Figure 1 Reporter-ChIP-nexus captures paused Pol II

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Contents

Description

After designing reporter-ChIP-nexus, the first thing we'd like to check is if paused Pol II can be observed on the reporter. To achieve this, we cloned the Super Core Promoter (SCP) into our reporter, transfected the reporter into *Drosophila melanogaster* Kc167 cells and performed ChIP-nexus.

SCP is a synthetic promoter designed by Kadonaga Lab. This promoter contains some of the well known core promoter elements including TATA, Inr, DPE, MTE and PB. This analysis will plot Pol II profile at SCP on the reporter.

Enviroment setup

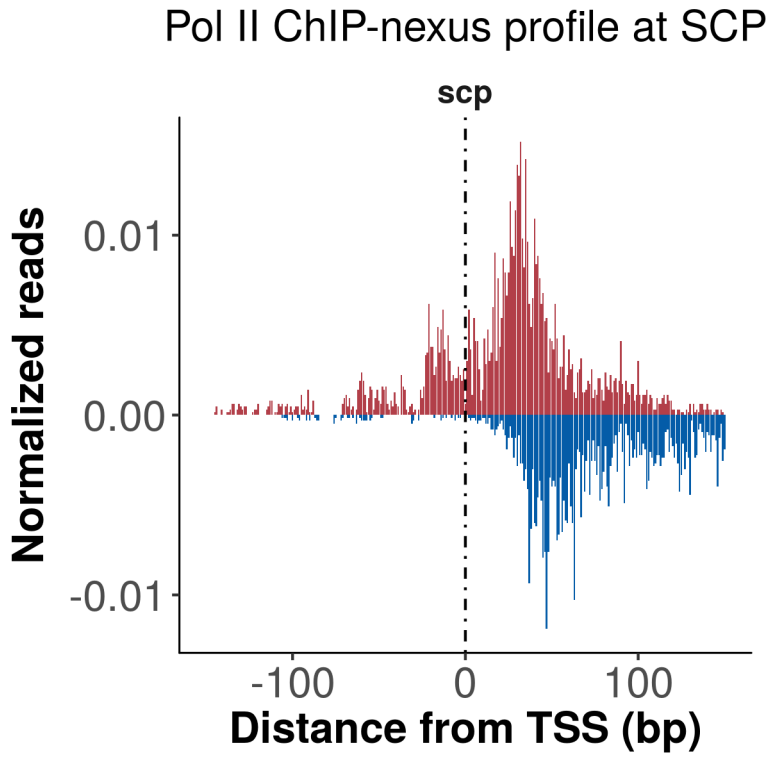
```
library(GenomicRanges, warn.conflicts=F)
library(magrittr)


setwd("/data/analysis_code")
options(knitr.figure_dir =
          "Figure1_reporter_chip_nexus_captures_paused_polii"
        )

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

Pol II ChIP-nexus profile at SCP

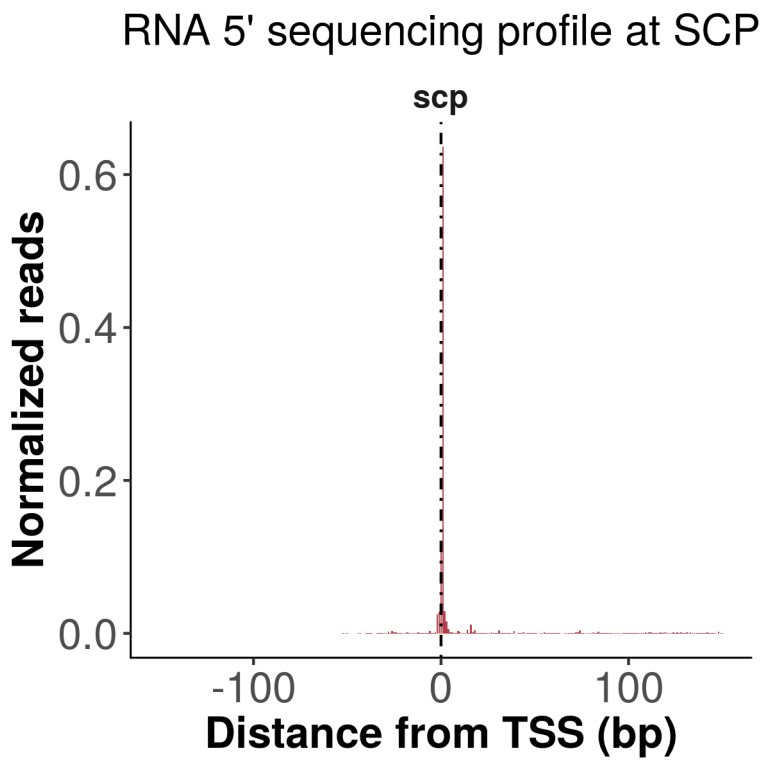
```
plasmid_annotations <- import("./plasmid_annotation.bed")

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$reads <- metapeak$reads / sum(abs(metapeak$reads))
      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$reads <- metapeak$reads / sum(abs(metapeak$reads))
      metapeak

    }
}


scp_metapeak <- get_exo_metapeak("reporter_scp_rpb3_chipnexus",
                                 upstream=150, downstream = 151)


plot_exo_single_gene <- function(metapeak, name, ncol = 1, scale = "free"){
  metapeak.p <- subset(metapeak, strand == "+")
  metapeak.n <- subset(metapeak, strand == "-")

  x <- ggplot(metapeak.p, aes(x=tss_distance, y=reads)) +
       geom_bar(fill="#B23F49", stat="identity") +
       geom_bar(data=metapeak.n, aes(x=tss_distance, y=reads),
                fill="#045CA8", stat="identity") +
```

```
        ggtitle(name)+
        xlab("Distance from TSS (bp)") +
        ylab("Normalized reads") +
        geom_vline(xintercept=0, linetype=4)+
        facet_wrap(facets = "sample_name", ncol =ncol, scale = scale)

    print(x)
}


nothing <- plot_exo_single_gene(scp_metapeak, "Pol II ChIP-nexus profile at SCP")
```



RNA 5' seq profile at SCP

```
scp_rna_metapeak <- get_exo_metapeak("reporter_scp_rna_5_sequencing",
                                     upstream=150, downstream = 151)

nothing <- plot_exo_single_gene(scp_rna_metapeak, "RNA 5' sequencing profile at SCP")
```



Session Info

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] lattice_0.20-35      reshape2_1.4.3       rtracklayer_1.38.3
```

```
##  [4] ggplot2_2.2.1        pander_0.6.1         magrittr_1.5
##  [7] GenomicRanges_1.30.3 GenomeInfoDb_1.14.0  IRanges_2.12.0
## [10] S4Vectors_0.16.0     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               plyr_1.8.4
##  [5] XVector_0.18.0             bitops_1.0-6
##  [7] tools_3.4.4                zlibbioc_1.24.0
##  [9] digest_0.6.15              evaluate_0.10.1
## [11] tibble_1.4.2               gtable_0.2.0
## [13] rlang_0.2.1                Matrix_1.2-14
## [15] DelayedArray_0.4.1         yaml_2.1.19
## [17] GenomeInfoDbData_1.0.0     stringr_1.3.1
## [19] knitr_1.20                 Biostrings_2.46.0
## [21] rprojroot_1.3-2            grid_3.4.4
## [23] Biobase_2.38.0             XML_3.98-1.11
## [25] BiocParallel_1.12.0        rmarkdown_1.10
## [27] matrixStats_0.53.1         backports_1.1.2
## [29] scales_0.5.0               Rsamtools_1.30.0
## [31] htmltools_0.3.6            GenomicAlignments_1.14.2
## [33] SummarizedExperiment_1.8.1 colorspace_1.3-2
## [35] labeling_0.3               stringi_1.2.3
## [37] RCurl_1.95-4.10            lazyeval_0.2.1
## [39] munsell_0.5.0
```