promoter_comparison

Promoters are upstream regions of all protein-coding genes

```
library(biomaRt)
mart = getParamart()
```

```
## Database connected
## biomart      ...      parasite_mart
## host         ...      https://parasite.wormbase.org:443/biomart/martservice
## dataset      ...      wbps_gene
```

```
UPSTREAM=1000
DOWNSTREAM=200
promoters = getCElegansPromoters(mart, upstream = UPSTREAM, downstream = DOWNSTREAM)
```

```
## getBM(filter = c("biotype", "species_id_1010"), value = list(
##     biotype = "protein_coding", species_id_1010 = "caelegprjna13758"),
##     attributes = c("wbps_gene_id", "external_gene_id", "chromosome_name",
##     "start_position", "end_position", "strand"))
```

```
promoters = trim(sort(promoters, ignore.strand=T)) # trim because one interval is chrIV:-359-840 at -10
head(promoters)
```

```
## GRanges object with 6 ranges and 2 metadata columns:
##       seqnames      ranges strand |   wbps_gene_id external_gene_id
##          <Rle>   <IRanges>  <Rle> |    <character>      <character>
##   [1]     chrI 10031-11230      - | WBGene00022277           homt-1
##   [2]     chrI 10495-11694      + | WBGene00022276           nlp-40
##   [3]     chrI 26582-27781      - | WBGene00022278           rcor-1
##   [4]     chrI 32951-34150      - | WBGene00022279           sesn-1
##   [5]     chrI 42733-43932      + | WBGene00022275            txt-7
##   [6]     chrI 46461-47660      + | WBGene00044345        Y48G1C.12
##   -------
##   seqinfo: 7 sequences (1 circular) from ce11 genome
```

```
selfOverlaps = findOverlaps(promoters, ignore.strand=T)
#head(selfOverlaps)

# selfOverlaps includes everything against itself + overlaps between promoters
# Filter out the self hits, and retain the "between" hits as "collisions".
collisions = selfOverlaps[!isSelfHit(selfOverlaps)]

overlappingPromoterRows = unique(c( from(collisions), to(collisions)))
length(overlappingPromoterRows)
```

```
## [1] 6749
```

```
sprintf("There are %d overlaps between %d promoters.", length(collisions), length(overlappingPromoterRo
```

```
## [1] "There are 8008 overlaps between 6749 promoters."
```

```
filtered.promoters = promoters[-which(seqnames(promoters) == 'chrM')]

# to remove overlapping promoters, uncomment below
nr.promoters = filtered.promoters[-overlappingPromoterRows]
sprintf("There are %d unambiguous promoters.", length(nr.promoters))
```

```
## [1] "There are 13246 unambiguous promoters."
```

```
# -500,+200
# "There are 4256 overlaps between 4067 promoters."
# "There are 15922 unambiguous promoters."

# -1000,+200
#"There are 8008 overlaps between 6749 promoters."
#"There are 13246 unambiguous promoters."
OUTPUT_03 = normalizePath("../03_output")
PROMOTOR_BED_PATH = sprintf("%s/filtered.promoters.minus%d_plus%d.bed", OUTPUT_03, UPSTREAM, DOWNSTREAM
write.table(filtered.promoters, PROMOTOR_BED_PATH, sep="\t", quote=F, row.names=F, col.names=F)

NR_PROMOTOR_BED_PATH = sprintf("%s/nr.promoters.minus%d_plus%d.bed", OUTPUT_03, UPSTREAM, DOWNSTREAM)
write.table(nr.promoters, PROMOTOR_BED_PATH, sep="\t", quote=F, row.names=F, col.names=F)
```

Setup a conda environment in your shell

I had to call my local setup script .zshrc, where I have initialized conda, to have access to the "base" environment, where I have installed wiggletools and ucsc user apps.

```
$ wiggletools apply_paste filtered.promoters.minus1000_plus200.df meanI maxI filtered.promoters.minus100
ELT2_LE_combined_subtracted.bw
```

The same can be done for the IDR peaks.

```
$ wiggletools apply_paste LE_IDR_peaks.df meanI maxI ELT2_LE_combined.IDR.bed ELT2_LE_combined_subtracte
```

```
PROMOTOR_DF_PATH = sprintf("%s/filtered.promoters.minus%d_plus%d.df", OUTPUT_03, UPSTREAM, DOWNSTREAM)
promoters.agg = read.table(PROMOTOR_DF_PATH)
colnames(promoters.agg) <- c("chrom", "start","end","len", "strand", "wbps_gene_id", "gene_name", "chip

IDR_peaks.agg = read.table(file.path(OUTPUT_03,"LE_IDR_peaks.df"))
IDR_peaks.agg$V4 = NULL
IDR_peaks.agg$V5 = NULL
IDR_peaks.agg$V6 = NULL
IDR_peaks.agg$V8 = NULL
colnames(IDR_peaks.agg) <- c("chrom", "start","end","intensity","nlogq","offset","signal.mean","signal.

gr.IDR = makeGRangesFromDataFrame(IDR_peaks.agg,keep.extra.columns = T)
seqinfo(gr.IDR) <- Seqinfo(genome="ce11")

gr.promoters = makeGRangesFromDataFrame(promoters.agg,keep.extra.columns = T)
seqinfo(gr.promoters) <- Seqinfo(genome="ce11")
```

```
chipmean.minval = min(gr.promoters$chip_signal_mean,na.rm=T)
chipmean.minval
```

```
## [1] -100.4667
```

```
chipmax.minval = min(gr.promoters$chip_signal_max,na.rm=T)
chipmax.minval
```

```
## [1] -80.85739
```

```
chipmean.log = log(-chipmean.minval + 1 + gr.promoters$chip_signal_mean,base=2)
chipmax.log = log(-chipmax.minval + 1 + gr.promoters$chip_signal_max,base=2)

gr.promoters$log_chip_signal_mean = chipmean.log
gr.promoters$log_chip_signal_max = chipmax.log
head(gr.promoters)
```

```
## GRanges object with 6 ranges and 7 metadata columns:
##       seqnames      ranges strand |       len   wbps_gene_id   gene_name
##          <Rle>   <IRanges>  <Rle> | <integer>    <character> <character>
##   [1]     chrI 26582-27781      - |      1200 WBGene00022278      rcor-1
##   [2]     chrI 32951-34150      - |      1200 WBGene00022279      sesn-1
##   [3]     chrI 42733-43932      + |      1200 WBGene00022275       txt-7
##   [4]     chrI 46461-47660      + |      1200 WBGene00044345   Y48G1C.12
##   [5]     chrI 48921-50120      + |      1200 WBGene00021677       pgs-1
##   [6]     chrI 63867-65066      - |      1200 WBGene00021678    Y48G1C.5
##       chip_signal_mean chip_signal_max log_chip_signal_mean log_chip_signal_max
##              <numeric>       <numeric>            <numeric>           <numeric>
##   [1]        116.59365       220.93678              7.76858             8.24219
##   [2]         23.56896        38.75358              6.96620             6.91422
##   [3]          7.16118        18.78316              6.76325             6.65307
##   [4]         26.93845        43.20576              7.00456             6.96651
##   [5]         11.93393        34.69149              6.82529             6.86479
##   [6]         -5.76947         9.25825              6.58041             6.50963
##   -------
##   seqinfo: 7 sequences (1 circular) from ce11 genome
```

```
# output file
LOG_PROMOTOR_DF_PATH = sprintf("%s/log_filtered.promoters.minus%d_plus%d.df", OUTPUT_03, UPSTREAM, DOWNS
write.table(as.data.frame(gr.promoters), file = LOG_PROMOTOR_DF_PATH,quote=F, row.names=F,sep="\t")
```

```
laps = findOverlaps(gr.promoters,gr.IDR, ignore.strand=T,minoverlap = 100)

head(laps)
```

```
## Hits object with 6 hits and 0 metadata columns:
##       queryHits subjectHits
##       <integer>   <integer>
##   [1]         1           4
##   [2]        29           7
##   [3]        31           8
##   [4]        32           9
##   [5]        38          14
##   [6]        42          16
##   -------
##   queryLength: 13246 / subjectLength: 4098
```

```
gr.promoters$IDR_mean = NaN
gr.promoters$IDR_max = NaN
gr.promoters$IDR_value = NaN
gr.promoters$nlogq = NaN
```

```
gr.promoters[from(laps)]$IDR_max = gr.IDR[to(laps)]$signal.max
gr.promoters[from(laps)]$IDR_mean = gr.IDR[to(laps)]$signal.mean
gr.promoters[from(laps)]$IDR_value = gr.IDR[to(laps)]$intensity
gr.promoters[from(laps)]$nlogq = gr.IDR[to(laps)]$nlogq
print("Number of promoters overlapping an IDR peak:")
```

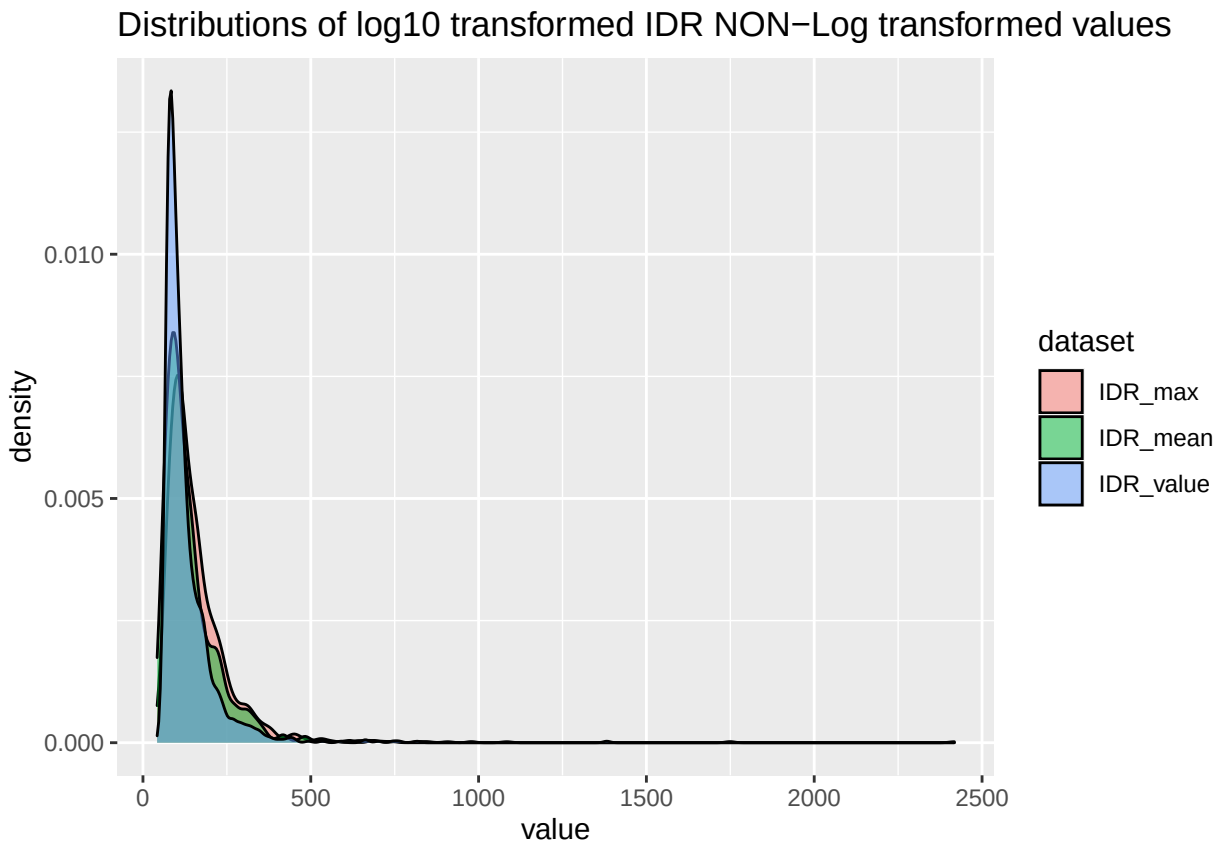
```
## [1] "Number of promoters overlapping an IDR peak:"
```

```
sum(!is.nan(gr.promoters$IDR_max))
```

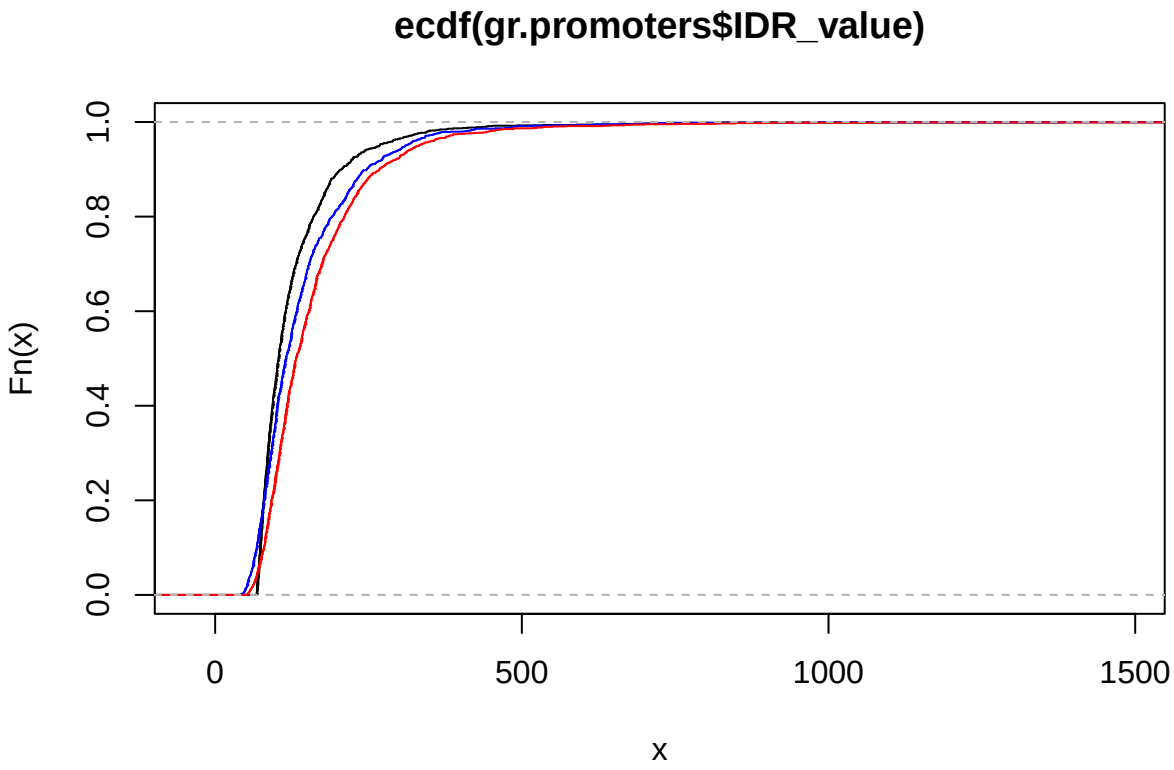
```
## [1] 1275
```

```
idr.nonlog = gather(as.data.frame(gr.promoters)[,c('IDR_value','IDR_mean','IDR_max')], key="dataset")
ggplot(idr.nonlog, aes(x=value, fill=dataset))  + geom_density(alpha=.5) + labs(title="Distributions of
```

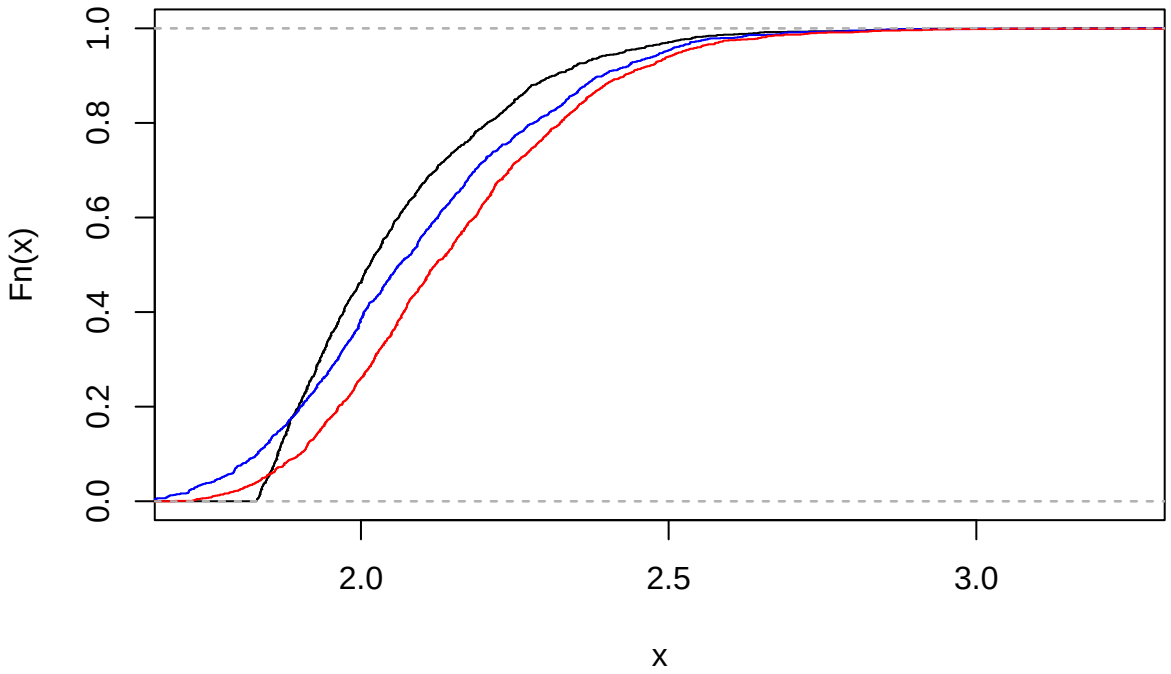
```
## Warning: Removed 35913 rows containing non-finite values (stat_density).
```




```
idr.val.ecdf = ecdf(gr.promoters$IDR_value)
idr.mean.ecdf = ecdf(gr.promoters$IDR_mean)
idr.max.ecdf = ecdf(gr.promoters$IDR_max)

plot(idr.val.ecdf)
lines(idr.mean.ecdf,col="blue")
lines(idr.max.ecdf,col="red")
```




```
# the data currently have all positive values, so no adjustment made for log
idr.val.log = log10(gr.promoters$IDR_value)
idr.mean.log = log10(gr.promoters$IDR_mean)
idr.max.log = log10(gr.promoters$IDR_max)

idr.val.log.ecdf = ecdf(idr.val.log)
idr.mean.log.ecdf = ecdf(idr.mean.log)
idr.max.log.ecdf = ecdf(idr.max.log)

plot(idr.val.log.ecdf)
lines(idr.mean.log.ecdf,col="blue")
lines(idr.max.log.ecdf,col="red")
```

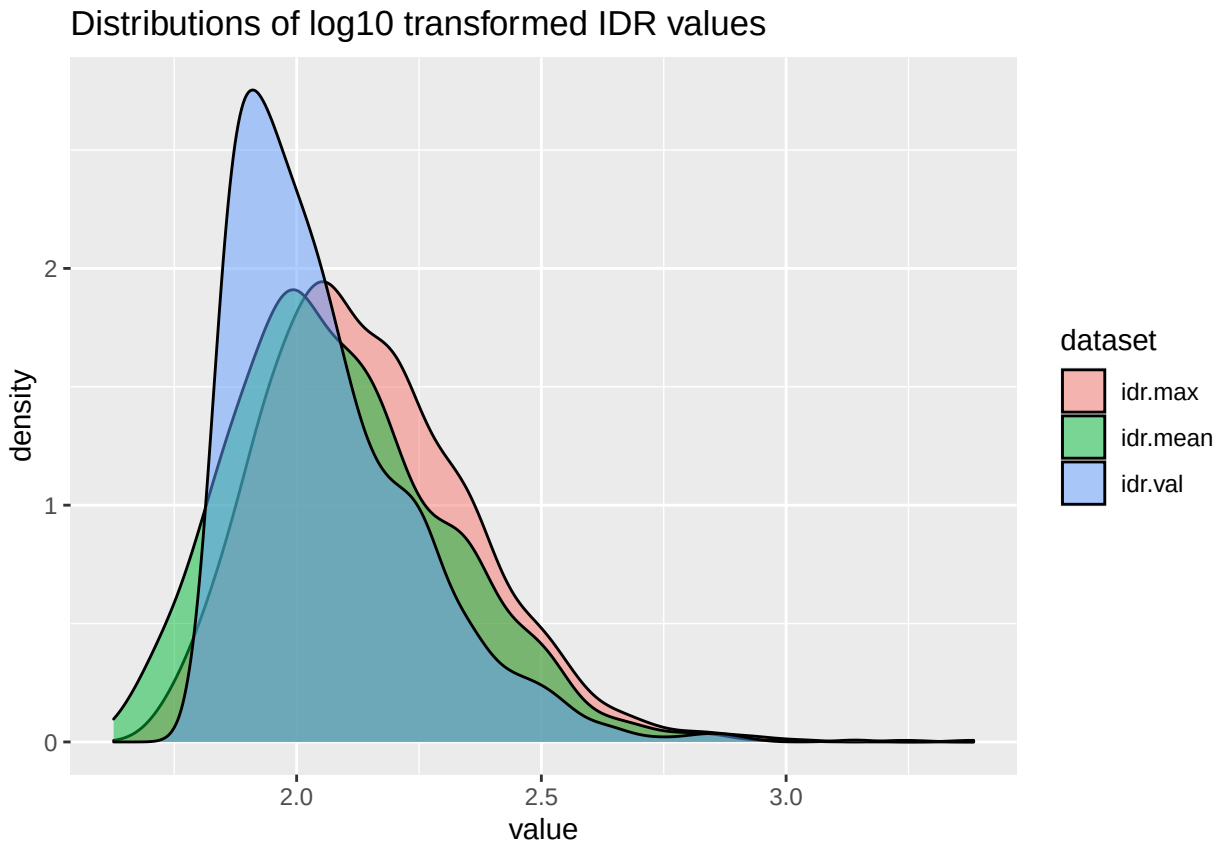



```
log.vals = data.frame(idr.mean = idr.mean.log, idr.val = idr.val.log, idr.max = idr.max.log)

long.log.vals = gather(log.vals, key="dataset")
head(long.log.vals)
```

```
##    dataset    value
## 1 idr.mean 2.288332
## 2 idr.mean      NaN
## 3 idr.mean      NaN
## 4 idr.mean      NaN
## 5 idr.mean      NaN
## 6 idr.mean      NaN
```

```
ggplot(long.log.vals, aes(x=value, fill=dataset))  + geom_density(alpha=.5) + labs(title="Distributions
```

```
## Warning: Removed 35913 rows containing non-finite values (stat_density).
```




```
gr.promoters$IDR_logTEN_max = idr.max.log
gr.promoters$IDR_logTEN_mean = idr.mean.log
gr.promoters$IDR_logTEN_value = idr.val.log
sum(idr.mean.log > 2.5, na.rm=T)
```

```
## [1] 59
```

```
# input file
#ROB_APEGLM_SHRINK
datapath = normalizePath('../../../Rob/02_embryo_intestine_RNAseq/03_output/DE_Results_GFPplus-vs-GFPmi
x = read.csv(datapath)
rownames(x) <- x$WBGeneID

# look at the number filtered by DESeq2
# as described by https://bioconductor.org/packages/release/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#
baseMean_is_zero = x$baseMean == 0
pval_na = is.na(x$pvalue)
padj_na = is.na(x$padj)
# case one
sum(baseMean_is_zero & pval_na & padj_na)
```

```
## [1] 0
```

```
# case two
sum(!baseMean_is_zero & pval_na & padj_na)
```

```
## [1] 52
```

```
# case three
sum(!pval_na & padj_na)
```

```
## [1] 3088
```

```
head(x)
```

```
##                      WBGeneID   baseMean log2FoldChange     lfcSE       pvalue
## WBGene00021406 WBGene00021406 114.300065      0.8215012 0.5991126 9.785429e-02
## WBGene00021407 WBGene00021407  16.099710      0.1920445 0.8458947 6.959493e-01
## WBGene00021408 WBGene00021408  19.527924      8.2813467 2.7319552 2.166056e-07
## WBGene00021405 WBGene00021405   2.279759      0.8617914 1.5230514 4.522192e-02
## WBGene00021409 WBGene00021409   2.405898      0.3925713 1.1231272 9.076469e-02
## WBGene00021404 WBGene00021404  20.940074      9.0546662 2.7383369 3.533428e-09
##                        padj
## WBGene00021406 1.890764e-01
## WBGene00021407 7.892656e-01
## WBGene00021408 2.186568e-06
## WBGene00021405           NA
## WBGene00021409           NA
## WBGene00021404 4.304637e-08
```

```
#x %>% filter(WBGeneID %in% gr.promoters$wbps_gene_id) -> x.coherent
mcols(gr.promoters) <- mcols(gr.promoters) %>% cbind(x[gr.promoters$wbps_gene_id,2:6])  %>% as.data.fra
head(gr.promoters)
```

```
## GRanges object with 6 ranges and 19 metadata columns:
##       seqnames      ranges strand |       len   wbps_gene_id   gene_name
##          <Rle>   <IRanges>  <Rle> | <integer>    <character> <character>
##   [1]     chrI 26582-27781      - |      1200 WBGene00022278      rcor-1
##   [2]     chrI 32951-34150      - |      1200 WBGene00022279      sesn-1
##   [3]     chrI 42733-43932      + |      1200 WBGene00022275       txt-7
##   [4]     chrI 46461-47660      + |      1200 WBGene00044345   Y48G1C.12
##   [5]     chrI 48921-50120      + |      1200 WBGene00021677       pgs-1
##   [6]     chrI 63867-65066      - |      1200 WBGene00021678    Y48G1C.5
##       chip_signal_mean chip_signal_max log_chip_signal_mean log_chip_signal_max
##              <numeric>       <numeric>            <numeric>           <numeric>
##   [1]        116.59365       220.93678              7.76858             8.24219
##   [2]         23.56896        38.75358              6.96620             6.91422
##   [3]          7.16118        18.78316              6.76325             6.65307
##   [4]         26.93845        43.20576              7.00456             6.96651
##   [5]         11.93393        34.69149              6.82529             6.86479
##   [6]         -5.76947         9.25825              6.58041             6.50963
##       IDR_mean   IDR_max IDR_value IDR_nlogq IDR_logTEN_max IDR_logTEN_mean
##      <numeric> <numeric> <numeric> <numeric>      <numeric>       <numeric>
##   [1]   194.237   220.937   199.906   3.57761        2.34427         2.28833
##   [2]       NaN       NaN       NaN       NaN            NaN             NaN
##   [3]       NaN       NaN       NaN       NaN            NaN             NaN
##   [4]       NaN       NaN       NaN       NaN            NaN             NaN
##   [5]       NaN       NaN       NaN       NaN            NaN             NaN
##   [6]       NaN       NaN       NaN       NaN            NaN             NaN
##       IDR_logTEN_value   baseMean log2FoldChange     lfcSE      pvalue
##              <numeric>  <numeric>      <numeric> <numeric>   <numeric>
##   [1]          2.30083 2412.21311     -0.9794165  0.309687 0.000866738
##   [2]              NaN 1373.99374     -0.5052768  0.265385 0.047391510
```

```
##   [3]              NaN   28.90608     -0.5703048  0.613899 0.247771859
##   [4]              NaN 1356.79181     -0.4399954  0.319295 0.144923448
##   [5]              NaN  548.04398      0.0544551  0.338998 0.867282462
##   [6]              NaN    6.18149      0.8158975  1.120646 0.151282869
##             padj
##        <numeric>
##   [1] 0.00420261
##   [2] 0.10943455
##   [3] 0.37766531
##   [4] 0.25395591
##   [5] 0.91338478
##   [6] 0.26219512
##   -------
##   seqinfo: 7 sequences (1 circular) from ce11 genome
```

```
names(gr.promoters) <- gr.promoters$wbps_gene_id

# sort promoters high to low by log2FC
gr.promoters = gr.promoters[order(gr.promoters$log2FoldChange,decreasing=T)]

# divide groups by peak and padj
enriched_intestine = gr.promoters$padj<.05 & !is.na(gr.promoters$padj)
has_peak = !is.nan(gr.promoters$IDR_max)
classA = enriched_intestine & has_peak
classB = !enriched_intestine & has_peak
classC = enriched_intestine & !has_peak
classD = !enriched_intestine & !has_peak

m = matrix( c(sum(classA),
              sum(classB),
              sum(classC),
              sum(classD)), ncol = 2)
m.chisq = chisq.test(m)

gr.promoters$class = "classA"
gr.promoters$class[classB] <- "classB"
gr.promoters$class[classC] <- "classC"
gr.promoters$class[classD] <- "classD"

promoters.hilo = as.data.frame(gr.promoters)

# BED format
write.table(promoters.hilo, file.path(OUTPUT_03, "promoters.hilo.bed"), quote=F, sep="\t", row.names=F,

# Matrix format readable into R
write.table(promoters.hilo, file.path(OUTPUT_03, "promoters.hilo.tsv"), quote=F, sep="\t", row.names=T,


write.table(promoters.hilo[classA,],
              file.path(OUTPUT_03, "promoters.hilo.classA.bed"), quote=F, sep="\t", row.names=F, col.name
write.table(promoters.hilo[classB,],
              file.path(OUTPUT_03, "promoters.hilo.classB.bed"), quote=F, sep="\t",
row.names=F, col.names=F)
write.table(promoters.hilo[classC,],
```

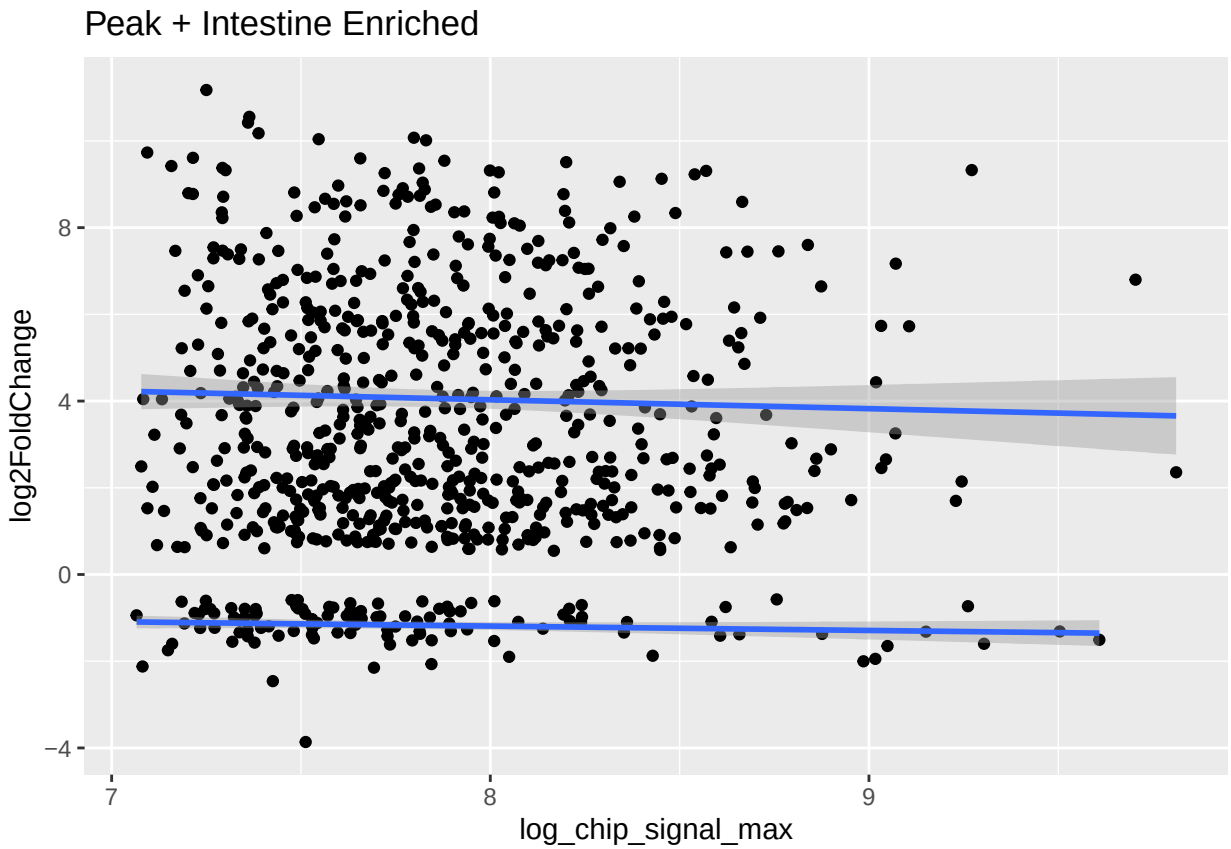
```
            file.path(OUTPUT_03, "promoters.hilo.classC.bed"), quote=F, sep="\t",
row.names=F, col.names=F)
write.table(promoters.hilo[classD,],
            file.path(OUTPUT_03, "promoters.hilo.classD.bed"), quote=F, sep="\t",
row.names=F, col.names=F)

# deeptooling up versus down only, no other filters
promoters.hilo.up = promoters.hilo %>% filter(log2FoldChange > 0)
promoters.hilo.down = promoters.hilo %>% filter(log2FoldChange < 0)

write.table(promoters.hilo.up,
            file.path(OUTPUT_03, "promoters.hilo.up.bed"),
            quote=F,
            sep="\t",
row.names=F, col.names=F)

write.table(promoters.hilo.down,
            file.path(OUTPUT_03, "promoters.hilo.down.bed"),
            quote=F,
            sep="\t",
row.names=F, col.names=F)
```

To produce the deeptools output, execute DEEPTOOLS.bash.

It will compute promoters.hilo.mx and promoters.hilo.pdf.

Deeptools PDFs indicate a font called dejavu, if you're tired of replacing it in Illustrator, install it from: https://sourceforge.net/projects/dejavu/

```
gr.promoters.classA = gr.promoters[classA]

# scatter plot with linear mods on logFC up and down separately
gr.promoters.classA %>% as.data.frame() %>%
  ggplot(
    aes(x=log_chip_signal_max,
        y=log2FoldChange,
        group=log2FoldChange>0)) + geom_point() +
        geom_smooth(method='lm', formula= y~x) +
        ggtitle("Peak + Intestine Enriched")
```




```
classA.up = promoters.hilo %>% as.data.frame() %>% filter(classA & log2FoldChange > 0)
up.table = classA.up[,c('log2FoldChange',
                        'chip_signal_mean',
                        'chip_signal_max',
                        'log_chip_signal_mean',
                        'log_chip_signal_max',
                        'IDR_mean',   'IDR_max', 'IDR_value')]

cor.up.table = cor(up.table)
options(digits=3)
knitr::kable(cor.up.table, caption="Pairwise correlations")
```

Table 1: Pairwise correlations

	log2FoldChange	chip_signal_mean	chip_signal_max	log_chip_signal_mean	log_chip_signal_max	IDR_mean	IDR_max	IDR_value
log2FoldChange	1.000	-0.076	-0.027	-0.089	-0.036	-0.023	-0.025	0.046
chip_signal_mean	-0.076	1.000	0.919	0.980	0.899	0.897	0.903	0.722
chip_signal_max	-0.027	0.919	1.000	0.888	0.969	0.981	0.990	0.851
log_chip_signal_mean	-0.089	0.980	0.888	1.000	0.912	0.870	0.869	0.681
log_chip_signal_max	-0.036	0.899	0.969	0.912	1.000	0.955	0.957	0.796
IDR_mean	-0.023	0.897	0.981	0.870	0.955	1.000	0.991	0.883
IDR_max	-0.025	0.903	0.990	0.869	0.957	0.991	1.000	0.860
IDR_value	0.046	0.722	0.851	0.681	0.796	0.883	0.860	1.000

```
cor.test(classA.up[,'log2FoldChange'],classA.up[,'IDR_mean'])
```

```
##
##  Pearson's product-moment correlation
##
## data:  classA.up[, "log2FoldChange"] and classA.up[, "IDR_mean"]
## t = -0.6, df = 638, p-value = 0.6
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.1004  0.0545
## sample estimates:
##     cor
## -0.0231
```

```
cor.test(classA.up[,'log2FoldChange'],classA.up[,'log_chip_signal_mean'])
```

```
##
##  Pearson's product-moment correlation
##
## data:  classA.up[, "log2FoldChange"] and classA.up[, "log_chip_signal_mean"]
## t = -2, df = 638, p-value = 0.02
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.1654 -0.0117
## sample estimates:
##     cor
## -0.0891
```

```
Sys.setenv(PROMOTOR_BED_PATH=PROMOTOR_BED_PATH,
           NR_PROMOTOR_BED_PATH=NR_PROMOTOR_BED_PATH)
```

```
source $HOME/.bash_profile
conda activate elt-2-rev
wiggletools
```

```
## WiggleTools
##
## Copyright [1999-2017] EMBL-European Bioinformatics Institute
## Development contact: Daniel Zerbino zerbino@ebi.ac.uk
##
## Citation: Zerbino DR, Johnson N, Juettemann T, Wilder SP and Flicek PR: WiggleTools: parallel proces
##
## This library parses wiggle files and executes various operations on them streaming through lazy evalu
##
## Inputs:
##  The program takes in Wig, BigWig, BedGraph, Bed, BigBed, Bam, VCF, and BCF files, which are distingu
##  Note that wiggletools assumes that every bam file has an index .bai file next to it.
##
## Outputs:
##  The program outputs a wiggle file in stdout unless the output is squashed
##
## Command line:
##  wiggletools --help
##  wiggletools program
##
```

```
## Program grammar:
##  program = (iterator) | do (iterator) | (extraction) | (statistic) | run (file)
##  iterator = (in_filename) | (unary_operator) (iterator) | (binary_operator) (iterator) (iterator) |
##  unary_operator = unit | coverage | write (output) | write_bg (ouput) | smooth (int) | abs | exp | l
##  output = (out_filename) | -
##  in_filename = *.wig | *.bw | *.bed | *.bb | *.bg | *.sam | *.bam | *.cram | read_count *.sam | read
##  statistic = (statistic_function) (iterator) | ndpearson (multiplex) (multiplex)
##  statistic_function = AUC | meanI | varI | minI | maxI | stddevI | CVI | energy (wavelength) | pears
##  binary_operator = diff | ratio | overlaps | trim | noverlaps | nearest | apply (statistic) | fillIn
##  reducer = cat | sum | product | mean | var | stddev | entropy | CV | median | min | max
##  setComparison = ttest | ftest | wilcoxon
##  multiplex_list = (multiplex) | (multiplex) : (multiplex_list)
##  multiplex = (iterator_list) | map (unary_operator) (multiplex) | strict (multiplex)
##  iterator_list = (iterator) | (iterator) : (iterator_list)
##  extraction = profile (output) (int) (iterator) (iterator) | profiles (output) (int) (iterator) (ite
##      | apply_paste (out_filename) (statistic) (bed_file) (iterator)
```