SCEPTRE Vs Seurat CHIP-seq Analysis

2023-03-20

```
#load required packages. Sometimes need to redownload ondisc
library(biomaRt)
library(plyranges)
```

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

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

```
## 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
```

```
## Loading required package: IRanges
```

```
## Loading required package: S4Vectors
```

```
## Loading required package: stats4
```

```
##
## Attaching package: 'S4Vectors'
```

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

```
## Loading required package: GenomicRanges
```

```
## Loading required package: GenomeInfoDb
```

```
##
## Attaching package: 'plyranges'
## The following object is masked from 'package:IRanges':
##
##     slice
```

```
## The following object is masked from 'package:biomaRt':
##
##     select
```

```
## The following object is masked from 'package:stats':
##
##     filter
```

```
library(GenomicRanges)
library(genomation)
```

```
## Loading required package: grid
```

```
## Warning: replacing previous import 'Biostrings::pattern' by 'grid::pattern'
## when loading 'genomation'
```

```
#devtools::install_github('timothy-barry/ondisc')
library(ondisc)
library(sceptre3)
library(BH)
library(varhandle)
```

```
##
## Attaching package: 'varhandle'
```

```
## The following object is masked from 'package:S4Vectors':
##
##     unfactor
```

```
library(kableExtra)
library(rjson)
library(ggplot2)
```

Goal

The goal of this report is to compare the genes that are found to be significantly affected by IRF1 perturbation via Seurat and SCEPTRE to CHIPseq data.

Read in Promoter Data

```r
#read in papalexi data
LOCAL_SCEPTRE2_DATA_DIR <-.get_config_path("LOCAL_SCEPTRE2_DATA_DIR")
papalexi_dir <- paste0(LOCAL_SCEPTRE2_DATA_DIR, "data/papalexi/eccite_screen/")
# gene info
gene_odm_fp <- paste0(papalexi_dir, "gene/matrix.odm")
gene_metadata_fp <- paste0(papalexi_dir, "gene/metadata_qc.rds")
gene_odm <- read_odm(odm_fp = gene_odm_fp, metadata_fp = gene_metadata_fp)
```

```r
#get TSS for each gene
ensembl <- useEnsembl(host = 'https://grch37.ensembl.org',biomart = "genes",
                      dataset = "hsapiens_gene_ensembl")
A = getBM(attributes=c("hgnc_symbol", "chromosome_name", "start_position",
                       "end_position", "strand"),
          filters=c('hgnc_symbol'),
          value = gene_odm |> get_feature_ids(), mart=ensembl) |>
  filter(chromosome_name %in% c(1:22, "X", "Y"))
```

```r
#get start and end site depending on whether the strand is postive or negative
TSS_start = rep(NA,nrow(A))
TSS_end = rep(NA,nrow(A))
for(j in c(1:nrow(A))){
  #if strand positive, use [start-500,start]
  if(A$strand[j]==1){
    TSS_end[j] = A$start_position[j]
    TSS_start[j] = A$start_position[j]-500
  }else{
    #if strand negative use [end,end + 500]
    TSS_start[j] = A$end_position[j]
    TSS_end[j] = A$end_position[j]+500
  }
}
#add to A matrix
A$TSS_start = TSS_start
A$TSS_end = TSS_end
#add chr to chromosome name
A$chromosome_name = paste0("chr",A$chromosome_name)
```

```r
#use A to make a promoter granges object
promoters <- GRanges(
  seqnames = A$chromosome_name,
  ranges = IRanges(start = A$TSS_start, end = A$TSS_end),
  TF = A$hgnc_symbol)
```

Read in CHIPseq Data and Join the Datasets

```r
#read in chipseq data as granges object
code_dir = .get_config_path("LOCAL_CODE_DIR")
data.dir = paste0(code_dir,"/sceptre2-manuscript/writeups/papalexi_analysis/")
chipseq.dir = paste0(data.dir,
              'GSM935549_hg19_wgEncodeSydhTfbsK562Irf1Ifng6hStdPk.narrowPeak')
chipseq_data = readNarrowPeak(chipseq.dir, track.line=FALSE, zero.based=TRUE)
```

```r
#get left join of promoters and chipseq peaks
direct_effects = plyranges::join_overlap_left(promoters,chipseq_data,
                                              minoverlap = 500)
#get overlapped genes
overlap_genes = direct_effects$TF[is.na(direct_effects$score)==F]
null_genes = direct_effects$TF[is.na(direct_effects$score)==T]
print(paste0("The number of promoters that overlap with Chipseq peaks is ",
             length(overlap_genes)))
```

```
## [1] "The number of promoters that overlap with Chipseq peaks is 1644"
```

Read in Seurat and SCEPTRE Results

```r
#read in sceptre and seurat analysis data
sceptre_path = paste0(data.dir,
                      'sceptre_full_mrna_results_with_effect_size.rds')
seurat_path = paste0(data.dir,
                     'seurat_all_perturbations_results.rds')
#read in sceptre and seurat results
sceptre = readRDS(sceptre_path)
seurat = readRDS(seurat_path)
#adjust pvalues according to BH
sceptre$p_value_adj = sceptre$p_value
for(val in unique(sceptre$grna_group)){
  ind = which(sceptre$grna_group == val)
  sceptre$p_value_adj[ind] = p.adjust(sceptre$p_value[ind],method = "BH")
}
for(j in c(1:length(seurat))){
  seurat[[j]]$p_val_adj = p.adjust(seurat[[j]]$p_val,method = "BH")
}
```

Filter to Get IRF1 Results

```r
#get sceptre and seurat IRF1 results
PRTB = 'IRF1'
sceptre_prtb = subset(sceptre,grna_group == PRTB)
seurat_prtb = seurat[[PRTB]]
```

Compare Significant Genes to CHIPseq Results

```r
alpha = 0.05
#get all significant genes from each method
sceptre_sig = unfactor(sceptre_prtb$response_id[sceptre_prtb$p_value_adj < alpha])
seurat_sig = rownames(seurat_prtb)[seurat_prtb$p_val_adj < alpha]
#get true positive sets
sceptre_true = sceptre_sig[sceptre_sig %in% overlap_genes]
seurat_true = seurat_sig[seurat_sig %in% overlap_genes]
```

Table 1: SCEPTRE Vs Seurat:IRF1 Perturbation True Positives

	Total Positive Genes	Unique to SCEPTRE	Unique to Seurat	Shared
True Positives	1644	66	44	160

```
#get number of shared and unique true postives
shared_true = sceptre_true[sceptre_true %in% seurat_true]
sceptre_true_unique = sceptre_true[(sceptre_true %in% seurat_true) == F]
seurat_true_unique = seurat_true[(seurat_true %in% sceptre_true) == F]

#get sensitivity
sceptre_sensitivity = length(sceptre_true)/length(overlap_genes)
seurat_sensitivity = length(seurat_true)/length(overlap_genes)

#get false positive sets
sceptre_false = sceptre_sig[sceptre_sig %in% null_genes]
seurat_false = seurat_sig[seurat_sig %in% null_genes]
#get number of shared and unique false postives
shared_false = sceptre_false[sceptre_false %in% seurat_false]
sceptre_false_unique = sceptre_false[(sceptre_false %in% seurat_false) == F]
seurat_false_unique = seurat_false[(seurat_false %in% sceptre_false) == F]


#get specificity
sceptre_specificity = 1-length(sceptre_false)/length(null_genes)
seurat_specificity = 1-length(seurat_false)/length(null_genes)
```

True Positive Table

```
#make table for true positives
true_pos = matrix(NA,1,4)
rownames(true_pos) = "True Positives"
colnames(true_pos) = c("Total Positive Genes","Unique to SCEPTRE",
                       "Unique to Seurat","Shared")
true_pos[1,] = c(length(overlap_genes),length(sceptre_true_unique),
                 length(seurat_true_unique),length(shared_true))

results_table = kable(true_pos,booktabs = TRUE, linesep = "",
    caption = paste0("SCEPTRE Vs Seurat:",PRTB," Perturbation True Positives"))
kable_styling(results_table,position = "center")
```

Table 2: SCEPTRE Vs Seurat:IRF1 Perturbation False Positives

	Total Null Genes	Unique to SCEPTRE	Unique to Seurat	Shared
False Positives	11483	433	258	1341

True Positive Table

```
#make table for false positives
false_pos = matrix(NA,1,4)
rownames(false_pos) = "False Positives"
colnames(false_pos) = c("Total Null Genes","Unique to SCEPTRE",
                        "Unique to Seurat","Shared")
false_pos[1,] = c(length(null_genes),length(sceptre_false_unique),
                 length(seurat_false_unique),length(shared_false))

results_table = kable(false_pos,booktabs = TRUE, linesep = "",
    caption = paste0("SCEPTRE Vs Seurat:",PRTB," Perturbation False Positives"))
kable_styling(results_table,position = "center")
```

Table 3: SCEPTRE Vs Seurat:IRF1 Perturbation Sensitivity and Specificity

	Sensitivity	Specificity
SCEPTRE	0.1374696	0.8455108
Seurat	0.1240876	0.8607507

Sensitivity Specificity Table

```r
#make table for sens and spec
sens_spec = matrix(NA,2,2)
rownames(sens_spec) = c("SCEPTRE","Seurat")
colnames(sens_spec) = c("Sensitivity","Specificity")
sens_spec[1,] = c(sceptre_sensitivity,sceptre_specificity)
sens_spec[2,] = c(seurat_sensitivity,seurat_specificity)

results_table = kable(sens_spec,booktabs = TRUE, linesep = "",
caption = paste0("SCEPTRE Vs Seurat:",PRTB,
                 " Perturbation Sensitivity and Specificity"))
kable_styling(results_table,position = "center")
```

Table 4: SCEPTRE Vs Seurat:IRF1 Perturbation CHIPseq and Reference Agreement

	Unique to CHIPseq	Unique to Reference	Shared
IRF1 Targets	492	346	8

Get Top Genes by Score

```
TF = direct_effects$TF
scores = direct_effects$pvalue
top_genes = sort(TF[order(scores,decreasing = T)][1:500])
```

Read in Reference Dataset For IRF1 TF Targets

```
data.dir = paste0(code_dir,"/sceptre2-manuscript/writeups/papalexi_analysis/")
targets.dir = paste0(data.dir,'IRF1_targets.json')
prtb_targets = fromJSON(file = targets.dir)
targets = c()
for(j in c(1:length(prtb_targets$associations))){
  targets = c(targets,prtb_targets$associations[[j]]$gene$symbol)
}
targets = targets[(targets%in%sceptre_prtb$response_id)]
null_targets =sceptre_prtb$response_id[(sceptre_prtb$response_id%in%targets)==F]
```

Table For Overlap Of Gene Sets

```
overlap_targets = top_genes[(top_genes%in% targets)]
CHIP_target_unique = top_genes[(top_genes%in% targets)==F]
ref_target_unique = targets[(targets %in% top_genes) == F]
#make table for true positives
chip_agree = matrix(NA,1,3)
rownames(chip_agree) = c("IRF1 Targets")
colnames(chip_agree ) = c("Unique to CHIPseq","Unique to Reference","Shared")
chip_agree [1,] = c(length(CHIP_target_unique),length(ref_target_unique),
                    length(overlap_targets))


results_table = kable(chip_agree ,booktabs = TRUE, linesep = "",
caption = paste0("SCEPTRE Vs Seurat:",PRTB,
                 " Perturbation CHIPseq and Reference Agreement"))
kable_styling(results_table,position = "center")
```

Compare Significant Gene Sets to Reference Data

```r
alpha = 0.05
#get all significant genes from each method
sceptre_sig = unfactor(sceptre_prtb$response_id[sceptre_prtb$p_value_adj < alpha])
seurat_sig = rownames(seurat_prtb)[seurat_prtb$p_val_adj < alpha]
#get true positive sets
sceptre_true = sceptre_sig[sceptre_sig %in% targets]
seurat_true = seurat_sig[seurat_sig %in% targets]
#get number of shared and unique true postives
shared_true = sceptre_true[sceptre_true %in% seurat_true]
sceptre_true_unique = sceptre_true[(sceptre_true %in% seurat_true) == F]
seurat_true_unique = seurat_true[(seurat_true %in% sceptre_true) == F]

#get sensitivity
sceptre_sensitivity = length(sceptre_true)/length(targets)
seurat_sensitivity = length(seurat_true)/length(targets)

#get false positive sets
sceptre_false = sceptre_sig[sceptre_sig %in% null_targets]
seurat_false = seurat_sig[seurat_sig %in% null_targets]
#get number of shared and unique false postives
shared_false = sceptre_false[sceptre_false %in% seurat_false]
sceptre_false_unique = sceptre_false[(sceptre_false %in% seurat_false) == F]
seurat_false_unique = seurat_false[(seurat_false %in% sceptre_false) == F]


#get specificity
sceptre_specificity = 1-length(sceptre_false)/length(null_targets)
seurat_specificity = 1-length(seurat_false)/length(null_targets)
```

True Positive Table

```r
#make table for true positives
true_pos = matrix(NA,1,4)
rownames(true_pos) = "True Positives"
colnames(true_pos) = c("Total Positive Genes","Unique to SCEPTRE",
                       "Unique to Seurat","Shared")
true_pos[1,] = c(length(targets),length(sceptre_true_unique),
                 length(seurat_true_unique),length(shared_true))

results_table = kable(true_pos,booktabs = TRUE, linesep = "",
    caption = paste0("SCEPTRE Vs Seurat:",PRTB,
                     " Perturbation Reference Target True Positives"))
kable_styling(results_table,position = "center")
```

Table 5: SCEPTRE Vs Seurat:IRF1 Perturbation Reference Target True Positives

	Total Positive Genes	Unique to SCEPTRE	Unique to Seurat	Shared
True Positives	354	11	7	98

Table 6: SCEPTRE Vs Seurat:IRF1 Perturbation Reference Target False Positives

	Total Null Genes	Unique to SCEPTRE	Unique to Seurat	Shared
False Positives	13658	507	301	1465

False Positive Table

```
#make table for true positives
false_pos = matrix(NA,1,4)
rownames(false_pos) = "False Positives"
colnames(false_pos) = c("Total Null Genes","Unique to SCEPTRE",
                        "Unique to Seurat","Shared")
false_pos[1,] = c(length(null_targets),length(sceptre_false_unique),
                 length(seurat_false_unique),length(shared_false))

results_table = kable(false_pos,booktabs = TRUE, linesep = "",
    caption = paste0("SCEPTRE Vs Seurat:",PRTB,
                     " Perturbation Reference Target False Positives"))
kable_styling(results_table,position = "center")
```

Table 7: SCEPTRE Vs Seurat:IRF1 Perturbation Reference Target Sensitivity and Specificity

	Sensitivity	Specificity
SCEPTRE	0.3079096	0.8556158
Seurat	0.2966102	0.8706985

Sensitivity and Specificity Table

```r
#make table for true positives
sens_spec = matrix(NA,2,2)
rownames(sens_spec) = c("SCEPTRE","Seurat")
colnames(sens_spec) = c("Sensitivity","Specificity")
sens_spec[1,] = c(sceptre_sensitivity,sceptre_specificity)
sens_spec[2,] = c(seurat_sensitivity,seurat_specificity)

results_table = kable(sens_spec,booktabs = TRUE, linesep = "",
caption = paste0("SCEPTRE Vs Seurat:",PRTB,
                 " Perturbation Reference Target Sensitivity and Specificity"))
kable_styling(results_table,position = "center")
```

Summary

The CHIPseq peaks that we are using do not agree with the database reference at all. This could be due to incorrect reading of the file. Seurat and SCEPTRE generally have similar sensitivity and specificity for both the CHIPseq data and the database reference although the sensitivity is much higher when compared to the database reference (30 percent vs 12 percent). In this case, around 10 percent of the total genes that are rejected differ between the two sets.

Bonus: Pvalue Scatterplot Between log Pvalues For Seurat and SCEPTRE

```r
sceptre_genes = unfactor(sceptre_prtb$response_id)
seurat_pvals = log(seurat_prtb[sceptre_genes,]$p_val,10)
sceptre_pvals = log(sceptre_prtb$p_value,10)
```

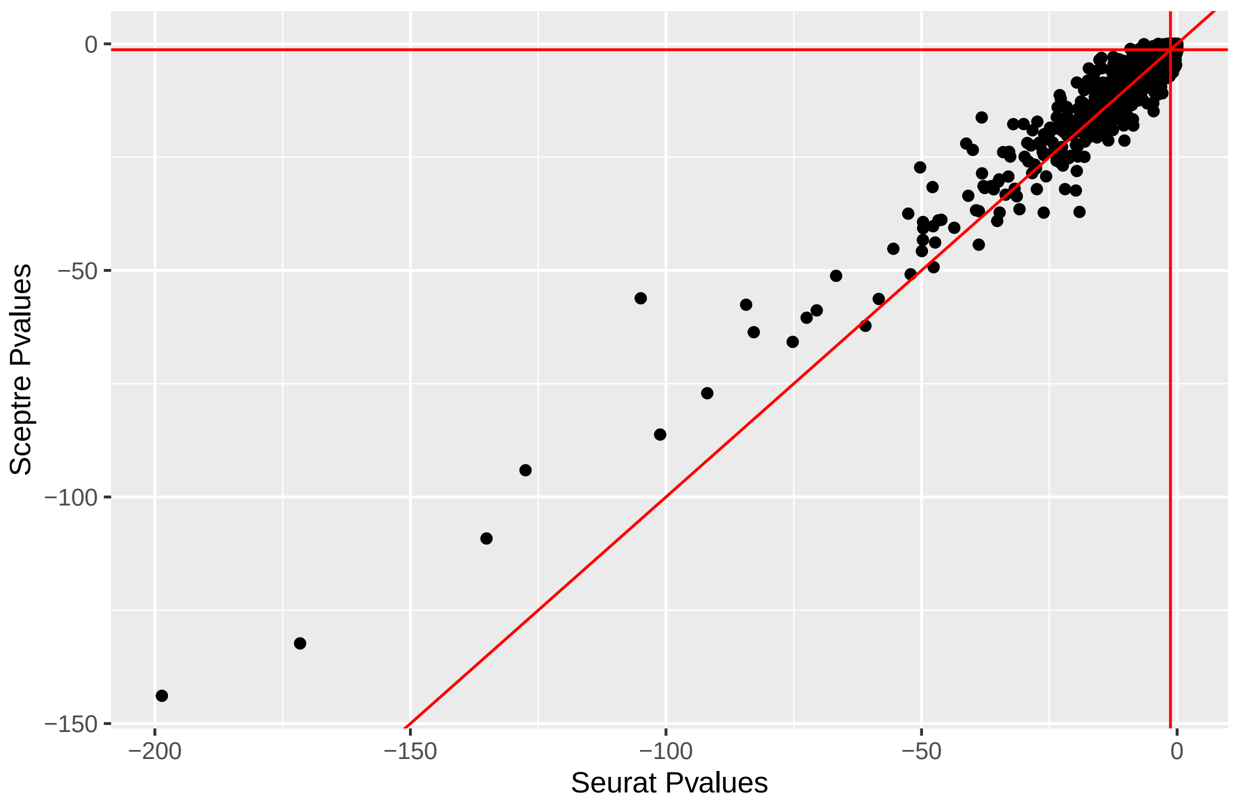
```
## Warning: NaNs produced
```

```r
#remove those with sceptre pval = 0
sceptre_0 = which(is.na(sceptre_pvals) == T)
sceptre_pvals = sceptre_pvals[-sceptre_0]
seurat_pvals = seurat_pvals[-sceptre_0]
```

```r
#merge pvalues
Pval = data.frame(seuratP = seurat_pvals,
                  sceptreP = sceptre_pvals)
```

```r
#volcano plot
ggplot(Pval,aes(x = seuratP,y = sceptreP)) + geom_point() +
  ggtitle('Identity Plot of CUL3 Pvalues: SCEPTRE vs  Unfiltered Seurat') +
  labs(y = 'Sceptre Pvalues',x = 'Seurat Pvalues')+
  geom_abline(slope=1, intercept = 0,color = 'red')+
  geom_vline(xintercept = log(0.05,10),color = 'red')+
  geom_hline(yintercept = log(0.05,10), color = 'red')
```

Identity Plot of CUL3 Pvalues: SCEPTRE vs Unfiltered Seurat


Sceptre Pvalues
Seurat Pvalues