# ExampleOfSignatureQBiC

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# Example of Signature-QBiC for HOXD13 and mutational signature SBS7a

This is the example shown in Figure 1b of Liu et al., Mutational Processes in Cancer Preferentially Affect Binding of Particular Transcription Factors.

## Load libraries

Note: you will need to install the github package PCAWG7 if not already installed. This contains the mutational signature profiles.

```
remotes::install_github("steverozen/PCAWG7")
```

```
library(PCAWG7)
library(tibble)
```

## Get information on mutational signatures

```
PCAWG_subs_signature <- PCAWG7::signature$genome$SBS96

mut.types <- lapply(row.names(PCAWG_subs_signature),function(x){
  return(paste(substring(x,1,3),
               paste(substring(x,1,1),
                     substring(x,4,4),
                     substring(x,3,3),sep=""),
               sep="_"))
})
mut.types <- row.names(PCAWG_subs_signature) <- unlist(mut.types)

## We used SBS7a as an example
signature <- "SBS7a"
sig <- PCAWG_subs_signature[ , signature, drop = FALSE]
```

## Gather the QBiC (protein binding microarray) information

We downloaded the QBiC scores table and p value table from http://qbic.genome.duke.edu/downloads (http://qbic.genome.duke.edu/downloads). The data are distributed across multiple files. For this example we used the “part-3” link to get the file ‘prediction_3.zip’. We changed ‘|’ characters in the file names to ‘!’ to avoid problems on MS Windows.

```
QBiC_score_file_path <-
  "../data-raw/prediction6mer.Homo_sapiens!M01252_1.94d!Barrera2016!HOXD13_I322L_R1.txt.gz"
QBiC_scores_table <-
  data.table::fread(QBiC_score_file_path, stringsAsFactors = F, fill = T)
# This gives a data frame with columns diff and z_score
# QBiC_scores_table contains NA for non-mutations, e.g AAAAAAAAAAA -> AAAAAAAAAAA

pvalue_file_path <-
  "../data-raw/pval6mer.Homo_sapiens!M01252_1.94d!Barrera2016!HOXD13_I322L_R1.csv.gz"
pvalue <- scan(pvalue_file_path)
# pvalue also contains NA for non-mutations
pvalue <- pvalue[!is.na(pvalue)]

all.possible.twelvemers <-
  tibble(readRDS("../data-raw/all.possible.twelvemers.rds"))
QBiC_score_info <-
  tibble(QBiC_mut = all.possible.twelvemers$seq,
         mut_type = all.possible.twelvemers$final_signature,
         scores   = QBiC_scores_table$z_score[!is.na(QBiC_scores_table$z_score)],
         p        = pvalue,
         q        = p.adjust(pvalue, method = "BH"))
rm(pvalue_file_path, QBiC_scores_table, QBiC_score_file_path)
```

# Plotting function for Figure 1

This function plots the distributions of QBiC scores assuming all mutations occur at equal frequencies and assuming mutations occur at frequencies in a particular mutational signature. See main text Figure 1c,e.7

```
TruncatedHist <- function(all.QBiC.scores,original.scores,weighted.prop,mutation.type){
  cut_off <- quantile(all.QBiC.scores,seq(0,1,0.01))[100] ##pile the 1% tail up
  original.scores[original.scores>cut_off] <- cut_off
  original.scores[original.scores<(-cut_off)] <- (-cut_off)
  weighted.hist <- original.hist <- hist(original.scores, breaks = seq(-(cut_off+0.5),
                                                                       (cut_off+0.5),0.5), pl
ot=F)
  weighted.hist$density <-  weighted.hist$density*weighted.prop
  plot(original.hist,freq = F,ylim=c(0,max(original.hist$density)+0.05),
       main=paste0("Original ",mutation.type),xaxt="n",yaxt="n")
  plot(weighted.hist,freq=F,ylim = c(0,max(original.hist$density)+0.05),
       main=paste0("Weighted ",mutation.type),xaxt="n",yaxt="n")
}
```

## The SignatureQBiC function.

This function can also be found in SigQBiC package. In the SigQBiC package, SignatureQBiC function returns Gain Ratio and Loss Ratio with the given QBiC scores, pvalues and signature. For tutorial purpose, here we split SignatureQBiC into several chunks to show how we calculate Gain Ratio and Loss Ratio.

```
SignatureQBiCExample <- function(QBiC_score_info,
                                 sig,
                                 plot.path = NULL) {

  max.score <- as.integer(max(QBiC_score_info$scores)) + 2
  # Guaranteed that the QBiC scores' distribution will be symmetric

  summary <-data.frame(matrix(ncol=5,nrow=0))
  my.breaks <- seq(-max.score,max.score,0.001)

  if(!is.null(plot.path)){
    all.weighted.freq <- 0
    if (!dir.exists(plot.path)) {
      if (!dir.create(plot.path, recursive = T))
        stop("Cannot create plotting directory ", plot.path)
    }
    png(filename = paste0(plot.path, "/", "hist%03d.png"))
    par(mar = c(1,1,1,1))
    par(mfrow = c(8,4))
  }

  for (mutation.type in mut.types) {
    stopifnot(mutation.type %in% QBiC_score_info$mut_type)
    # Scores for the given mutation.type
    tmp.scores <-
      QBiC_score_info$scores[QBiC_score_info$mut_type==mutation.type] ##the scores were put i
nto bins

    dist.hist <- hist(tmp.scores, breaks = my.breaks, plot=F)
    w.dist.hist <- dist.hist
    w.dist.hist$counts <- dist.hist$counts * sig[mutation.type, ]

    partial.summary <-
      data.frame(scores         = dist.hist$mids,
                 frequency      = dist.hist$counts,
                 mut_type       = mutation.type,
                 signature_freq = sig[mutation.type, ],
                 weighted.freq  = dist.hist$counts * sig[mutation.type, ])
    ##multiply the counts of each bin by the frequency of mutations in a signature

    if(!is.null(plot.path)){
      #collect 'weighted.freq' for each mutation type for the whole distribution plotting
      all.weighted.freq <- all.weighted.freq + dist.hist$counts * sig[mutation.type, ]
      TruncatedHist(QBiC_score_info$scores,
                    original.scores = tmp.scores,
                    weighted.prop = sig[mutation.type, ],
                    mutation.type=mutation.type)
    }
    summary <- rbind(summary, partial.summary)
  }
  if(!is.null(plot.path)){
    all.scores <-  QBiC_score_info$scores
    cut_off <- quantile(all.scores,seq(0,1,0.01))[100] ##pile the 1% tail up
    weighted.hist <- original.hist <- hist(all.scores, breaks = my.breaks, plot=F)
    weighted.hist$counts <-
      all.weighted.freq*sum(original.hist$counts)/sum(all.weighted.freq)
    plot(original.hist,main = "Original Distribution")
```

```
    plot(weighted.hist,main = "Weighted Distribution")
    dev.off()
  }

  return(summary)
}
```

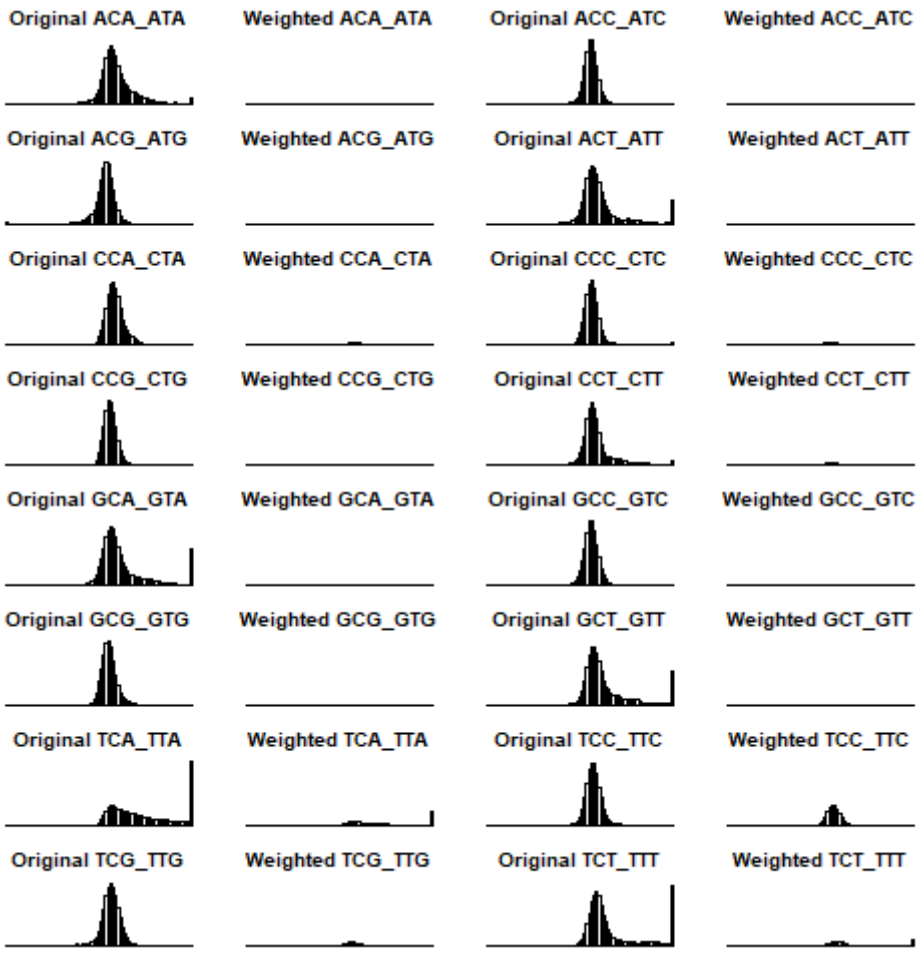
Run SignatureQBiC.

```
if (file.exists("ss.rds")) {
  summaryofscores <- readRDS("ss.rds")
} else {
  summaryofscores <-
    SignatureQBiCExample(QBiC_score_info = QBiC_score_info,
                         sig = sig,
                         plot.path = "./png.dir")
  saveRDS(summaryofscores, "ss.rds")
}
```

# Example of part of plotting output from SignatureQBicExample

This figure shows the distributions of QBiC scores for mutations centered at C>T For each histogram, the vertical axis indicates the density, and horizontal axis indicates the QBiC scores. The ‘weighted TCA_TTA’ plot in the first column, second plot from the bottom) shows an example of the case in which that particular mutation (TCA to TTA) is expected to generate many mutations with high QBiC scores (high bar at the far right of the plot).

```
knitr::include_graphics("./png.dir/hist003.png")
```



# Normalize the weighted frequencies.

After multiplying with signature probability, the weighted frequency is less than the original frequency. To compare the weighted distribution with original distribution, the frequency of two distribution should be normalized to the same.

```
summaryofscores$weighted.freq <-
  summaryofscores$weighted.freq *
  sum(summaryofscores$frequency)/sum(summaryofscores$weighted.freq)
```

# Find the p-value cutoff for $D_{Pos}$ and $D_{Neg}$

We first show the example for $D_{Pos}$.

```
pos.sig.QBiC_score_info <-
  QBiC_score_info[QBiC_score_info$q < 0.1 & QBiC_score_info$scores>0,] #select Dpos

##get the cutoff of QBiC scores based on BH FDR (indicated as T in paper)
qvalue.cutoff.score <- min(pos.sig.QBiC_score_info$scores)
```

Select $D'_{Pos}$ ($D'_{Neg}$) and $D_{Pos}$ ($D_{Neg}$) to calculate GR and LR This part is included in SigQBiC::SignatureQBiC. We show this part separately for tutorial purpose. Generate Dpos and D'pos based on T, and calculate Gain Ratio (GR). GR > 1 stands for area(D'pos) > area(Dpos)

# Compute $D'_{Pos}$ and the Gain Ratio, GR

```
summaryofscores.Dpos <-
  summaryofscores[summaryofscores$scores>qvalue.cutoff.score, ] ##Select Dpos

Dpos <- rep(summaryofscores.Dpos$scores,
            summaryofscores.Dpos$frequency) #Transform the information from histogram to a nu
meric vector
Dprimepos <- rep(summaryofscores.Dpos$scores,
                 round(summaryofscores.Dpos$weighted.freq, digits = 0))
GR = sum(Dprimepos)/sum(Dpos)
GR ##GR = 2.952
```

```
## [1] 2.951748
```

Generate Dneg and D'neg based on T, and calculate Loss Ratio (LR) LR > 1 stands for area(D'neg) > area(Dneg)

```
summaryofscores.Dneg <-
  summaryofscores[summaryofscores$scores<(-qvalue.cutoff.score), ] ##Select Dneg

Dneg <- rep(summaryofscores.Dneg$scores,
            summaryofscores.Dneg$frequency)

Dprimeneg <- rep(summaryofscores.Dneg$scores,
                 round(summaryofscores.Dneg$weighted.freq, digits = 0))
LR = sum(Dprimeneg)/sum(Dneg) ##LR = 0.058
LR
```

```
## [1] 0.05772716
```

# Calculate whether area($D'_{Pos}$) is statistically > area($D_{Pos}$)

For the GR and LR predicted for a TF-signature pair, we did this statistical test when the GR>1 or LR>1. So in this case, we only perform the statistical test for GR. Example of generating one set of random mutations with equal frequency. We generated 1000 sets of random mutations for statistical test

```
ResampleMutationFrequency <- function(i){
  set.seed(i)
  ##Generate mutations based on 96 trinucleotide based with equal frequency
  resampling.of.mut.type <-
    table(sample(c(1:96),size=nrow(all.possible.twelvemers),replace=T))

  names(resampling.of.mut.type) <- mut.types

  resampling.of.mut.type <- resampling.of.mut.type/sum(resampling.of.mut.type) #Normalize num
ber of mutations to sum of 1
  return(resampling.of.mut.type)
}
```

Generate GR and LR for random mutations assuming all mutations occur at equal frequency.

```
random.mut.freq <- data.frame(ResampleMutationFrequency(123),row.names = 1)

summaryofscores.test <-
  SignatureQBiCExample(QBiC_score_info = QBiC_score_info,
                       sig = random.mut.freq)

summaryofscores.test$weighted.freq <-
  summaryofscores.test$weighted.freq *
  sum(summaryofscores.test$frequency)/sum(summaryofscores.test$weighted.freq)
```

# Calculate whether area($D'_{Pos}$) of resampled mutations with equal frequency is larger than area($D'_{Pos}$) of mutations with signature frequency

```
##Calculate GR and LR. Similar as above
summaryofscores.test.Dpos <-
  summaryofscores.test[summaryofscores.test$scores>qvalue.cutoff.score, ] ##Select Dpos
Dprimepos_resampled <- rep(summaryofscores.test.Dpos$scores,
                 round(summaryofscores.test.Dpos$weighted.freq, digits = 0))
GR.resample <- sum(Dprimepos)/sum(Dprimepos_resampled)
GR.resample
```

```
## [1] 2.951748
```

GR.resample = 2.95 tells that area({$D*_{Pos}$) is larger than area($D'_{Pos}$) of the resampled mutations with equal frequency.