Figure 4B to D and S8 TATA insertion may reduce Pol II pausing

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Contents

Description

Previous studies suggest that the TATA box is highly enriched at promoters with short pausing, however, it is not clear if the TATA box functionally contribute to pause release. To test the role of the TATA box in Pol II pausing, we take a few paused promoters inluding *pk*, *comm2* and *dve*, and inserted either the canonical TATA box sequence (TATAAAA) or replace its entire upstream sequence with that of a TATA containing promoter *Act5C*. hanges in paused Pol II stability were probed by performing Pol II ChIP-nexus at control and Triptolide (TRI) treated conditions.

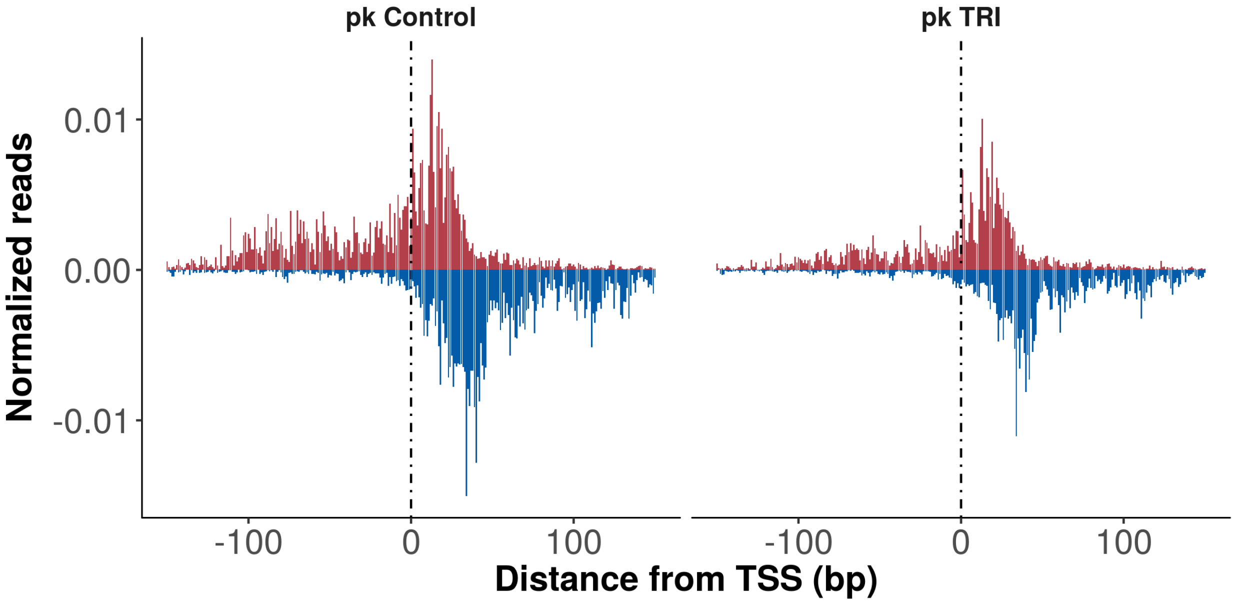
Enviroment setup

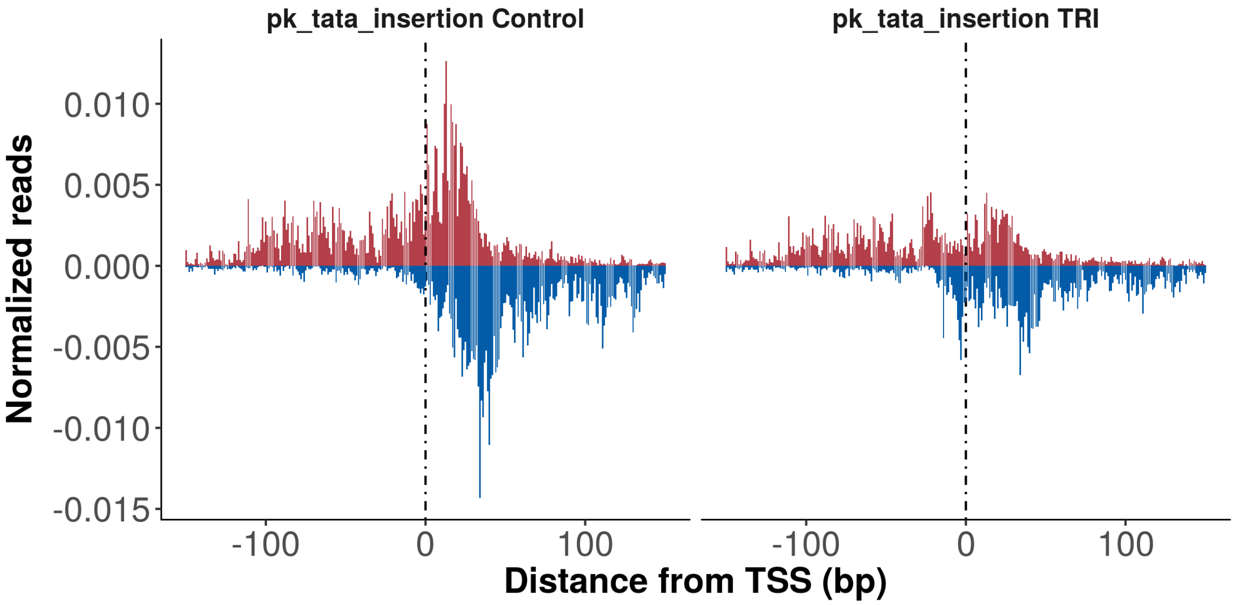
```r
library(GenomicRanges, warn.conflicts=F)
library(magrittr)
library(Rmisc)

setwd("/data/analysis_code")
options(knitr.figure_dir =
          "Figure4B_to_D_S8_tata_insertion_may_reduce_polii_pausing")

source("shared_code/knitr_common.r")
source("shared_code/ggplot_common.r")
source("shared_code/granges_common.r")
source("shared_code/metapeak_common.r")
source("shared_code/sample_common.r")
```

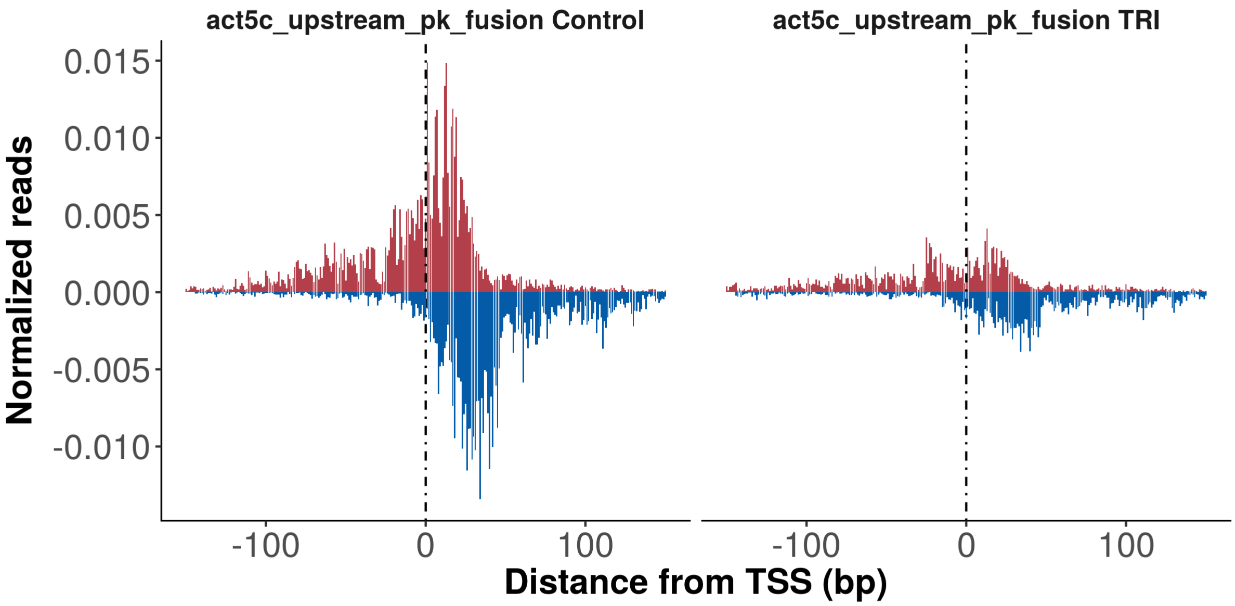
Analysis

TATA box promotes pause release

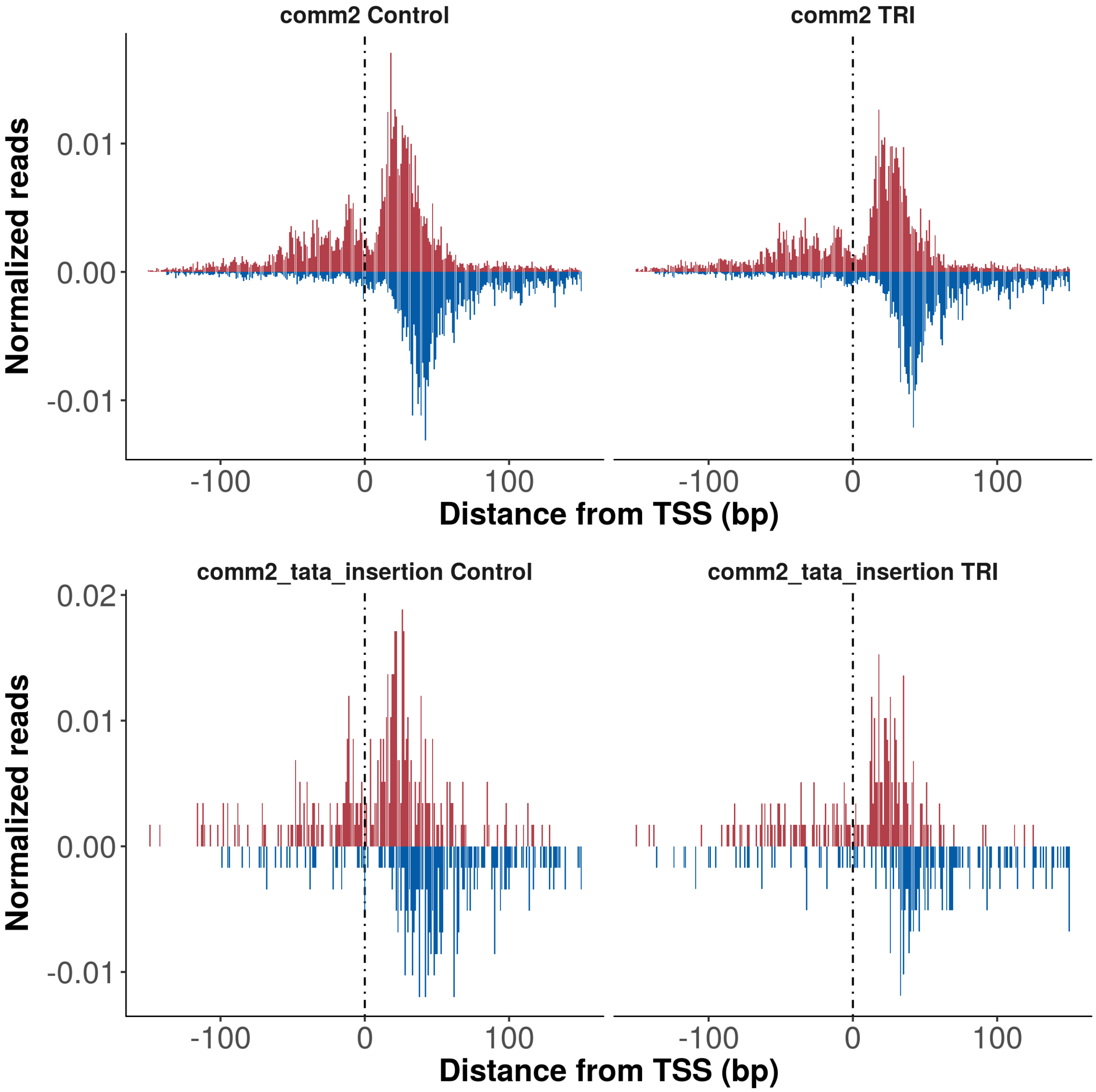
```
plasmid_annotations <- import("./plasmid_annotation.bed")

get_exo_metapeak <- function(sample, upstream=100, downstream=101,
                             smooth=NA, endogeneous = F, dps_sample_path=NULL){

  gene <- as.character(subset(sample_list, sample_name == sample)$gene)
    chromosome <- as.character(subset(sample_list, sample_name == sample )$chromosome)

    if(endogeneous ==F){

        sample_path <- load_bigwig(sample)
        region <- plasmid_annotations[seqnames(plasmid_annotations) == chromosome &
                                      plasmid_annotations$name == gene] %>%
                  resize(., 1, "start")
        seqlevels(region) <- chromosome
      metapeak <- exo_metapeak(region, sample_path,
                               upstream=upstream, downstream=downstream,
                               sample_name=gene, smooth=smooth)
      metapeak$sample <- paste(metapeak$sample_name, metapeak$strand)
      metapeak

    }else{

      region <- genome_annotations[grep(gene, genome_annotations$name, ignore.case = T)]
      seqlevels(region) <- as.character(seqnames(region))
      metapeak <- exo_metapeak(region, dps_sample_path,
                               upstream=upstream, downstream=downstream,
                               sample_name=gene, smooth=smooth)
      metapeak$sample <- paste(metapeak$sample_name, metapeak$strand)
      metapeak

    }
}


compare_dmso_and_tri <- function(dmso, tri, name,  plotting = T){

  plasmid_dmso <- get_exo_metapeak(dmso, upstream=150, downstream = 151)
  plasmid_tri <- get_exo_metapeak(tri, upstream=150, downstream = 151)

  plasmid_tri$reads <- plasmid_tri$reads / sum(abs(plasmid_dmso$reads))
  plasmid_dmso$reads <- plasmid_dmso$reads / sum(abs(plasmid_dmso$reads))


  plasmid_dmso$sample_name <- paste(name, "Control")
  plasmid_tri$sample_name <- paste(name, "TRI")

  if(plotting){
    metapeak <- rbind(plasmid_dmso, plasmid_tri)
    metapeak.p <- subset(metapeak, strand == "+")
    metapeak.n <- subset(metapeak, strand == "-")

  plot <- ggplot(metapeak.p, aes(x = tss_distance, y = reads, fill = strand))+
          geom_bar(fill="#B23F49", stat="identity") +
          geom_vline(xintercept =0, linetype = "dotdash")+
          geom_bar(data=metapeak.n, aes(x=tss_distance, y=reads),
                   fill="#045CA8", stat="identity")+
          xlab("Distance from TSS (bp)")+ ylab("Normalized reads")+
          facet_wrap(facets = "sample_name", ncol =2 )
  print(plot)
  }

  dmso_sig <- subset(plasmid_dmso, tss_distance >0 & tss_distance <= 80)$reads %>%
              abs() %>% sum()
  tri_sig <-  subset(plasmid_tri, tss_distance >0 & tss_distance <= 80)$reads%>%
              abs() %>% sum()

  sig_df <- data.frame(condition = c("dmso", "tri"),
                       paused_polii = c(dmso_sig, tri_sig),
                       name = name)
  sig_df$paused_pol_norm <- sig_df$paused_polii / sig_df$paused_polii[1]
  sig_df
}



name_list <-c("pk", "pk_tata_insertion", "act5c_upstream_pk_fusion")

pk_pol_sig_rep1 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list, "_rpb3_chipnexus_rep1"),
       paste0("reporter_triptolide_1h_dps_", name_list, "_rpb3_chipnexus_rep1"),
       name_list,list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)

pk_pol_sig_rep2 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list, "_rpb3_chipnexus_rep2"),
       paste0("reporter_triptolide_1h_dps_", name_list, "_rpb3_chipnexus_rep2"),
       name_list, SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)
```

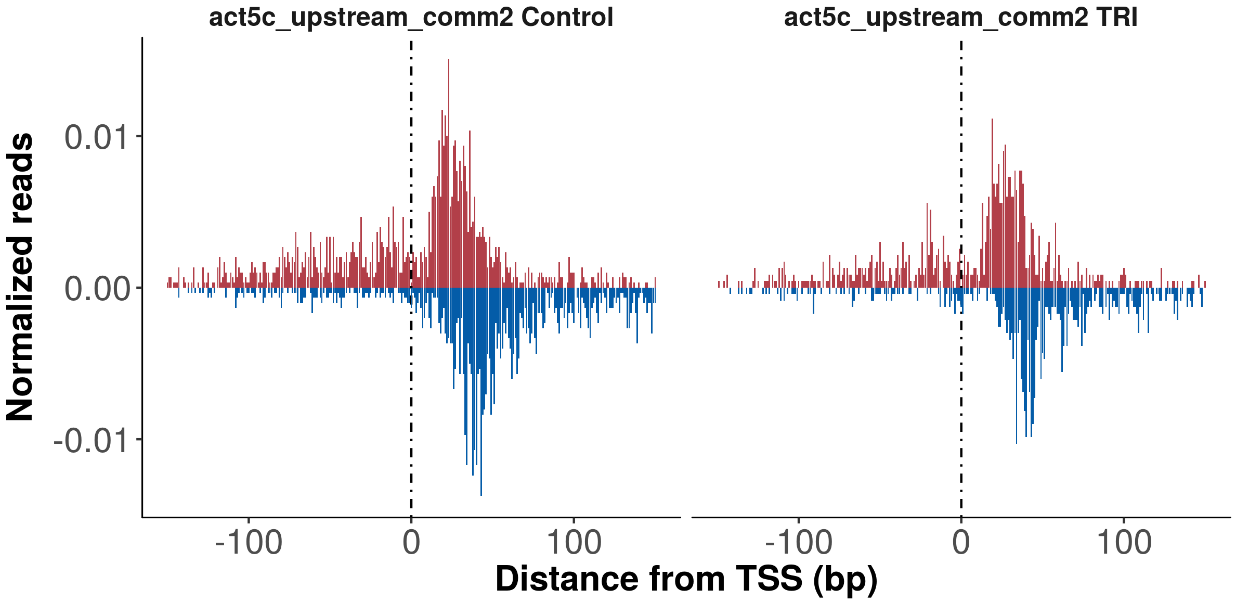

pk Control
pk TRI
Normalized reads
0.01
0.00
-0.01
-100
0
100
-100
0
100
Distance from TSS (bp)


pk_tata_insertion Control
pk_tata_insertion TRI
Normalized reads
0.010
0.005
0.000
-0.005
-0.010
-0.015
-100
0
100
-100
0
100
Distance from TSS (bp)

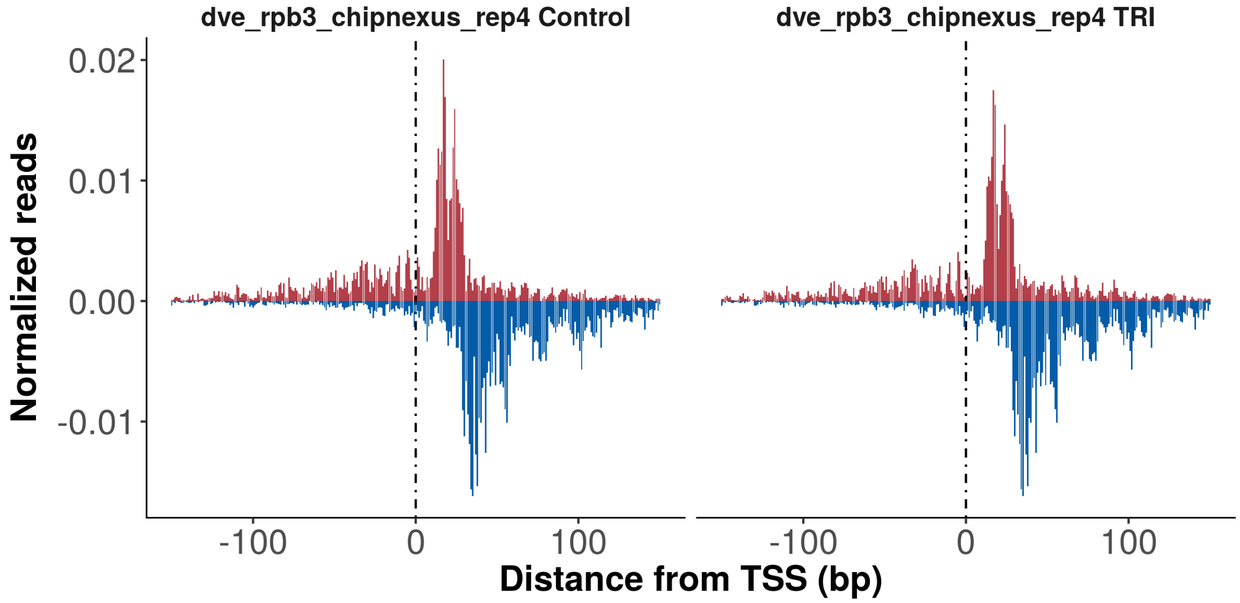


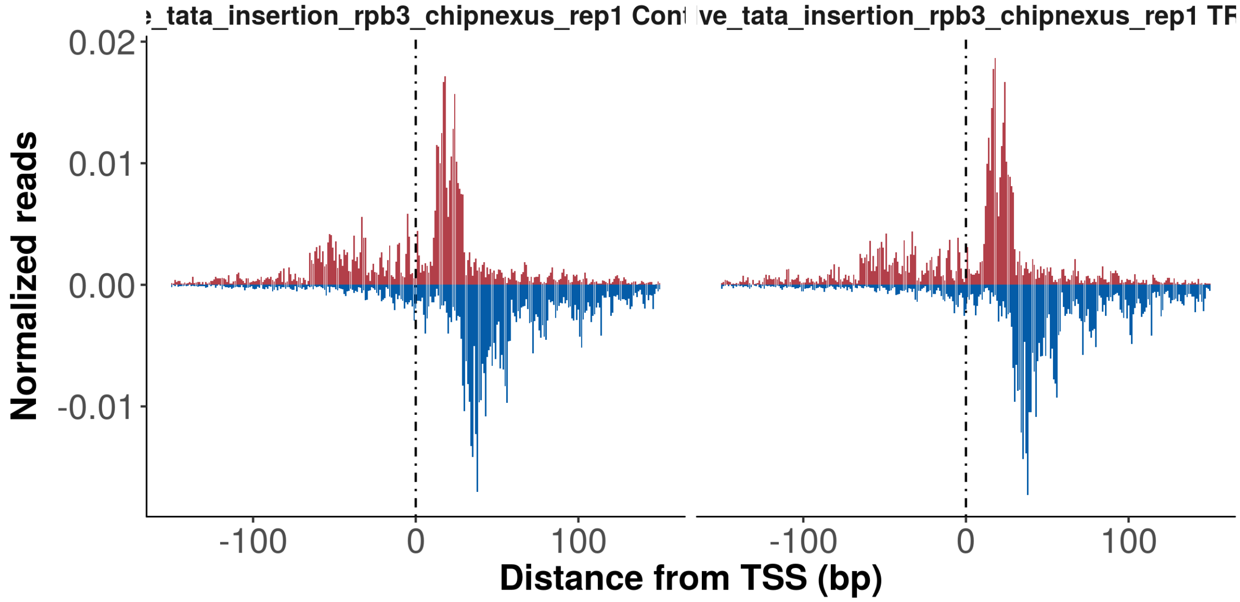
```
pk_pol_sig_rep3 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list, "_rpb3_chipnexus_rep3"),
       paste0("reporter_triptolide_1h_dps_", name_list, "_rpb3_chipnexus_rep3"),
       name_list, list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)


name_list2 <-c("comm2", "comm2_tata_insertion", "act5c_upstream_comm2")

comm2_pol_sig_rep1 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_40m_dps_", name_list2, "_rpb3_chipnexus_rep1"),
       paste0("reporter_triptolide_40m_dps_", name_list2, "_rpb3_chipnexus_rep1"),
       name_list2, SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)
```

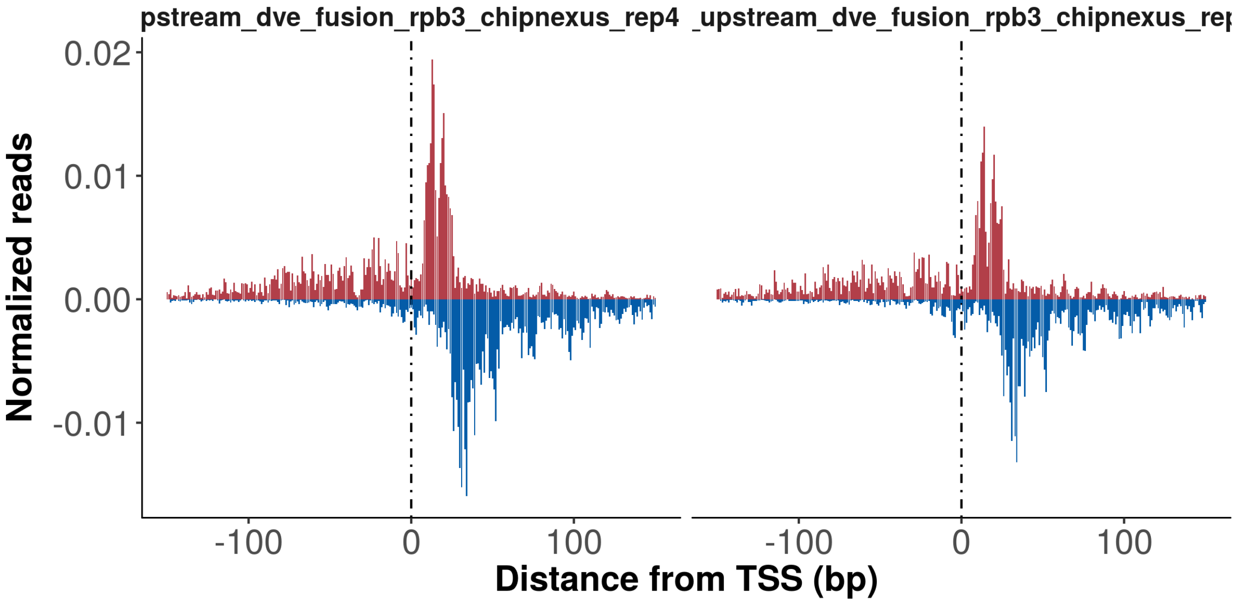

comm2 Control
comm2 TRI
comm2_tata_insertion Control
comm2_tata_insertion TRI
Normalized reads
0.01
0.00
-0.01
0.02
-100
0
100
Distance from TSS (bp)



```
comm2_pol_sig_rep2 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_40m_dps_", name_list2[1:2], "_rpb3_chipnexus_rep2"),
       paste0("reporter_triptolide_40m_dps_", name_list2[1:2], "_rpb3_chipnexus_rep2"),
       name_list2[1:2], list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)

comm2_pol_sig_rep3 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_40m_dps_", name_list2[c(1,3)], "_rpb3_chipnexus_rep3"),
       paste0("reporter_triptolide_40m_dps_", name_list2[c(1,3)], "_rpb3_chipnexus_rep3"),
       name_list2[c(1,3)], list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)

name_list3 <-c("dve_rpb3_chipnexus_rep4",
               "dve_tata_insertion_rpb3_chipnexus_rep1",
               "act5c_upstream_dve_fusion_rpb3_chipnexus_rep4")

dve_pol_sig_rep1 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list3),
       paste0("reporter_triptolide_1h_dps_", name_list3),
       name_list3, SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)
```


dve_rpb3_chipnexus_rep4 Control
dve_rpb3_chipnexus_rep4 TRI
Normalized reads
0.02
0.01
0.00
-0.01
-100
0
100
-100
0
100
Distance from TSS (bp)

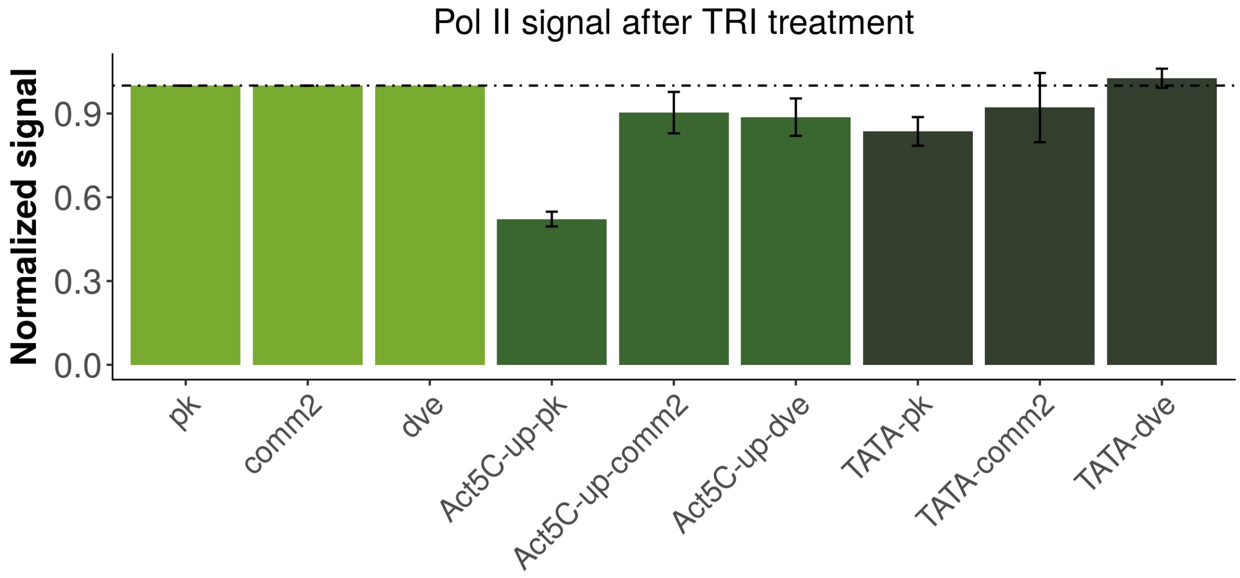

_tata_insertion_rpb3_chipnexus_rep1 Cont
ve_tata_insertion_rpb3_chipnexus_rep1 TF
Normalized reads
0.02
0.01
0.00
-0.01
-100
0
100
-100
0
100
Distance from TSS (bp)



```
name_list4 <-c("dve_rpb3_chipnexus_rep3",
               "dve_tata_insertion_rpb3_chipnexus_rep2",
               "act5c_upstream_dve_fusion_rpb3_chipnexus_rep3")

dve_pol_sig_rep2 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list4),
       paste0("reporter_triptolide_1h_dps_", name_list4),
       name_list4, list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)


name_list5 <-c("dve_rpb3_chipnexus_rep1",
               "act5c_upstream_dve_fusion_rpb3_chipnexus_rep1")


dve_pol_sig_rep3 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list5),
       paste0("reporter_triptolide_1h_dps_", name_list5),
       name_list5, list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)

name_list6 <-c("dve_rpb3_chipnexus_rep2",
               "act5c_upstream_dve_fusion_rpb3_chipnexus_rep2")

dve_pol_sig_rep4 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list6),
       paste0("reporter_triptolide_1h_dps_", name_list6),
       name_list6, list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)
```

Quantify Pol II changes

```
process_pol_sig <- function(df, control_n = 1){
  df_sub <- subset(df, condition == "tri")
  df_sub$paused_pol_norm <- df_sub$paused_pol_norm /  df_sub$paused_pol_norm[control_n]
  df_sub
}

sig_list <- list(pk_pol_sig_rep1, pk_pol_sig_rep2, pk_pol_sig_rep3,
                 comm2_pol_sig_rep1, comm2_pol_sig_rep2,comm2_pol_sig_rep3,
                 dve_pol_sig_rep1, dve_pol_sig_rep2, dve_pol_sig_rep3, dve_pol_sig_rep4)

sig_list_norm <- lapply(sig_list, process_pol_sig) %>% do.call(rbind, .)
sig_list_norm$name <- gsub("_rpb3.*", "", sig_list_norm$name)

summary_df <- summarySE(sig_list_norm, measurevar="paused_pol_norm",
                        groupvars=c("name", "condition"))

summary_df$name <- factor(summary_df$name, levels = c("pk", "comm2", "dve",
                    "act5c_upstream_pk_fusion", "act5c_upstream_comm2", "act5c_upstream_dve_fusion",
                    "pk_tata_insertion", "comm2_tata_insertion", "dve_tata_insertion"))


ggplot(summary_df, aes(x=name, y=paused_pol_norm)) +
  geom_bar(stat= "identity", position = "dodge",
          fill = rep(c("#78AB30", "#3A662F", "#333E2F"), each = 3)) +
  geom_errorbar(aes(ymin=paused_pol_norm-se, ymax=paused_pol_norm+se),
               width=.1, position=position_dodge(.9)) +
  ggtitle("Pol II signal after TRI treatment")+
  ylab("Normalized signal")+
  scale_x_discrete(labels=c("pk", "comm2", "dve",
                           "Act5C-up-pk", "Act5C-up-comm2", "Act5C-up-dve",
                           "TATA-pk","TATA-comm2", "TATA-dve")) +
  xlab("")+
  geom_hline(yintercept = 1, lty  = 4)+
  theme(axis.text.x = element_text(size=14, angle = 45, hjust = 1))
```



statistical test

```
wt <- c("pk", "comm2", "dve")
upstream_mut <- c("act5c_upstream_pk_fusion", "act5c_upstream_comm2",
                  "act5c_upstream_dve_fusion")
tata_mut <- c("pk_tata_insertion","comm2_tata_insertion", "dve_tata_insertion")

wt_values <- subset(sig_list_norm, name %in% wt)
upstream_values <-  subset(sig_list_norm, name %in% upstream_mut )
tata_values <-  subset(sig_list_norm, name %in% tata_mut )

t.test(wt_values$paused_pol_norm, upstream_values$paused_pol_norm, alternative = c("greater"))
```

```
##
##  Welch Two Sample t-test
##
## data:  wt_values$paused_pol_norm and upstream_values$paused_pol_norm
## t = 3.3448, df = 8, p-value = 0.005079
## alternative hypothesis: true difference in means is greater than 0
## 95 percent confidence interval:
##  0.1026404       Inf
## sample estimates:
## mean of x mean of y
## 1.0000000 0.7688546
```

```
t.test(wt_values$paused_pol_norm, tata_values$paused_pol_norm,  alternative = c("greater"))
```

```
##
##  Welch Two Sample t-test
##
## data:  wt_values$paused_pol_norm and tata_values$paused_pol_norm
## t = 1.8189, df = 6, p-value = 0.0594
## alternative hypothesis: true difference in means is greater than 0
## 95 percent confidence interval:
##  -0.005832168          Inf
## sample estimates:
## mean of x mean of y
## 1.0000000 0.9146436
```

SessionInfo

This analysis was performed with the following R/Bioconductor session:

```
sessionInfo()
```

```
## R version 3.4.4 (2018-03-15)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.4 LTS
##
## Matrix products: default
## BLAS: /usr/lib/libblas/libblas.so.3.6.0
## LAPACK: /usr/lib/lapack/liblapack.so.3.6.0
##
## locale:
```

```
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] reshape2_1.4.3       rtracklayer_1.38.3   ggplot2_2.2.1
##  [4] pander_0.6.1         Rmisc_1.5            plyr_1.8.4
##  [7] lattice_0.20-35      magrittr_1.5         GenomicRanges_1.30.3
## [10] GenomeInfoDb_1.14.0  IRanges_2.12.0       S4Vectors_0.16.0
## [13] BiocGenerics_0.24.0
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.17               compiler_3.4.4
##  [3] pillar_1.2.3               XVector_0.18.0
##  [5] bitops_1.0-6               tools_3.4.4
##  [7] zlibbioc_1.24.0            digest_0.6.15
##  [9] evaluate_0.10.1            tibble_1.4.2
## [11] gtable_0.2.0               rlang_0.2.1
## [13] Matrix_1.2-14              DelayedArray_0.4.1
## [15] yaml_2.1.19                GenomeInfoDbData_1.0.0
## [17] stringr_1.3.1              knitr_1.20
## [19] Biostrings_2.46.0          rprojroot_1.3-2
## [21] grid_3.4.4                 Biobase_2.38.0
## [23] XML_3.98-1.11              BiocParallel_1.12.0
## [25] rmarkdown_1.10             matrixStats_0.53.1
## [27] GenomicAlignments_1.14.2   backports_1.1.2
## [29] scales_0.5.0               Rsamtools_1.30.0
## [31] htmltools_0.3.6            SummarizedExperiment_1.8.1
## [33] colorspace_1.3-2           labeling_0.3
## [35] stringi_1.2.3              RCurl_1.95-4.10
## [37] lazyeval_0.2.1             munsell_0.5.0
```