

target: An R Package to Predict Combined Function of Transcription Factors

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Abstract Abstracts should be up to 300 words and provide a succinct summary of the article. Although the abstract should explain why the article might be interesting, care should be taken not to inappropriately over-emphasise the importance of the work described in the article. Citations should not be used in the abstract, and the use of abbreviations should be minimized.

Keywords

transcription-factors; DNA-binding; gene-expression; r-package; bioconductor; workflow

R version: R version 3.6.1 (2019-07-05)

Bioconductor version: 3.9

Introduction

The introduction provides context as to why the software tool was developed and what need it addresses. It is good scholarly practice to mention previously developed tools that address similar needs, and why the current tool is needed.

Methods

Implementation

For software tool papers, this section should address how the tool works and any relevant technical details required for implementation of the tool by other developers.

Operation

This part of the methods should include the minimal system requirements needed to run the software and an overview of the workflow for users of the tool.

Use Cases

Table 1. Expression and binding data of YY1 and YY2 in HeLa cells.

GEO ID	Data Type	Design	Ref.
GSE14964	Microarrays	YY#-knockdown	Chen et al. [1]
GSE31417	ChIP-Seq	YY1 vs input	Michaud et al. [2]
GSE96878	ChIP-Seq	YY2 vs input	Wu et al. [3]

Table 1

```
# load required libraries
library(tidyverse)
library(reshape2)
library(broom)
library(cowplot)
library(ggupset)
library(seqLogo)
library(ggplotify)
library(rtracklayer)
library(GenomicRanges)
library(Biostrings)
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
library(BSgenome.Hsapiens.UCSC.hg19)
library(org.Hs.eg.db)
library(BCRANK)
library(target)
```

Preparing the binding data

```
# locate the peaks bed files
peak_files <- c(YY1 = 'data/Oth.Utr.05.YY1.AllCell.bed',
               YY2 = 'data/Oth.Utr.05.YY2.AllCell.bed')

# load the peaks bed files as GRanges
peaks <- map(peak_files, ~GRanges(import.bed(.x)))
```

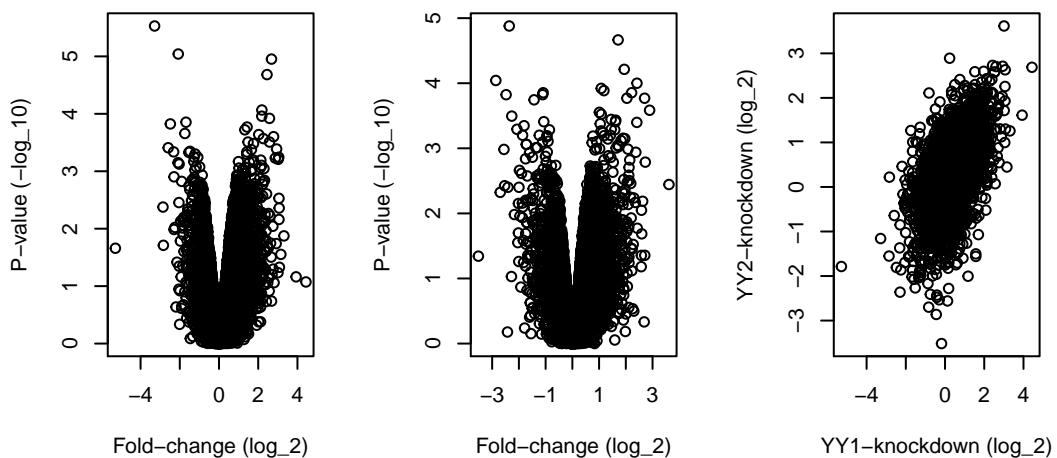


Figure 1. Differential expression between factor knockdown and wild-type in HeLa cells.

Preparing the expression data

```
# locate the expression text files
expression_files <- c(YY1 = 'data/DataSet_01_18.tsv',
                     YY2 = 'data/DataSet_01_19.tsv')

# load the expression text files
express <- map(expression_files,
  ~read_tsv(.x, col_names = FALSE) %>%
    dplyr::select(2,3, 7, 9) %>% #9
    setNames(c('tf', 'gene', 'fc', 'pvalue')) %>%
    filter(tf %in% c('YY1', 'YY2')) %>%
    na.omit())
```

Figure 1

```
par(mfrow = c(1, 3))
plot(express$YY1$fc,
      -log10(express$YY1$pvalue),
      xlab = 'Fold-change (log2)',
      ylab = 'P-value (-log10)')
plot(express$YY2$fc,
      -log10(express$YY2$pvalue),
      xlab = 'Fold-change (log2)',
      ylab = 'P-value (-log10)')
plot(express$YY1$fc[order(express$YY1$gene)],
      express$YY2$fc[order(express$YY2$gene)],
      xlab = 'YY1-knockdown (log2)',
      ylab = 'YY2-knockdown (log2)')
```

Preparing genome annotation

```
# load genome data
symbol_entrez <- select(org.Hs.eg.db,
  unique(c(express$YY1$gene)),
  'ENTREZID',
  'SYMBOL') %>%
  setNames(c('gene', 'gene_id'))
```

```
# format genome to join with express
genome <- transcripts(TxDb.Hsapiens.UCSC.hg19.knownGene,
                      filter = list(gene_id = symbol_entrez$gene_id),
                      columns = c('tx_id', 'tx_name', 'gene_id')) %>%
  promoters(upstream = 100000) %>%
  as_tibble() %>%
  filter(length(gene_id) > 1) %>%
  mutate(gene_id = as.character(gene_id))

# make regions by merging the genome and express data
regions <- map(express,
  ~inner_join(genome, symbol_entrez) %>%
    inner_join(.x) %>%
    makeGRangesFromDataFrame(keep.extra.columns = TRUE))
```

Predicting gene targets of individual factors

```
# get associated peaks
ap <- map2(peaks, regions,
  ~associated_peaks(peaks=.x,
    regions = .y,
    regions_col = 'tx_id'))

# get direct targets
dt <- map2(peaks, regions,
  ~direct_targets(peaks=.x,
    regions = .y,
    regions_col = 'tx_id',
    stats_col = 'fc'))
```

Figure 2

```
par(mfrow = c(2, 2))
map(ap, ~plot(.x$distance,
  .x$peak_score,
  xlab = 'Distance',
  ylab = 'Peak Score'))

## $YY1
## NULL
##
## $YY2
## NULL

labs <- c('down', 'none', 'up')
cols <- c('green', 'gray', 'red')

groups <- map(dt,
  ~cut(.x$stat,
    breaks = quantile(.x$stat, c(0, .1, .9, 1)),
    labels = labs))

map2(dt, groups,
  ~{plot_predictions(. $score_rank,
    group = .y,
    colors = cols,
    labels = labs,
    xlab = 'Regulatory Potential',
    ylab = 'ECDF')

  invisible(NULL)
})
```

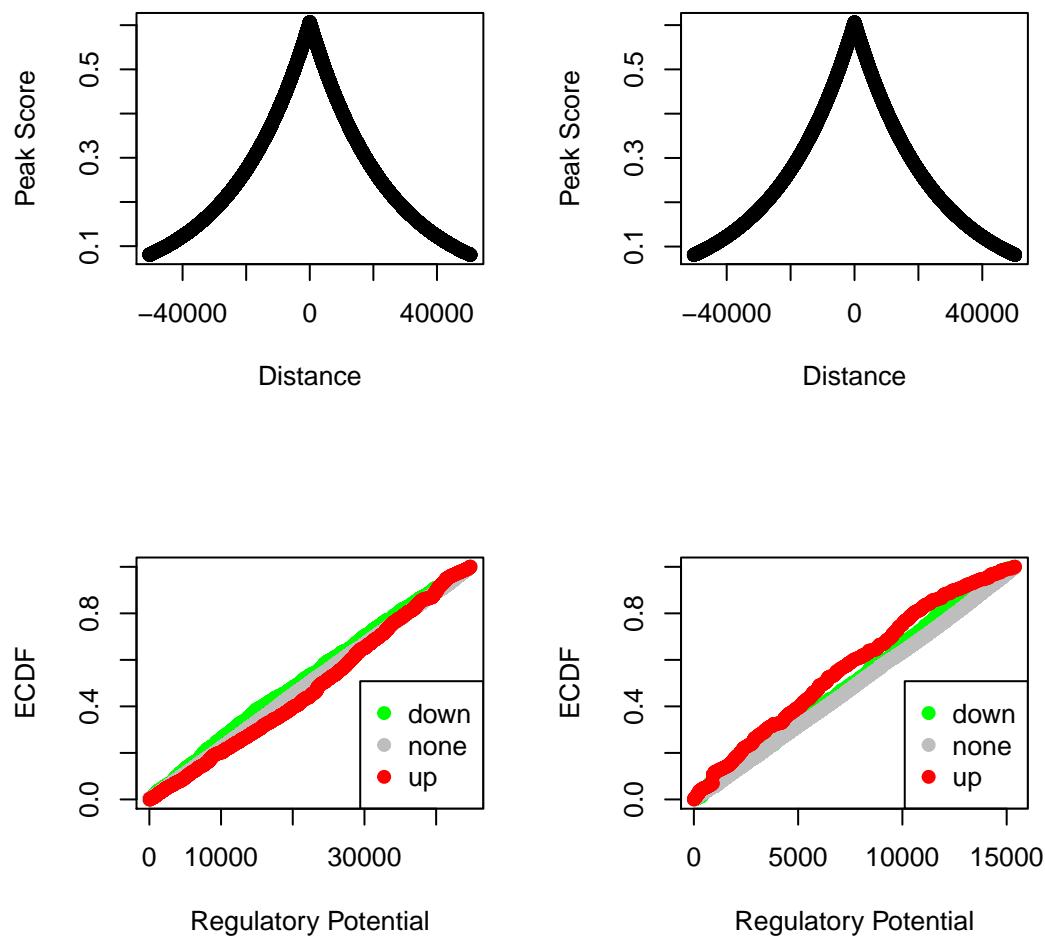


Figure 2. Predicted functions of YY1 and YY2 on their specific targets.

Table 2. Testing for statistical significance of the regulated gene groups.

Factor	statistic	p.value	method	alternative
YY1	0.2236994	0	Two-sample Kolmogorov-Smirnov test	two-sided
YY2	0.1492537	0	Two-sample Kolmogorov-Smirnov test	two-sided

```
## $YY1
## NULL
##
## $YY2
## NULL

map2_df(dt, groups,
  ~test_predictions(.x$rank,
    group = .y,
    compare = c('down', 'up')) %>%
  tidy(),
  .id = 'Factor') %>%
knitr::kable(
  caption = 'Testing for statistical significance of the regulated gene groups.',
  label = 'tests')
```

Table 2

Predicting the shared targets of two factors

```
# merge and name peaks
common_peaks <- reduce(subsetByOverlaps(peaks$YY1, peaks$YY2))
common_peaks$name <- paste0('common_peak_', 1:length(common_peaks))

# bind express tables into one
both_express <- bind_rows(express) %>%
  nest(fc, pvalue, .key = 'values_col') %>%
  spread(tf, values_col) %>%
  unnest(YY1, YY2, .sep = '_')

# make regions using genome and expression data of both factors
both_regions <- inner_join(genome, symbol_entrez) %>%
  inner_join(both_express) %>%
  makeGRangesFromDataFrame(keep.extra.columns = TRUE)

# get associated peaks with both factors
common_ap <- associated_peaks(peaks = common_peaks,
  regions = both_regions,
  regions_col = 'tx_id')

# get direct targets of both factors
common_dt <- direct_targets(peaks = common_peaks,
  regions = both_regions,
  regions_col = 'tx_id',
  stats_col = c('YY1_fc', 'YY2_fc'))
```

Figure 3

```
par(mfrow = c(1, 2))
plot(common_ap$distance,
  common_ap$peak_score,
  xlab = 'Distance',
  ylab = 'Peak Score')

labs <- c('competitive', 'none', 'cooperative')
```

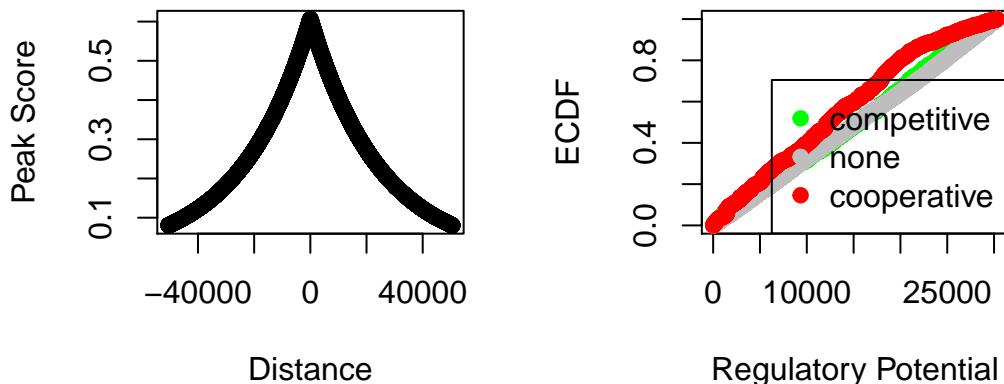


Figure 3. Predicted function of YY1 and YY2 on their shared targets.

```

cols <- c('green', 'gray', 'red')

common_groups <- cut(common_dt$stat,
                      breaks = quantile(common_dt$stat, c(0, .1, .9, 1)),
                      labels = labs)

plot_predictions(common_dt$score_rank,
                 group = common_groups,
                 colors = cols,
                 labels = labs,
                 xlab = 'Regulatory Potential',
                 ylab = 'ECDF')

```

Binding motif analysis

```

# group peaks by their assigned targets
peak_groups <- split(common_dt$tx_id, common_groups)

# reorder peaks and get top n peaks
peak_groups <- lapply(peak_groups, function(x) {
  # get peaks in x targets group
  p <- common_ap[common_ap$assigned_region %in% unique(x)]

  # order peaks by score
  p <- p[order(p$peak_score, decreasing = TRUE)]

  # get n top peaks
  #n <- length(p)
  n <- 100
  p[1:n]
})

bcout <- map(peak_groups[c('competitive', 'cooperative')], ~{
  # make a temporary file
  tmp_fasta <- tempfile()

  # extract sequences of top peaks from the hg19 genome
  pseq <- getSeq(BSgenome.Hsapiens.UCSC.hg19,

```

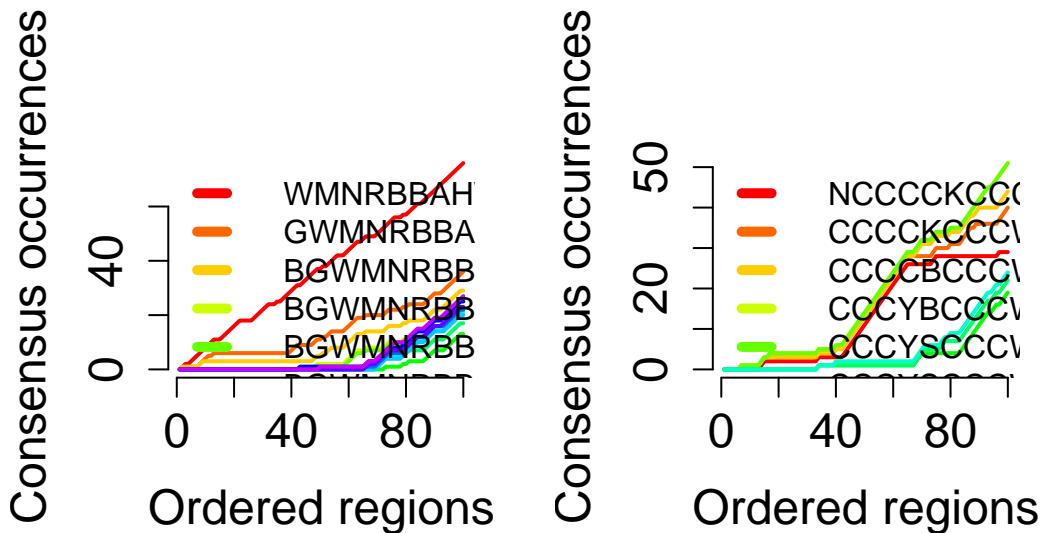


Figure 4. Occurrences of consensus sequences in the ranked regions.

```

    names = .x)

# write sequences to fasta file
writeXStringSet(pseq, tmp_fasta)

# set random see
set.seed(123)

# call bcrank with the fasta file
bcrank(tmp_fasta, silent = TRUE)
})

```

Figure 4

```
par(mfrow = c(1, 2))
map(bcout, ~plot(toptable(.x, 1)))
```

```
## $competitive
## $competitive$rect
## $competitive$rect$w
## [1] 176.837
##
## $competitive$rect$h
## [1] 198.1241
##
## $competitive$rect$left
## [1] -2.96
##
## $competitive$rect$top
## [1] 79.04
##
## $competitive$text
## $competitive$text$x
## [1] 37.26069 37.26069 37.26069 37.26069 37.26069 37.26069 37.26069
## [8] 37.26069 37.26069 37.26069 37.26069 37.26069 37.26069 37.26069
##
## $competitive$text$y
## [1] 64.888276 50.736552 36.584828 22.433103 8.281379
## [6] -5.870345 -20.022069 -34.173793 -48.325517 -62.477241
```

```

## [11] -76.628966 -90.780690 -104.932414
##
##
##
## $cooperative
## $cooperative$rect
## $cooperative$rect$w
## [1] 128.4046
##
## $cooperative$rect$h
## [1] 85.46897
##
## $cooperative$rect$left
## [1] -2.96
##
## $cooperative$rect$top
## [1] 53.04
##
##
## $cooperative$text
## $cooperative$text$x
## [1] 37.26069 37.26069 37.26069 37.26069 37.26069 37.26069 37.26069
## [1] 43.543448 34.046897 24.550345 15.053793 5.557241 -3.939310
## [7] -13.435862 -22.932414

```

Figure 5

```

map(bcout, ~seqLogo(pwm(toptable(.x, 1)))

# solution: https://support.bioconductor.org/p/35240/
mySeqLogo = seqLogo::seqLogo

bad = (sapply( body(mySeqLogo), "==" , "grid.newpage()") |
       sapply( body(mySeqLogo), "==" , "par.ask = FALSE"))
body(mySeqLogo)[bad] = NULL

grid.newpage()
pushViewport(viewport(layout = grid.layout(1,2)))
pushViewport(viewport(layout.pos.col = 1, layout.pos.row = 1))
mySeqLogo(pwm(toptable(bcout$competitive, 1)))
popViewport(1)

pushViewport(viewport(layout.pos.col = 2, layout.pos.row = 1))
mySeqLogo(pwm(toptable(bcout$cooperative, 1)))
popViewport(1)

```

Summary

This section is required if the paper does not include novel data or analyses. It allows authors to briefly summarize the key points from the article.

Software availability

This section will be generated by the Editorial Office before publication. Authors are asked to provide some initial information to assist the Editorial Office, as detailed below.

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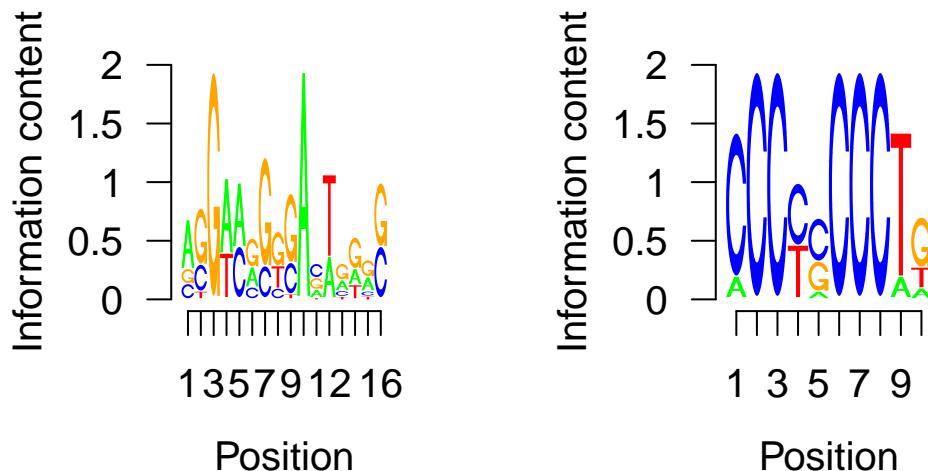


Figure 5. Predicted motifs of the cooperative and competitive binding sites.

3. Link to source code as at time of publication (*F1000Research* TO GENERATE)
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Acknowledgments

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Please do not list grant funding in this section.

References

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