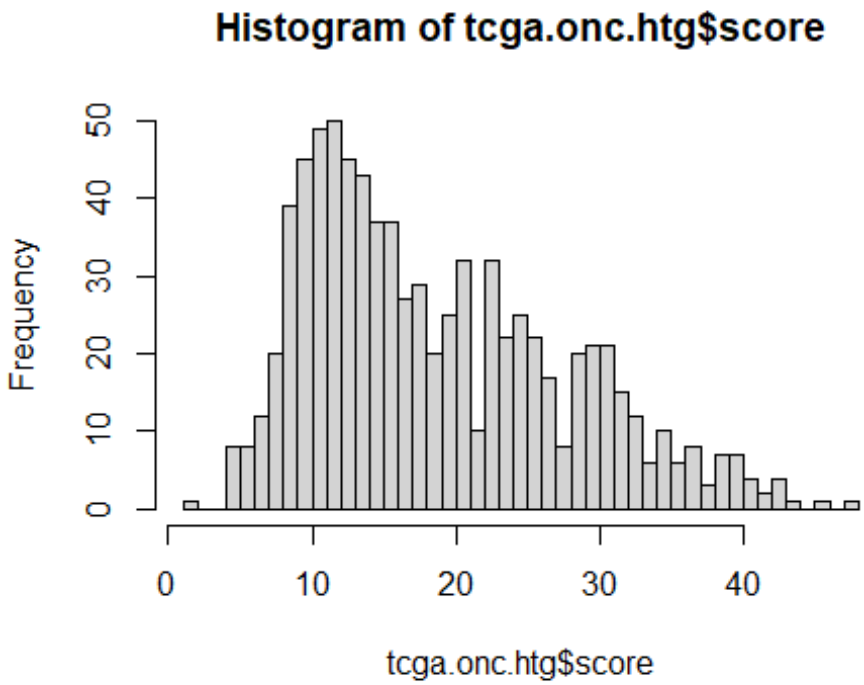

```r
cor(tcga.onc.ctsv$score, tcga.onc.htg$score)
```

```
## [1] 0.9839576
```

```r
# Interpretation: Some minor differences when GRB7 is omitted (oncotypedxHTG),
# but still very good consistency with R=0.98

hist(tcga.onc.ctsv$score, breaks = 40)
```




```r
hist(tcga.onc.htg$score, breaks = 40)
```




```r
# Distributions of oncotype recurrences scores
# match published data (PMID 32565552)

table(tcga.onc.ctsv$risk)
```

```
##
##   0 0.5   1
## 137 476 199

table(tcga.onc.htg$risk)

##
##   0 0.5   1
## 182 456 174

# risk groups similar

# ************************************************************************
```

```r
# Genomic Grade Index ####

# ********************************
#  Calculate GGI for TCGA samples *
# ********************************
#

# # Import GGI genelist from genefu package
# # Has already been performed in DATA IMPORT section above !
# library(genefu)
# data(sig.ggi)

# count number of GGI genes with HUGO.gene.symbol
sum(!is.na(sig.ggi$HUGO.gene.symbol))
```

```
## [1] 112
```

```r
# 112 genes

# count number of GGI genes in HTG-panel
sum(sig.ggi$HUGO.gene.symbol %in% htgprobes)
```

```
## [1] 56
```

```r
# 56 genes available

# Use HUGO.gene.symbol as probe name for TCGA mapping
library(dplyr)
library(tibble)
HUGO.sig.ggi <- sig.ggi %>%
  filter(!is.na(HUGO.gene.symbol)) %>%  # keep only probes with HUGO gene symbol
  mutate(probe = HUGO.gene.symbol) %>%  # replace Affy probes with gene symbol
  distinct(probe, .keep_all = TRUE) %>% # remove duplicates
  mutate(weight = grade-2) %>%          # weights: +1 / -1
  dplyr::select(probe, weight, HUGO.gene.symbol,
                EntrezGene.ID) %>%      # select cols
  remove_rownames() %>%                 # remove Affy-rownames
  arrange(., probe)                     # order rows by gene names



# Get TCGA RNA-seq data of the breast cancer cohort for
# the genes from GGI gene list:

# Define genelist
genelist <- HUGO.sig.ggi$probe

# RNAseq for all TCGA-BRCA (including ERneg)
tcga.ggi.Rseq <- tcgaRseqGenelist(genelist)
```

```
## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'
```

```r
# RNAseq for ERpos TCGA-BRCA
tcga.ggi.Rseq.ERpos <- tcgaRseqGenelistERpos(genelist)
```

```
## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'
```

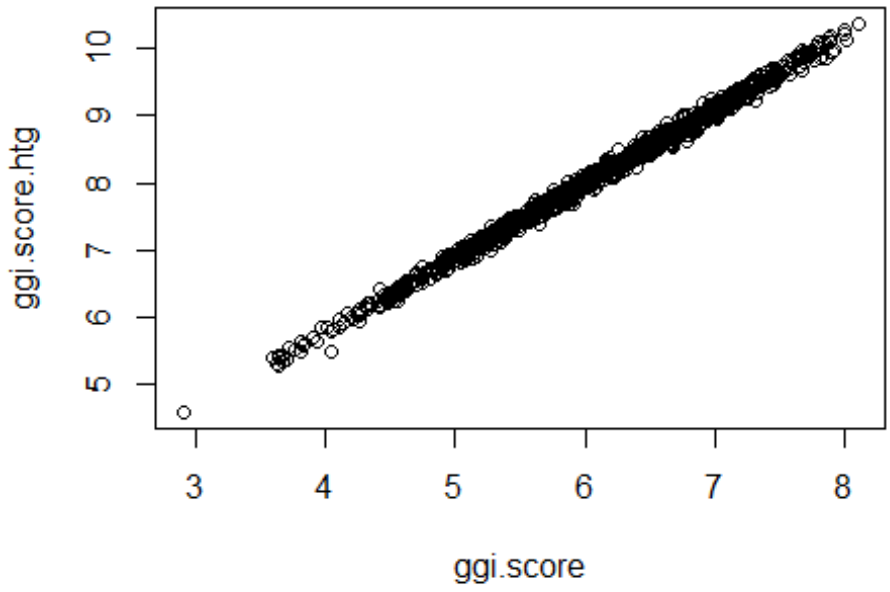
Calculate GGI all TCGA-BRCA (including ERneg)

```
# Transpose the count matrix of ALL TCGA samples
tcga <- t(tcga.ggi.Rseq)

# log2 transform count data
log2tcga <- log2(tcga + 1)

# order columns by gene names
log2tcga <- log2tcga[,order(colnames(log2tcga))]


# *** Use all 85 ggi-genes available in TCGA dataset: ***

tcga.sig.ggi <- HUGO.sig.ggi %>%
  filter(probe %in% colnames(log2tcga))

# check identity:
stopifnot(colnames(log2tcga) == tcga.sig.ggi$probe)

# Compute GGI for TCGA
ggi.score <- rep(NA,nrow(log2tcga)) # empty vector for results
names(ggi.score) <- rownames(log2tcga)
for (i in 1:nrow(log2tcga)){
  ggi.score[i] <- mean(log2tcga[i,]*tcga.sig.ggi$weight)
}


# *** Use only 46 ggi-genes available in HTG and TCGA dataset: ***

tcga.sig.ggi.htg <- HUGO.sig.ggi %>%
  filter(probe %in% colnames(log2tcga)) %>%
  filter(probe %in% htgprobes)

# TCGA RNA-Seq or subset of 46 HTG ggi-genes
log2tcga.htg <- log2tcga[, colnames(log2tcga) %in% htgprobes]

# check identity:
stopifnot(colnames(log2tcga.htg) == tcga.sig.ggi.htg$probe)

# Compute GGI for TCGA using only HTG genes
ggi.score.htg <- rep(NA,nrow(log2tcga.htg)) # empty vector for results
names(ggi.score.htg) <- rownames(log2tcga.htg)
for (i in 1:nrow(log2tcga.htg)){
  ggi.score.htg[i] <- mean(log2tcga.htg[i,]*tcga.sig.ggi.htg$weight)
}
```

Calculate GGI for TCGA-ERpos-Only

```
# Transpose the count matrix of ERpos TCGA samples
tcga <- t(tcga.ggi.Rseq.ERpos)
```

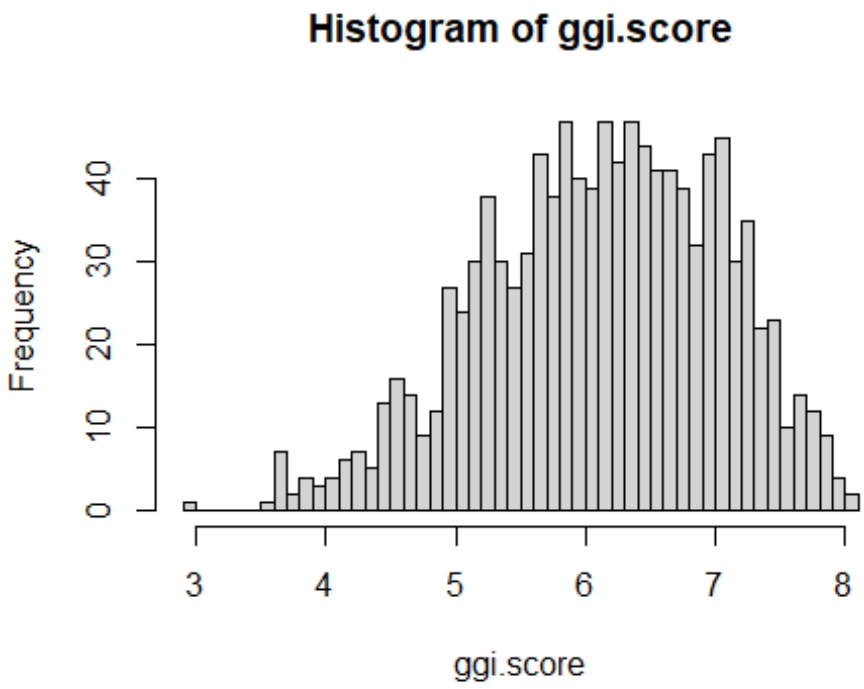
```r
# log2 transform count data
log2tcga <- log2(tcga + 1)

# order columns by gene names
log2tcga <- log2tcga[,order(colnames(log2tcga))]

# *** Use only 46 ggi-genes available in HTG and TCGA dataset: ***

tcga.sig.ggi.htg <- HUGO.sig.ggi %>%
  filter(probe %in% colnames(log2tcga)) %>%
  filter(probe %in% htgprobes)

# TCGA RNA-Seq or subset of 46 HTG ggi-genes
log2tcga.htg <- log2tcga[, colnames(log2tcga) %in% htgprobes]

# check identity:
stopifnot(colnames(log2tcga.htg) == tcga.sig.ggi.htg$probe)

# Compute GGI for TCGA using only HTG genes
ggi.score.htg.ERpos <- rep(NA,nrow(log2tcga.htg)) # empty vector for results
names(ggi.score.htg.ERpos) <- rownames(log2tcga.htg)
for (i in 1:nrow(log2tcga.htg)){
  ggi.score.htg.ERpos[i] <- mean(log2tcga.htg[i,]*tcga.sig.ggi.htg$weight)
}


# COMPARE OBTAINED RESULTS FOR GGI ####

# *** ggi from 85 total genes and 46 htg genes in ALL TCGA BRCA: ***

plot(ggi.score, ggi.score.htg)
```



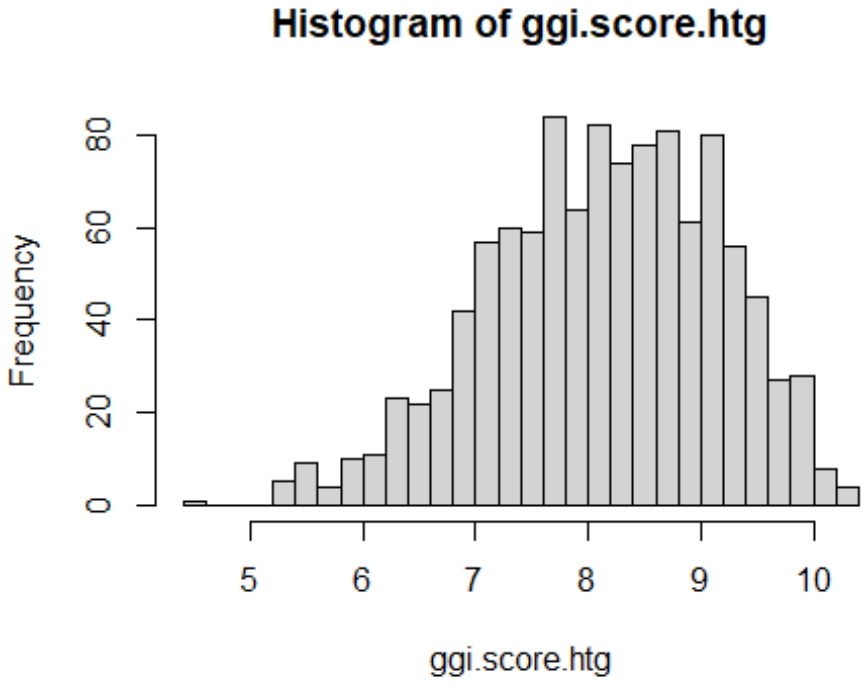
```r
cor(ggi.score, ggi.score.htg)
```

```
## [1] 0.9974926
```

```r
# Interpretation: nearly identical, R=0,997

hist(ggi.score, breaks = 40)
```




```r
hist(ggi.score.htg, breaks = 40)
```

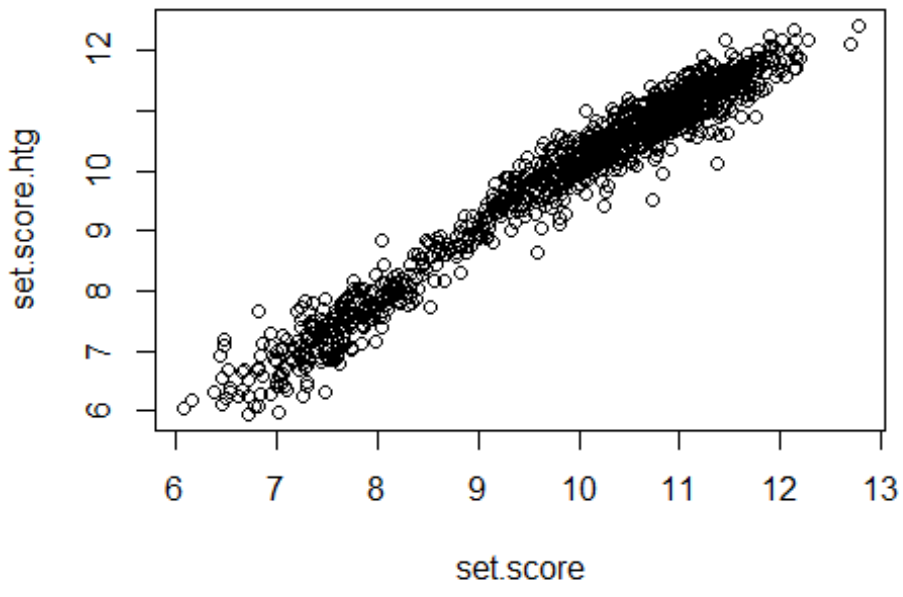


```
# SET Index ####

# ******************************************
#  Calculate SET-ER/PR Index for TCGA samples *
# ******************************************
#

# # Import SET-ER/PR index genelist from PMID 31231679 (Sinn 2019) Suppl.Table 2
# # Has already been performed in DATA IMPORT section above !
# sig.set18 <-  pull(read.table("SET-ERPR-genes_PMID 31231679.txt",
#                               header=FALSE, sep=","))



sig.set18.htg <- sig.set18[sig.set18 %in% htgprobes]
# 12 genes available

# SET18 genes missing:
sig.set18[!(sig.set18 %in% htgprobes)]

## [1] "NPY1R"  "AZGP1"  "ABAT"   "ADCY1"  "MRPS30" "KCNE4"

# "NPY1R"  "AZGP1"  "ABAT"   "ADCY1"  "MRPS30" "KCNE4"


##################################### #
# Get TCGA RNA-seq data of the breast cancer cohort for
# the genes from SET-ER/PR index gene list:

# Define genelist
genelist <- sig.set18

tcga.set18.Rseq <- tcgaRseqGenelist(genelist)  # including ERneg

## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'

tcga.set18.Rseq.ERpos <- tcgaRseqGenelistERpos(genelist)

## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'
```

SET index for ALL TCGA-BRCA (including ERneg)

```
# Transpose the count matrix of ALL TCGA samples
tcga <- t(tcga.set18.Rseq)

# log2 transform count data
log2tcga <- log2(tcga + 1)

# order columns by gene names
log2tcga <- log2tcga[,order(colnames(log2tcga))]


# *** Use all 18 SET-ER/PR index genes available in TCGA dataset: ***
```

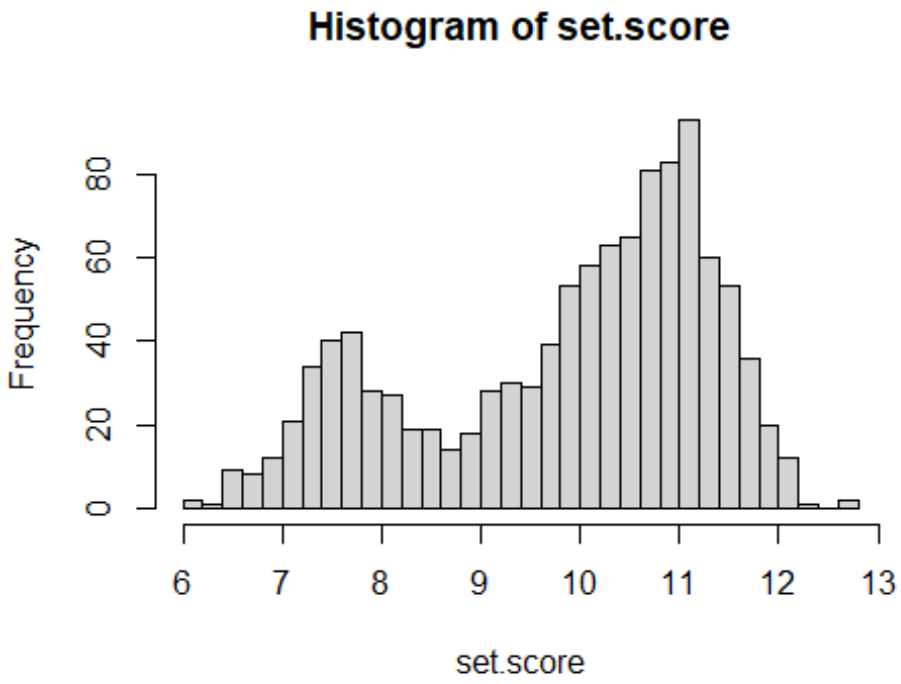
```r
# Compute mean of set18 for TCGA
set.score <- rep(NA,nrow(log2tcga)) # empty vector for results
names(set.score) <- rownames(log2tcga)
for (i in 1:nrow(log2tcga)){
  set.score[i] <- mean(log2tcga[i,])
}


# *** Use only the 12 SET-ER/PR-index genes available in HTG panel: ***

# TCGA RNA-Seq or subset of 46 HTG ggi-genes
log2tcga.htg <- log2tcga[, colnames(log2tcga) %in% sig.set18.htg]

# Compute mean of set18.htg for TCGA using only HTG genes
set.score.htg <- rep(NA,nrow(log2tcga.htg)) # empty vector for results
names(set.score.htg) <- rownames(log2tcga.htg)
for (i in 1:nrow(log2tcga.htg)){
  set.score.htg[i] <- mean(log2tcga.htg[i,])
}


# COMPARE OBTAINED RESULTS FOR SET18 ####

# *** SET18 from 18 total genes and 12 htg genes in TCGA dataset: ***
#     (including ERneg)

plot(set.score, set.score.htg)
```



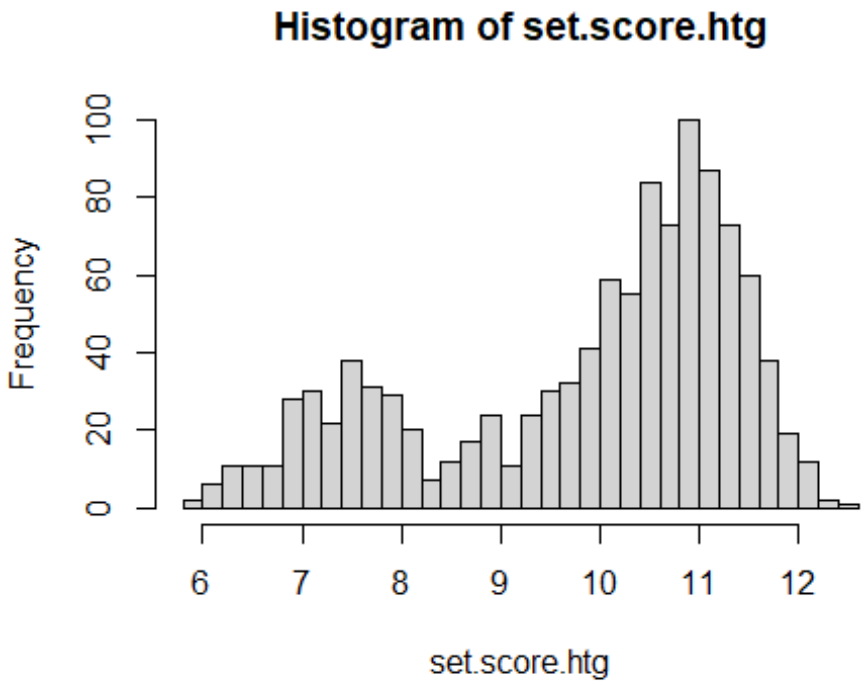

```r
cor(set.score, set.score.htg)
```

```
## [1] 0.9795561
```

```r
# Interpretation: nearly identical, R=0.98

hist(set.score, breaks = 40)
```

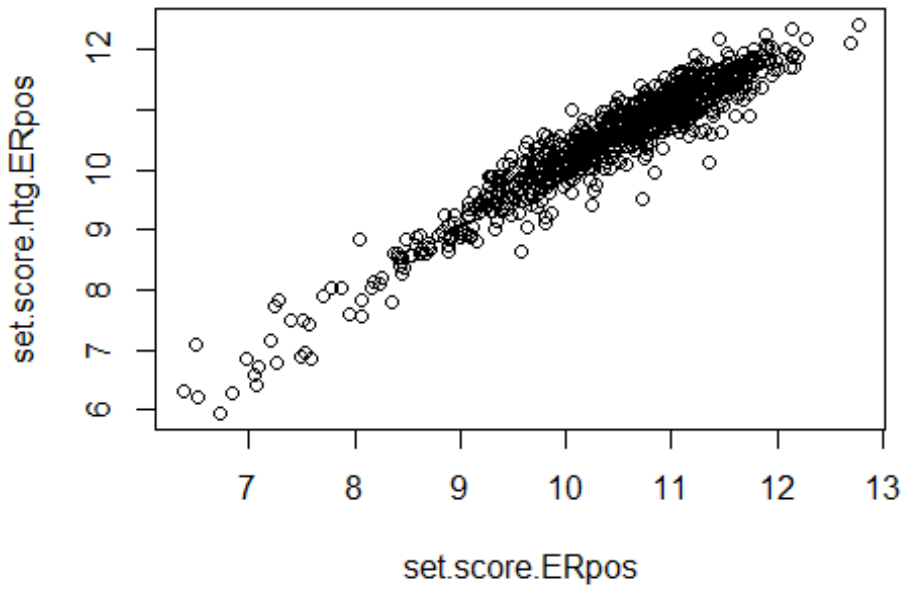



```r
hist(set.score.htg, breaks = 40)
```

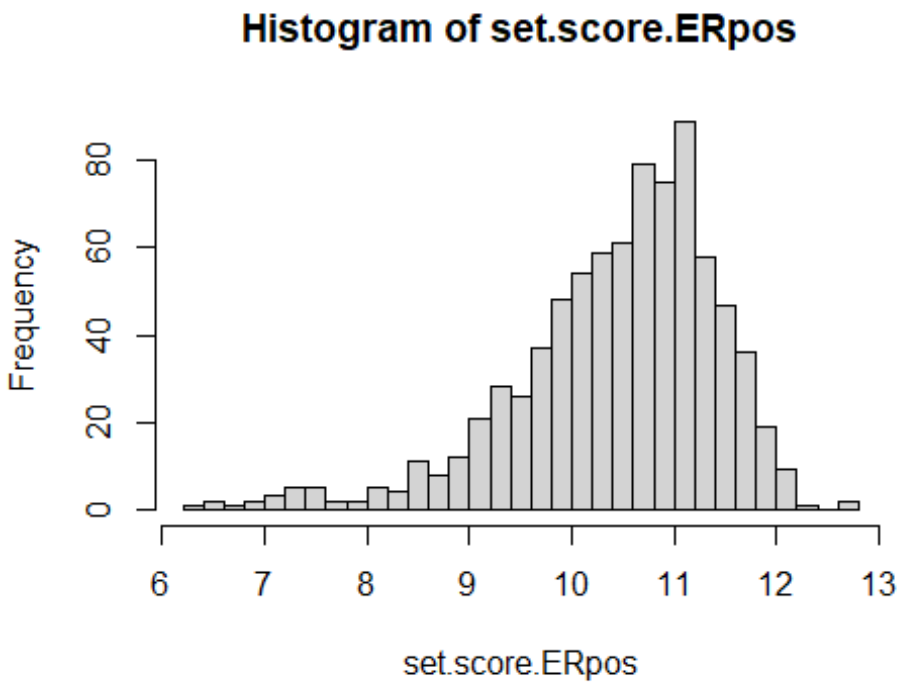



```r
# Plot data only for ERpos-BRCA

set.score.ERpos <- set.score[names(set.score)
```

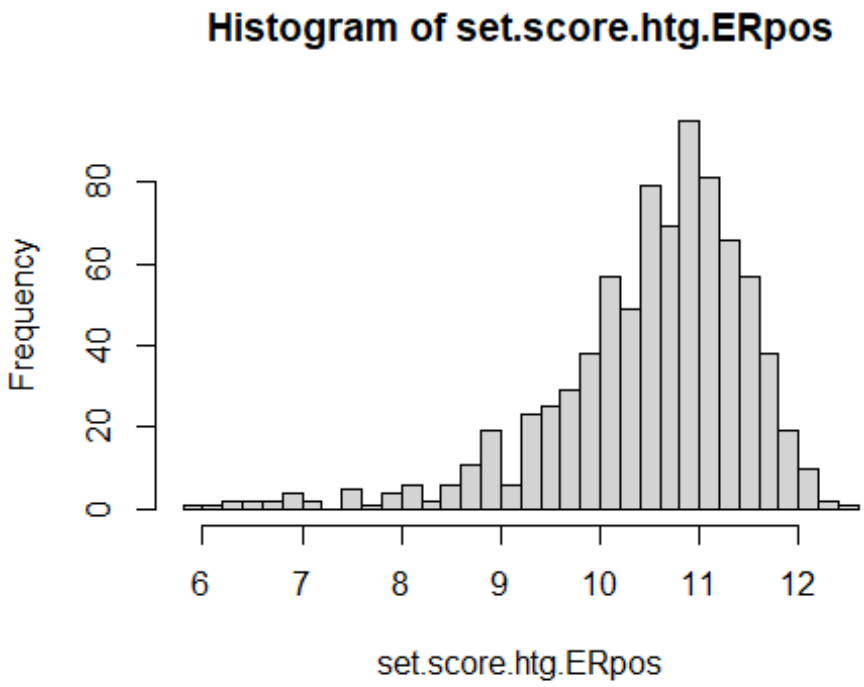
```
                              %in% colnames(tcga.set18.Rseq.ERpos)]

set.score.htg.ERpos <- set.score.htg[names(set.score.htg)
                                     %in% colnames(tcga.set18.Rseq.ERpos)]

plot(set.score.ERpos, set.score.htg.ERpos)
```




```
cor(set.score.ERpos, set.score.htg.ERpos)

## [1] 0.9556287

# Interpretation: strong correlation, R=0.956


hist(set.score.ERpos, breaks = 40)
```



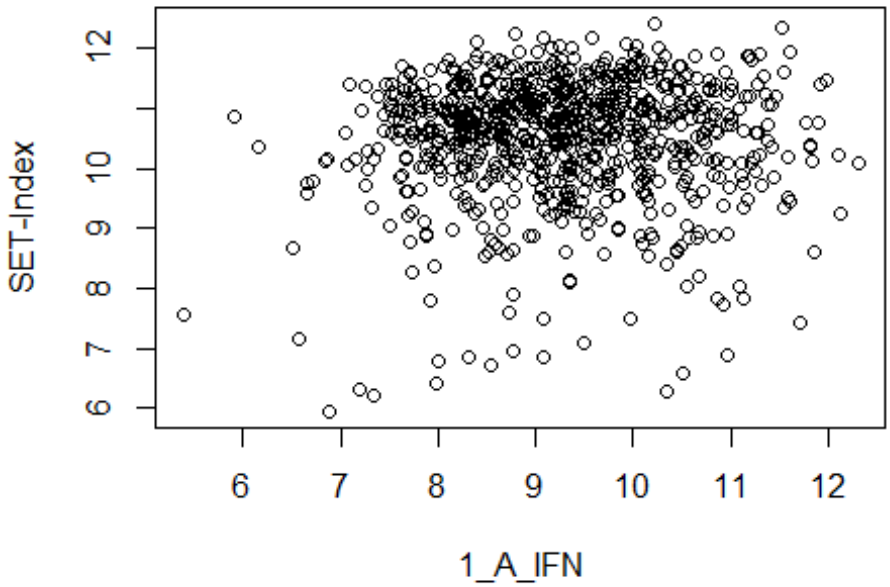

```r
hist(set.score.htg.ERpos, breaks = 40)
```

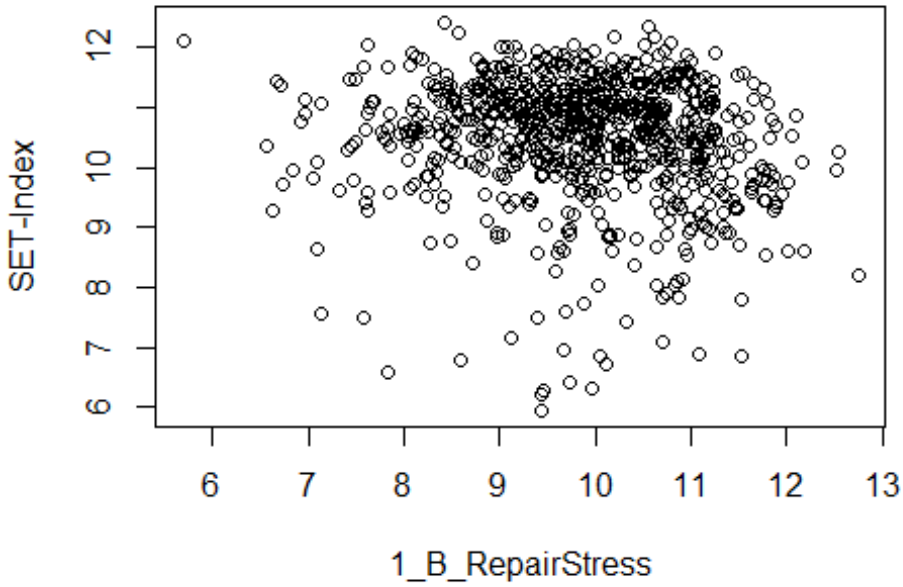


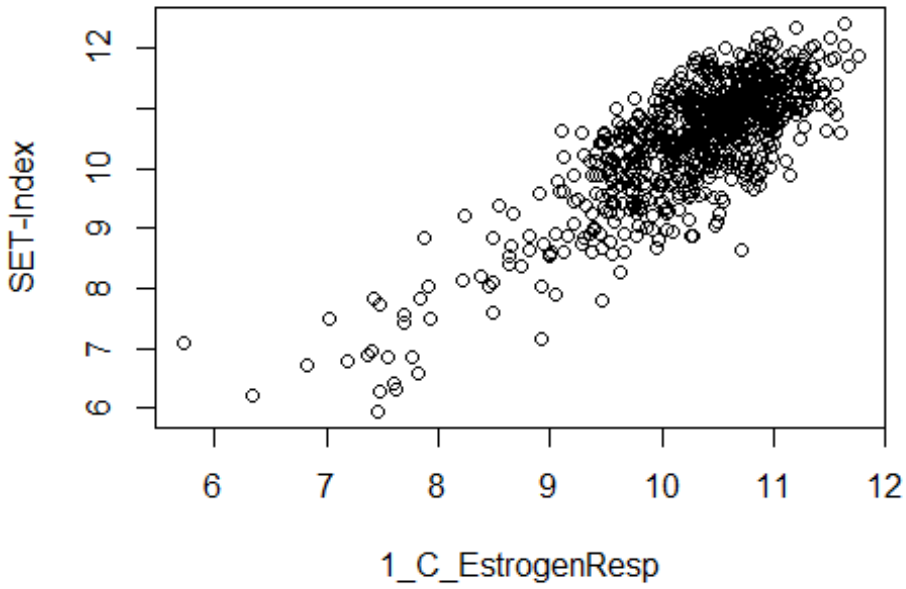
```r
# PENELOPE-Signature ####

# ******************************************
#  Define subsets of 355 Penelope signature   *
# ******************************************
#

# # Import 355 Penelope signature infos
# # Has already been performed in DATA IMPORT section above !
# sig.pen355 <-  read.table("Penelope_n355genes_info.txt",
#                               header=TRUE, sep="\t")


# Use TCGA RNA-Seq data as dataset:

# Get TCGA RNA-seq data of the breast cancer cohort for
# the genes from SET-ER/PR index gene list:

# Define genelist
genelist <- sig.pen355$Gene

tcga.pen355.Rseq <- tcgaRseqGenelist(genelist)

## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'

tcga.pen355.Rseq.ERpos <- tcgaRseqGenelistERpos(genelist)

## harmonizing input:
##   removing 8 colData rownames not in sampleMap 'primary'

# Only 323 of the 355 genes are available in the TCGA dataset from cBioPortal

sig.pen323 <- sig.pen355[sig.pen355$Gene %in% rownames(tcga.pen355.Rseq.ERpos),]

# re-order the genes in rows of RNA-Seq matrix according to sig.pen.323
tcga.pen355.Rseq.ERpos <- tcga.pen355.Rseq.ERpos[sig.pen323$Gene,]

# calculate signature scores
stopifnot(sig.pen323$Gene == rownames(tcga.pen355.Rseq.ERpos))


# Transpose the count matrix of ALL TCGA samples
tcga <- t(tcga.pen355.Rseq.ERpos)

# log2 transform count data
log2tcga <- log2(tcga + 1)




#### Calculate the subcluster mean values for all samples in dataset:

# Define Subcluster signatures to calculate:
```

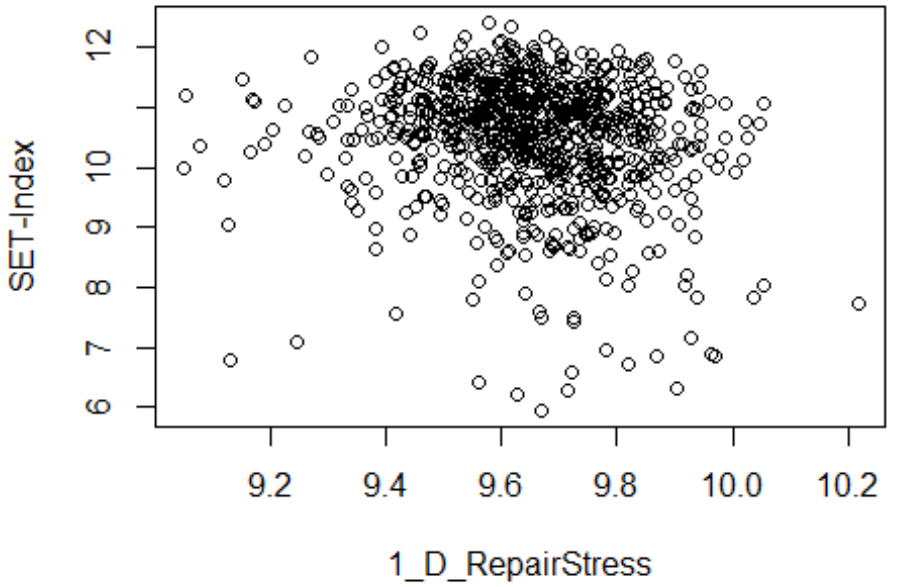
```r
pen322.subclu <- unique(sig.pen323$SubCluster_1080pairedSamples)

# Define RNAseq data:
rseqdata <- log2tcga


# define dataframe for results

rseqdata.pen322.subclu.scores <- data.frame(matrix(NA,
                                                   nrow = nrow(rseqdata),
                                                   ncol = length(pen322.subclu)))
rownames(rseqdata.pen322.subclu.scores) <- rownames(rseqdata)
colnames(rseqdata.pen322.subclu.scores) <- pen322.subclu

# Loop over all pen322 subcluster with second inner loop to calculate
#   mean-score of subclusters for all samples in rseqdata

for (i in 1:length(pen322.subclu)) {               # loop over pen322 subclusters
  genes <- sig.pen323$Gene[sig.pen323$SubCluster_1080pairedSamples
                           %in% pen322.subclu[i]]   # select genes of subcluster
    for (k in 1:nrow(rseqdata)) {  # loop over samples to caclulate score
      rseqdata.pen322.subclu.scores[k,i] <- mean(rseqdata[k, genes], na.rm = TRUE)
    }
}

# Save re-named result:

tcgaERpos.pen322.subclu.scores <- rseqdata.pen322.subclu.scores


##############################################  #
```

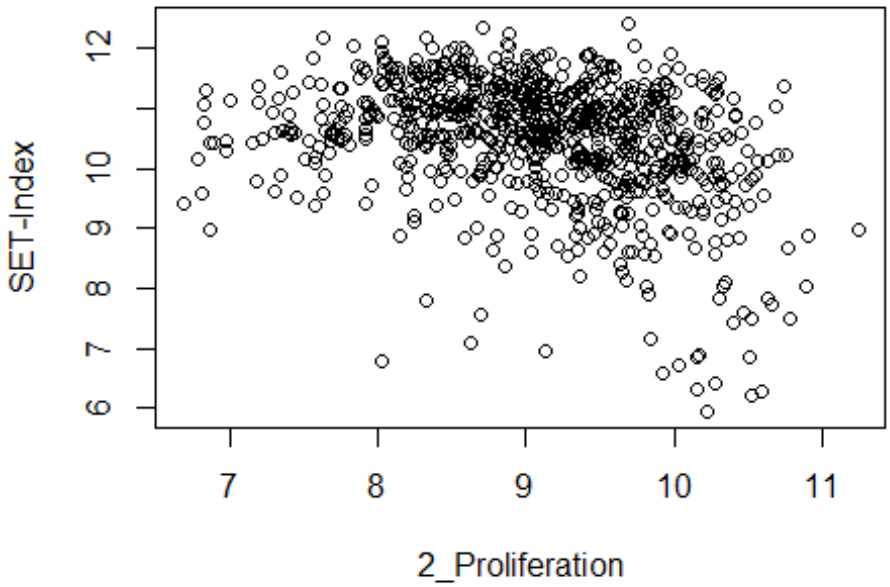
```r
# Compare PENELOPE-Subcluster-Signatures to other GeneSignatures ####


# SET-Index:

stopifnot(rownames(tcgaERpos.pen322.subclu.scores) == names(set.score.htg.ERpos))

for (i in 1:length(pen322.subclu)) {
  plot(tcgaERpos.pen322.subclu.scores[,i], set.score.htg.ERpos,
       xlab = pen322.subclu[i], ylab = "SET-Index")
}
```

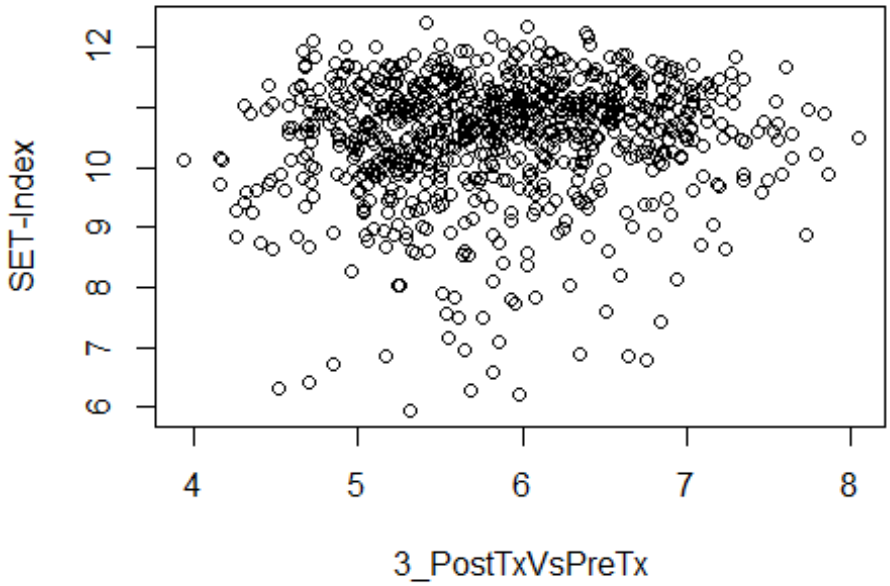

SET-Index
6
7
8
9
10
11
12
6
7
8
9
10
11
12
1_A_IFN

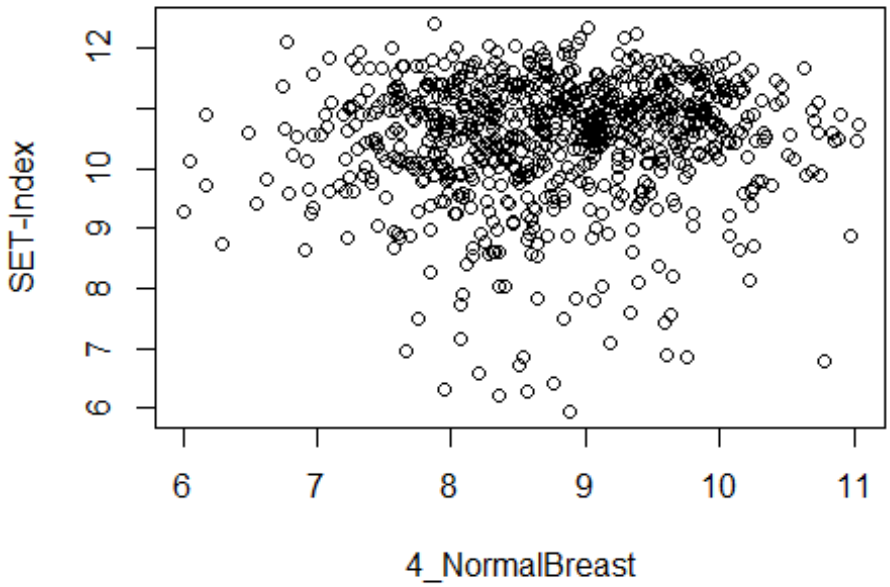

SET-Index
6
7
8
9
10
11
12
6
7
8
9
10
11
12
13
1_B_RepairStress


SET-Index
6
7
8
9
10
11
12
1_C_EstrogenResp

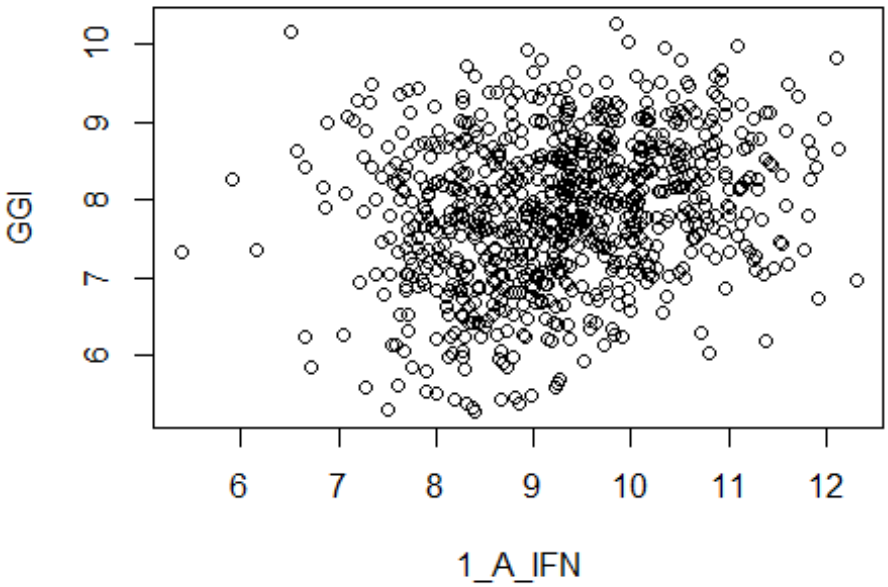

SET-Index
6
7
8
9
10
11
12
9.2
9.4
9.6
9.8
10.0
10.2
1_D_RepairStress

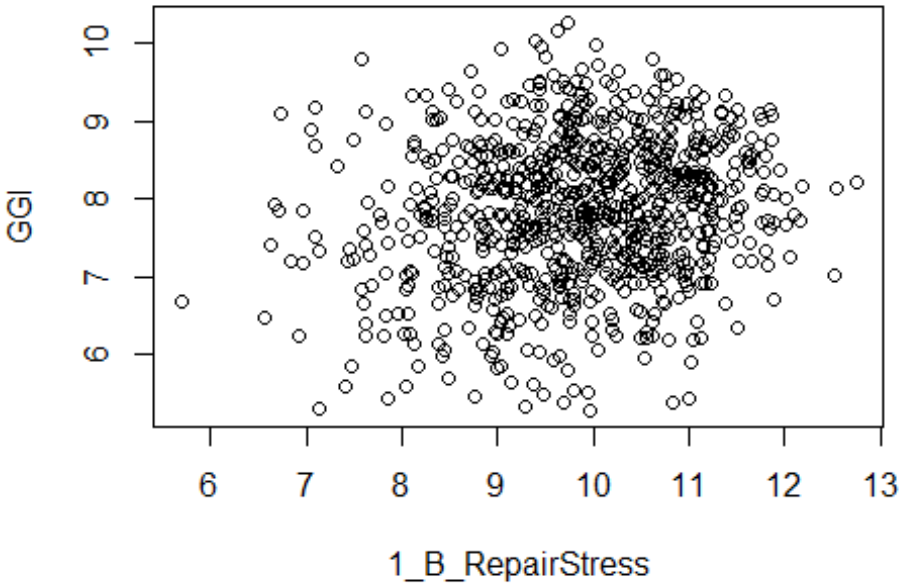

SET-Index
6
7
8
9
10
12
7
8
9
10
11
2_Proliferation

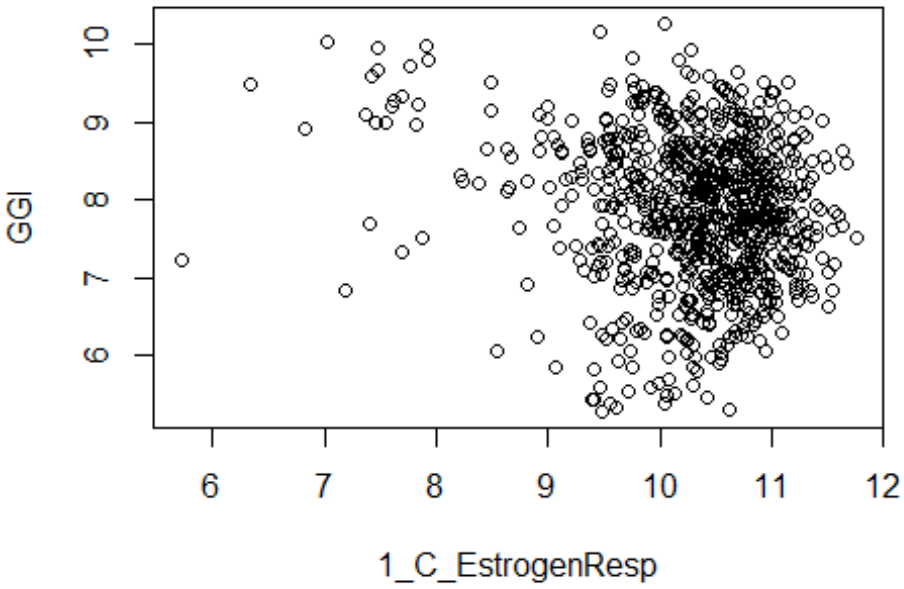

SET-Index
6
7
8
9
10
12
4
5
6
7
8
3_PostTxVsPreTx

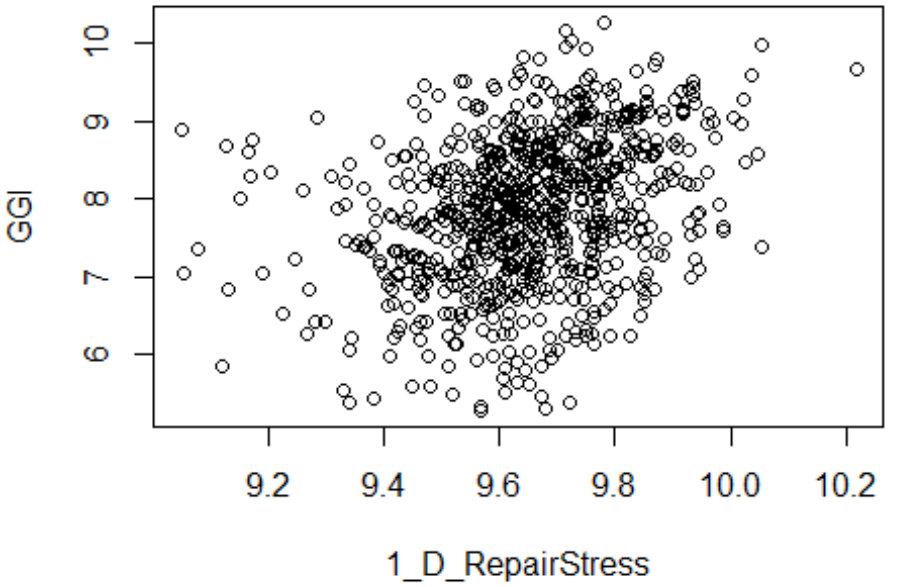


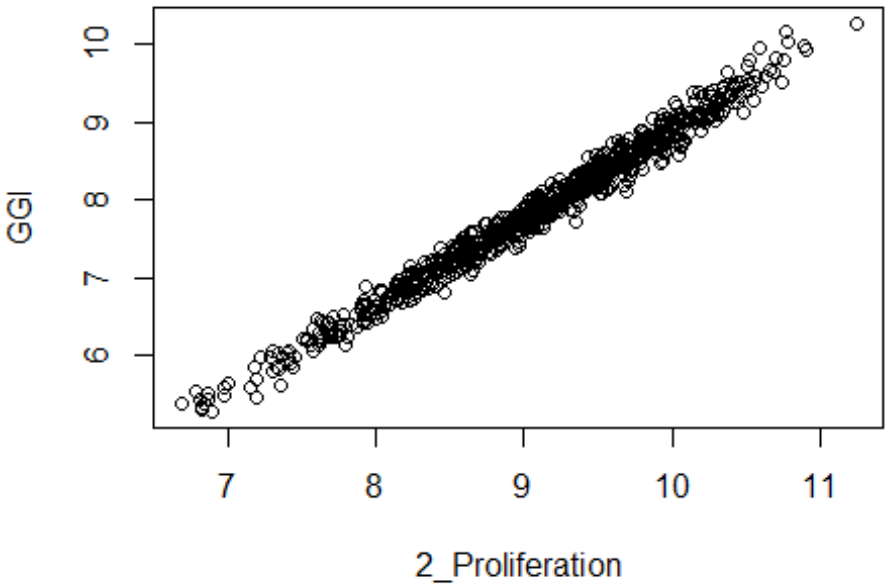

```
# GGI:

stopifnot(rownames(tcgaERpos.pen322.subclu.scores) == names(ggi.score.htg.ERpos))

for (i in 1:length(pen322.subclu)) {
  plot(tcgaERpos.pen322.subclu.scores[,i], ggi.score.htg.ERpos,
       xlab = pen322.subclu[i], ylab = "GGI")
}
```

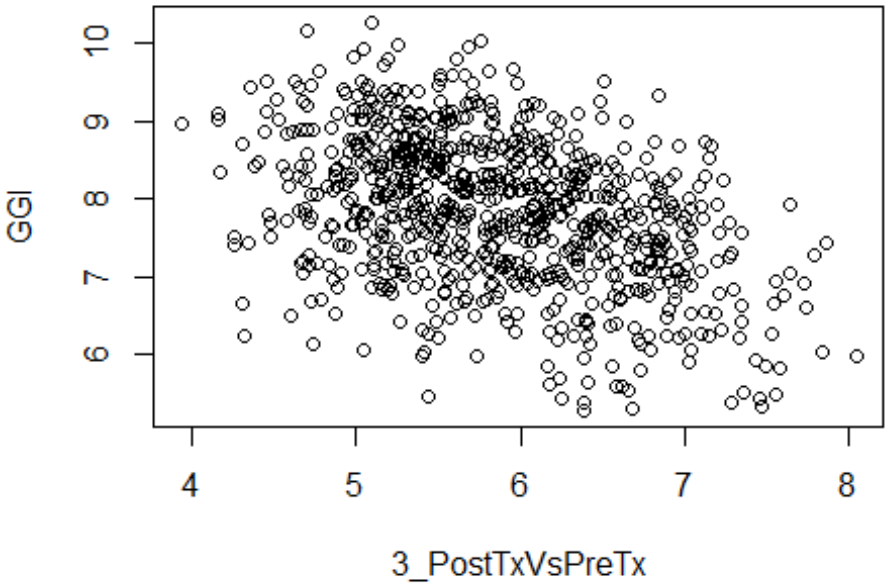

GGI
6
7
8
9
10
6
7
8
9
10
11
12
1_A_IFN

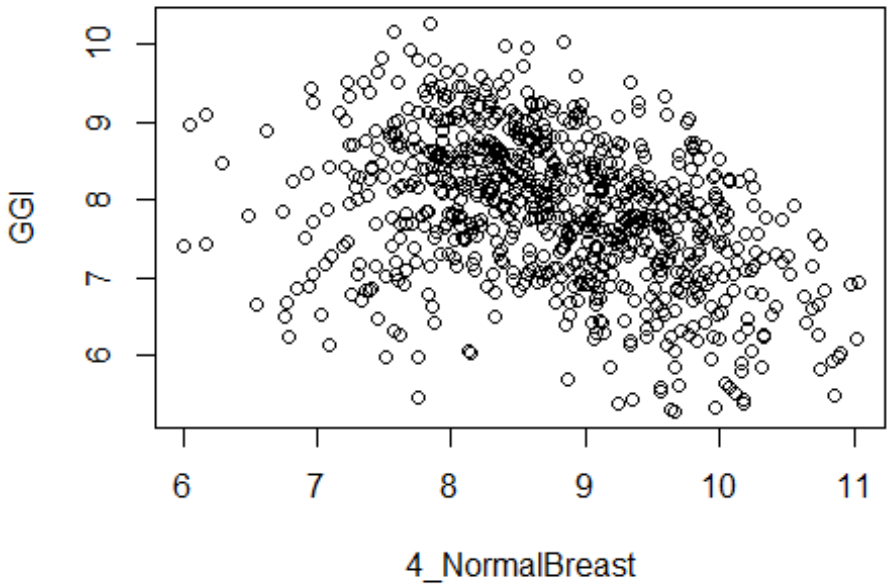

GGI
6
7
8
9
10
6
7
8
9
10
11
12
13
1_B_RepairStress


GGI
6
7
8
9
10
6
7
8
9
10
11
12
1_C_EstrogenResp

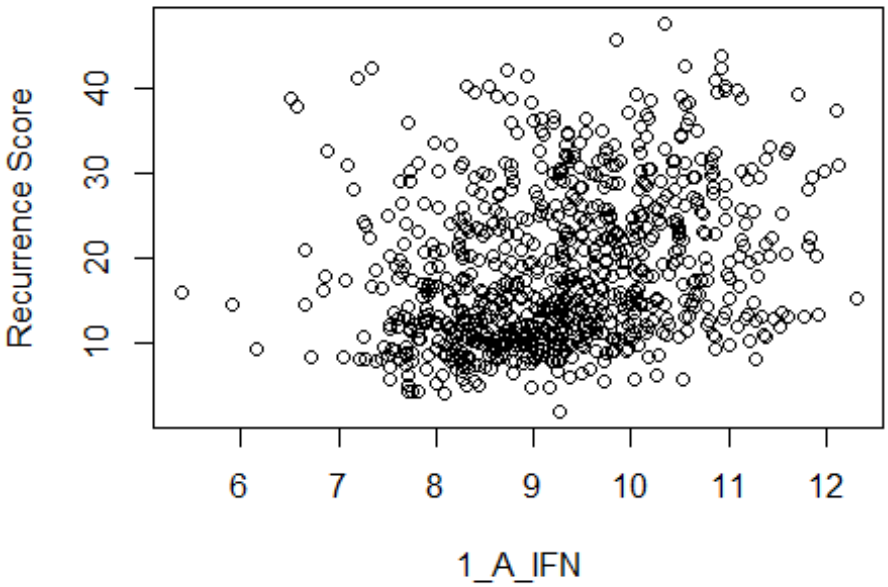

GGI
6
7
8
9
10
9.2
9.4
9.6
9.8
10.0
10.2
1_D_RepairStress

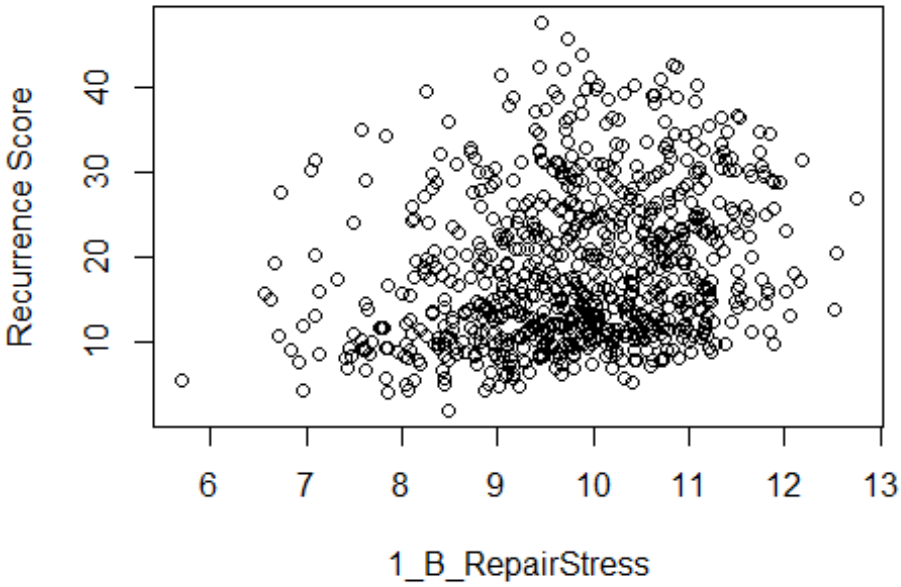

GGI
6
7
8
9
10
7
8
9
10
11
2_Proliferation

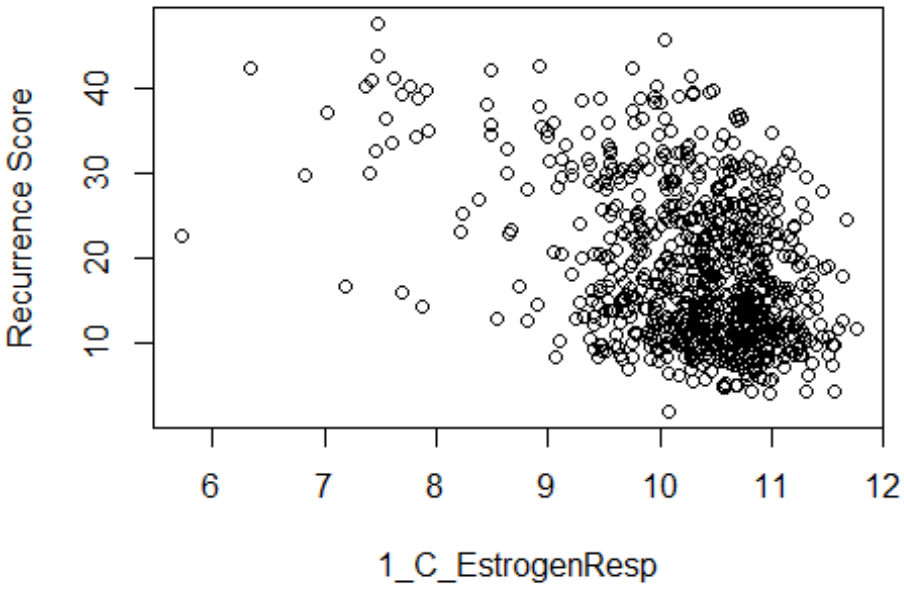

GGI
6
7
8
9
10
4
5
6
7
8
3_PostTxVsPreTx

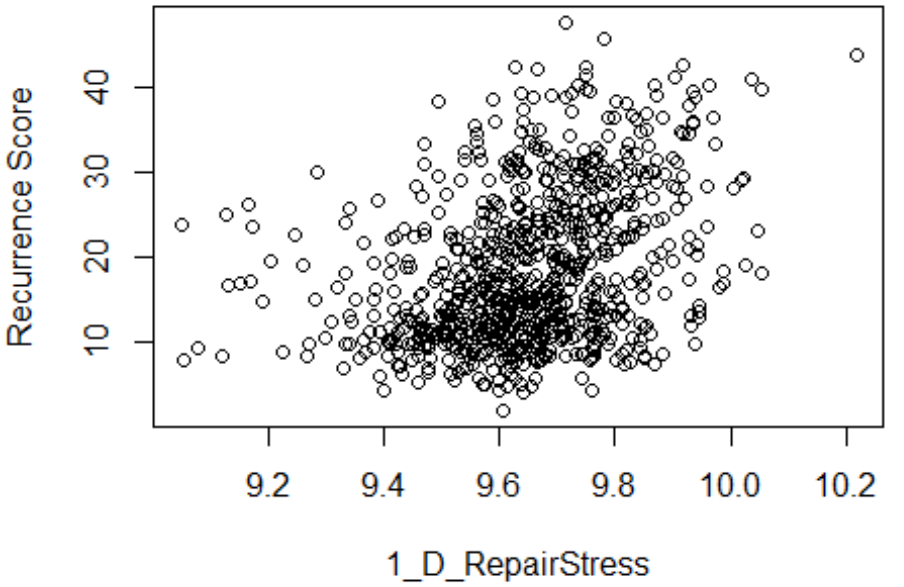


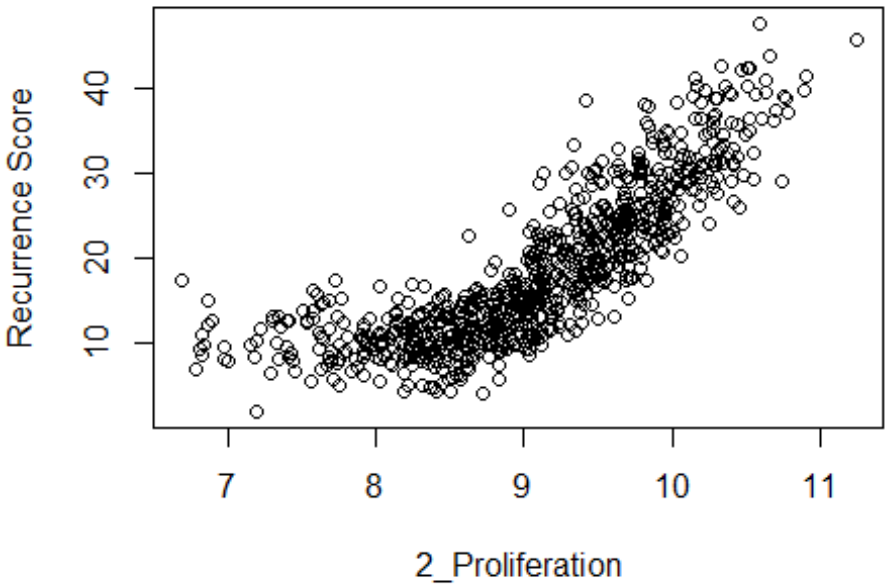

```r
# Recurrence Score:

stopifnot(rownames(tcgaERpos.pen322.subclu.scores) == names(tcga.onc.htg$score))

for (i in 1:length(pen322.subclu)) {
  plot(tcgaERpos.pen322.subclu.scores[,i], tcga.onc.htg$score,
       xlab = pen322.subclu[i], ylab = "Recurrence Score")
}
```

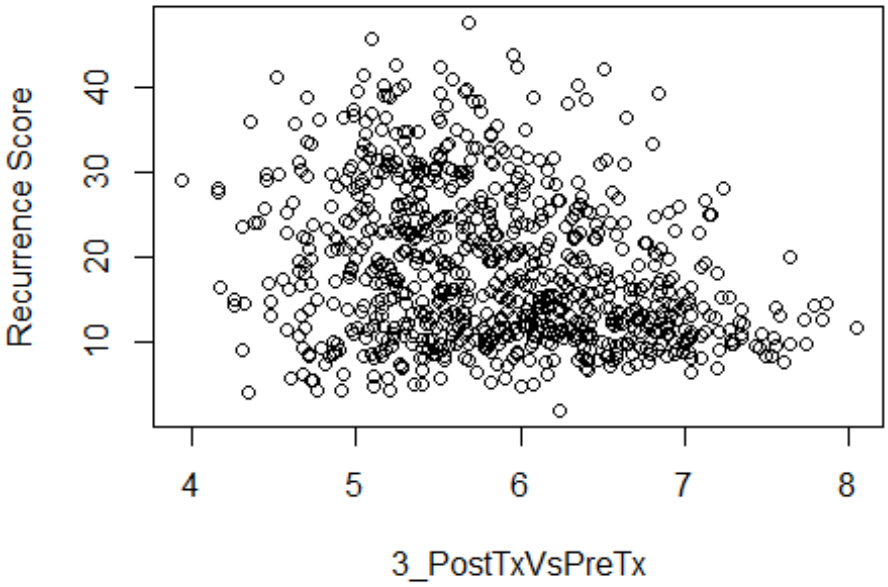

Recurrence Score
10
20
30
40
6
7
8
9
10
11
12
1_A_IFN

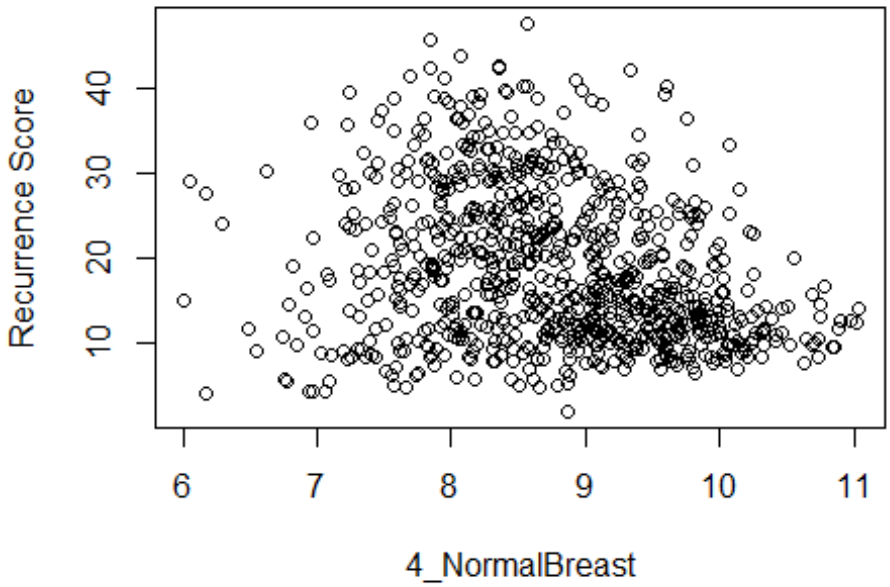

Recurrence Score
10
20
30
40
6
7
8
9
10
11
12
13
1_B_RepairStress


Recurrence Score
10
20
30
40
6
7
8
9
10
11
12
1_C_EstrogenResp


Recurrence Score
10
20
30
40
9.2
9.4
9.6
9.8
10.0
10.2
1_D_RepairStress


Recurrence Score
10
20
30
40
7
8
9
10
11
2_Proliferation


Recurrence Score
10
20
30
40
4
5
6
7
8
3_PostTxVsPreTx


Recurrence Score
10
20
30
40
6
7
8
9
10
11
4_NormalBreast

```r
# SESSION INFO ####
sessionInfo()
```

```
## R version 4.3.0 (2023-04-21 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 11 x64 (build 22631)
##
## Matrix products: default
##
##
## locale:
## [1] LC_COLLATE=German_Germany.utf8  LC_CTYPE=German_Germany.utf8
## [3] LC_MONETARY=German_Germany.utf8 LC_NUMERIC=C
## [5] LC_TIME=German_Germany.utf8
##
## time zone: Europe/Berlin
## tzcode source: internal
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] tibble_3.2.1                cBioPortalData_2.12.0
##  [3] MultiAssayExperiment_1.26.0 SummarizedExperiment_1.30.2
##  [5] GenomicRanges_1.52.0        GenomeInfoDb_1.36.1
##  [7] IRanges_2.34.1              S4Vectors_0.38.1
##  [9] MatrixGenerics_1.12.3       matrixStats_1.0.0
## [11] AnVIL_1.12.3                genefu_2.32.0
## [13] AIMS_1.32.0                 Biobase_2.60.0
## [15] BiocGenerics_0.46.0         e1071_1.7-13
## [17] iC10_1.5                    iC10TrainingData_1.3.1
## [19] impute_1.74.1               pamr_1.56.1
## [21] cluster_2.1.4               biomaRt_2.56.1
## [23] survcomp_1.50.0             prodlim_2023.03.31
## [25] survival_3.5-5              dplyr_1.1.2
##
## loaded via a namespace (and not attached):
##   [1] rstudioapi_0.15.0         jsonlite_1.8.5
##   [3] magrittr_2.0.3            GenomicFeatures_1.52.1
##   [5] SuppDists_1.1-9.7         rmarkdown_2.22
##   [7] BiocIO_1.10.0             zlibbioc_1.46.0
##   [9] vctrs_0.6.2               Rsamtools_2.16.0
##  [11] memoise_2.0.1             RCurl_1.98-1.12
##  [13] htmltools_0.5.5           S4Arrays_1.0.5
##  [15] progress_1.2.2            lambda.r_1.2.4
##  [17] curl_5.0.2                parallelly_1.36.0
##  [19] KernSmooth_2.23-20        htmlwidgets_1.6.2
##  [21] futile.options_1.0.1      cachem_1.0.8
##  [23] GenomicAlignments_1.36.0  mime_0.12
##  [25] lifecycle_1.0.3           pkgconfig_2.0.3
##  [27] Matrix_1.6-1              R6_2.5.1
##  [29] fastmap_1.1.1             GenomeInfoDbData_1.2.10
##  [31] future_1.33.0             shiny_1.7.4.1
##  [33] digest_0.6.31             RaggedExperiment_1.24.0
##  [35] AnnotationDbi_1.62.2      RSQLite_2.3.1
```

```
##  [37] filelock_1.0.2           RTCGAToolbox_2.30.0
##  [39] fansi_1.0.4              RJSONIO_1.3-1.8
##  [41] httr_1.4.6               abind_1.4-5
##  [43] compiler_4.3.0           proxy_0.4-27
##  [45] withr_2.5.0              bit64_4.0.5
##  [47] BiocParallel_1.34.2      DBI_1.1.3
##  [49] highr_0.10               lava_1.7.2.1
##  [51] rappdirs_0.3.3           DelayedArray_0.26.7
##  [53] rjson_0.2.21             tools_4.3.0
##  [55] httpuv_1.6.11            future.apply_1.11.0
##  [57] bootstrap_2019.6         glue_1.6.2
##  [59] restfulr_0.0.15          promises_1.2.0.1
##  [61] grid_4.3.0               generics_0.1.3
##  [63] tzdb_0.4.0               class_7.3-21
##  [65] tidyr_1.3.0              data.table_1.14.8
##  [67] hms_1.1.3                xml2_1.3.5
##  [69] utf8_1.2.3               XVector_0.40.0
##  [71] pillar_1.9.0             stringr_1.5.0
##  [73] RCircos_1.2.2            limma_3.56.2
##  [75] later_1.3.1              splines_4.3.0
##  [77] BiocFileCache_2.8.0      lattice_0.21-8
##  [79] rtracklayer_1.60.0       bit_4.0.5
##  [81] tidyselect_1.2.0         Biostrings_2.68.1
##  [83] miniUI_0.1.1.1           knitr_1.43
##  [85] futile.logger_1.4.3      xfun_0.39
##  [87] DT_0.29                  stringi_1.7.12
##  [89] yaml_2.3.7               evaluate_0.21
##  [91] codetools_0.2-19         cli_3.6.1
##  [93] survivalROC_1.0.3.1      xtable_1.8-4
##  [95] Rcpp_1.0.10              GenomicDataCommons_1.24.2
##  [97] rmeta_3.0                globals_0.16.2
##  [99] dbplyr_2.3.3             png_0.1-8
## [101] XML_3.99-0.14            rapiclient_0.1.3
## [103] parallel_4.3.0           TCGAutils_1.20.2
## [105] ellipsis_0.3.2           readr_2.1.4
## [107] blob_1.2.4               prettyunits_1.1.1
## [109] mclust_6.0.0             bitops_1.0-7
## [111] listenv_0.9.0            purrr_1.0.1
## [113] crayon_1.5.2             rlang_1.1.1
## [115] rvest_1.0.3              KEGGREST_1.40.0
## [117] formatR_1.14
```