

Motif enrichment

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```
#Load libraries
library(tidyverse)
library(ggplot2)
setwd("D:/PhD/TSS cluster/H99/TRASS_d17/")

#read data
# TC motif
TC_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E30S.tsv",sep = "\t",head=1)
TC_ESharp<-TC_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #252

TC_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E30B.tsv",sep = "\t",head=1)
TC_EBroad<-TC_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #421

TC_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S30S.tsv",sep = "\t",head=1)
TC_SSharp<-TC_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #282

TC_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S30B.tsv",sep = "\t",head=1)
TC_SBroad<-TC_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #432

TC_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E37S.tsv",sep = "\t",head=1)
TC_E37Sharp<-TC_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #247

TC_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E37B.tsv",sep = "\t",head=1)
TC_E37Broad<-TC_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #346
```

```

TC_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S37S.tsv",sep = "\t",header=TRUE)
TC_S37Sharp<-TC_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #221

TC_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S37B.tsv",sep = "\t",header=TRUE)
TC_S37Broad<-TC_S37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #388

#GG motif
GG_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E30S.tsv",sep = "\t",header=TRUE)
GG_ESharp<-GG_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #23

GG_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E30B.tsv",sep = "\t",header=TRUE)
GG_EBroad<- GG_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #27

GG_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S30S.tsv",sep = "\t",header=TRUE)
GG_SSharp<-GG_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #21

GG_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S30B.tsv",sep = "\t",header=TRUE)
GG_SBroad<-GG_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #64

GG_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E37S.tsv",sep = "\t",header=TRUE)
GG_E37Sharp<- GG_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #23

GG_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E37B.tsv",sep = "\t",header=TRUE)
GG_E37Broad<-GG_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #30

GG_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S37S.tsv",sep = "\t",header=TRUE)
GG_S37Sharp<-GG_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%

```

```

mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #13

GG_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S37B.tsv",sep = "\t",h
GG_S37Broad<-GG_S37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #49

#TATA motif
TATA_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E30S.tsv",sep = "\
TATA_ESharp<- TATA_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #46

TATA_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E30B.tsv",sep = "\
TATA_EBroad<- TATA_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #58

TATA_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S30S.tsv",sep = "\
TATA_SSharp<- TATA_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #45

TATA_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S30B.tsv",sep = "\
TATA_SBroad<- TATA_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #77

TATA_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E37S.tsv",sep = \
TATA_E37Sharp<- TATA_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #43

TATA_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E37B.tsv",sep = \
TATA_E37Broad<- TATA_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #67

TATA_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S37S.tsv",sep = \
TATA_S37Sharp<- TATA_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #36

TATA_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S37B.tsv",sep = \
TATA_S37Broad<- TATA_S37Broad%>%filter(p.value<0.0005)%>%

```

```

mutate(position_to_TSS=start-100)%>%
mutate(motif="TATA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #91

#AC motif
AC_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E30S.tsv",sep = "\t",head=1)
AC_ESharp<- AC_ESharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #9

AC_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E30B.tsv",sep = "\t",head=1)
AC_EBroad<- AC_EBroad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #61

AC_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S30S.tsv",sep = "\t",head=1)
AC_SSharp<- AC_SSharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #13

AC_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S30B.tsv",sep = "\t",head=1)
AC_SBroad<- AC_SBroad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #78

AC_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E37S.tsv",sep = "\t",head=1)
AC_E37Sharp<- AC_E37Sharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #19

AC_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E37B.tsv",sep = "\t",head=1)
AC_E37Broad<- AC_E37Broad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #57

AC_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S37S.tsv",sep = "\t",head=1)
AC_S37Sharp<- AC_S37Sharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #16

AC_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S37B.tsv",sep = "\t",head=1)
AC_S37Broad<- AC_S37Broad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #52

```

#TTAC motif

```
TTAC_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E30S.tsv",sep = "\n")
TTAC_ESharp<- TTAC_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #11
```

```
TTAC_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E30B.tsv",sep = "\n")
TTAC_EBroad<- TTAC_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #50
```

```
TTAC_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S30S.tsv",sep = "\n")
TTAC_SSharp<- TTAC_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #15
```

```
TTAC_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S30B.tsv",sep = "\n")
TTAC_SBroad<- TTAC_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #48
```

```
TTAC_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E37S.tsv",sep = "\n")
TTAC_E37Sharp<- TTAC_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #15
```

```
TTAC_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E37B.tsv",sep = "\n")
TTAC_E37Broad<- TTAC_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #37
```

```
TTAC_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S37S.tsv",sep = "\n")
TTAC_S37Sharp<- TTAC_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #11
```

```
TTAC_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S37B.tsv",sep = "\n")
TTAC_S37Broad<- TTAC_S37Broad%>%filter(p.value<0.0005)%>%
```

```

mutate(position_to_TSS=start-100)%>%
mutate(motif="TTAC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #47

#GA motif
GA_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E30S.tsv",sep = "\t",head=1)
GA_ESharp<- GA_ESharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #12

GA_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E30B.tsv",sep = "\t",head=1)
GA_EBroad<- GA_EBroad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #39

GA_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA S30S.tsv",sep = "\t",head=1)
GA_SSharp<- GA_SSharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #10

GA_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA S30B.tsv",sep = "\t",head=1)
GA_SBroad<- GA_SBroad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #33

GA_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E37S.tsv",sep = "\t",head=1)
GA_E37Sharp<- GA_E37Sharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #11

GA_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E37B.tsv",sep = "\t",head=1)
GA_E37Broad<- GA_E37Broad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #27

GA_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA S37S.tsv",sep = "\t",head=1)
GA_S37Sharp<- GA_S37Sharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #6

```

```
GA_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA_S37B.tsv", sep = "\t", header = TRUE)
GA_S37Broad <- GA_S37Broad %>% filter(p.value < 0.0005) %>%
  mutate(position_to_TSS = start - 100) %>%
  mutate(motif = "GA") %>%
  mutate(Id = str_match(sequence_name, "(\\b.+):")[, 2]) #36
```

```
#Position relative to TSS
```

```
#position of motif in Expo Sharp cluster
```

```
ESharp_motif <- bind_rows(TC_ESharp, GG_ESharp, TATA_ESharp, AC_ESharp, TTAC_ESharp, GA_ESharp)
```

```
#remove gene as identify in Percentage small set All cluster
```

```
gene_reject_E30 <- read.delim("D:/PhD/TSS cluster/H99/TRASS_d17/gene_reject_E30.txt")
```

```
ESharp_motif <- ESharp_motif %>% filter(!(Id %in% gene_reject_E30$gene_reject_E30))
```

```
neworder <- c("AC", "TTAC", "GG", "GA", "TATA", "TC")
```

```
table(ESharp_motif$motif)
```

```
##
```

```
## AC GA GG TATA TC TTAC
```

```
## 9 10 21 41 241 11
```

```
ESharp_motif <- ESharp_motif %>%
  mutate(motif = factor(motif, levels = neworder)) %>%
  arrange(motif)
```

```
Sharp_plot <- ESharp_motif %>% ggplot(aes(x = position_to_TSS)) +
  geom_density() +
  facet_wrap(vars(motif), nrow = 1) +
  scale_y_continuous(limits = c(0, 0.09)) +
  theme_bw() +
  theme(panel.spacing = unit(0.5, "cm")) +
  theme(plot.title = element_text(size = 23, vjust = 4),
        axis.title.x.bottom = element_blank(),
        axis.title.y.left = element_blank(),
        axis.text.x.bottom = element_text(size = 11),
        axis.text.y.left = element_text(size = 11))
```

```
#Position of motif in Expo Broad cluster
```

```
EBroad_motif <- bind_rows(TC_EBroad, GG_EBroad, TATA_EBroad, AC_EBroad, TTAC_EBroad, GA_EBroad)
```

```
#remove gene as identify in Percentage small set All cluster
```

```
EBroad_motif <- EBroad_motif %>% filter(!(Id %in% gene_reject_E30$gene_reject_E30))
```

```
table(EBroad_motif$motif)
```

```
##
```

```
## AC GA GG TATA TC TTAC
```

```
## 60 39 27 55 392 47
```



```

neworder <- c("AC", "TTAC", "GG", "GA", "TATA", "TC")

EBroad_motif<-EBroad_motif%>%
  mutate(motif=factor(motif, levels=neworder))%>%
  arrange(motif)

Broad_plot<-EBroad_motif%>%ggplot(aes(x=position_to_TSS))+
  geom_density()+
  facet_wrap(vars(motif), nrow=1)+
  scale_y_continuous(limits = c(0, 0.09))+
  xlab("Position relative to TSS")+
  theme_bw()+
  theme(panel.spacing = unit(0.5, "cm"))+
  theme(plot.title = element_text(size=23, vjust=4),
        axis.title.x.bottom = element_text(size=15),
        axis.title.y.left = element_blank(),
        axis.text.x.bottom = element_text(size=11),
        axis.text.y.left = element_text(size=11))

library(gridExtra)

##
## Attaching package: 'gridExtra'

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

grid.arrange(Sharp_plot, Broad_plot, nrow=2)

```


