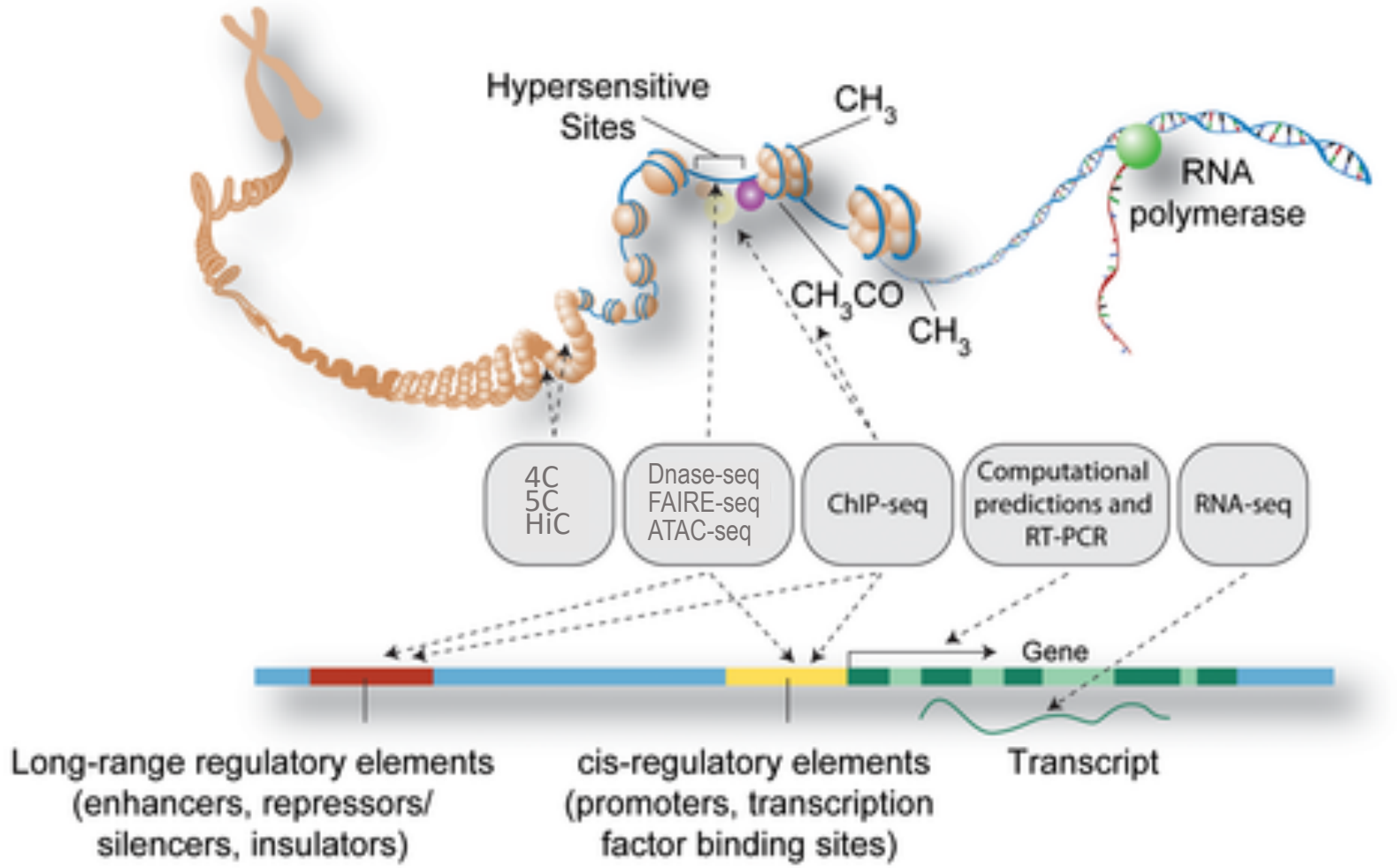
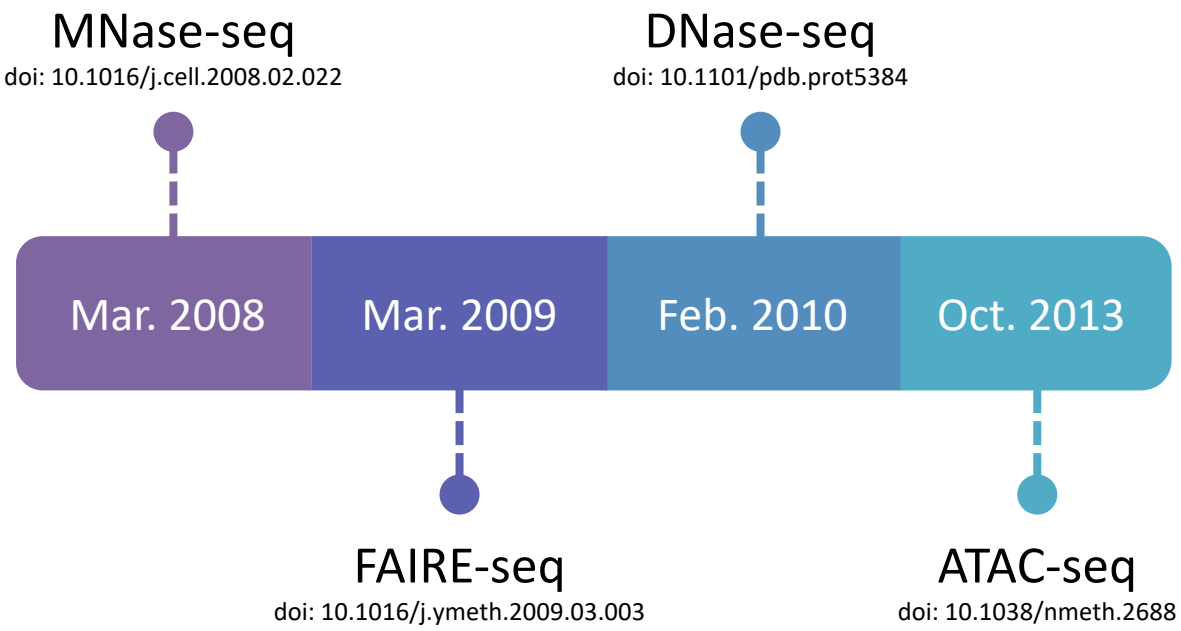
Jianhong Ou
Haibo Liu
Julie Zhu

Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

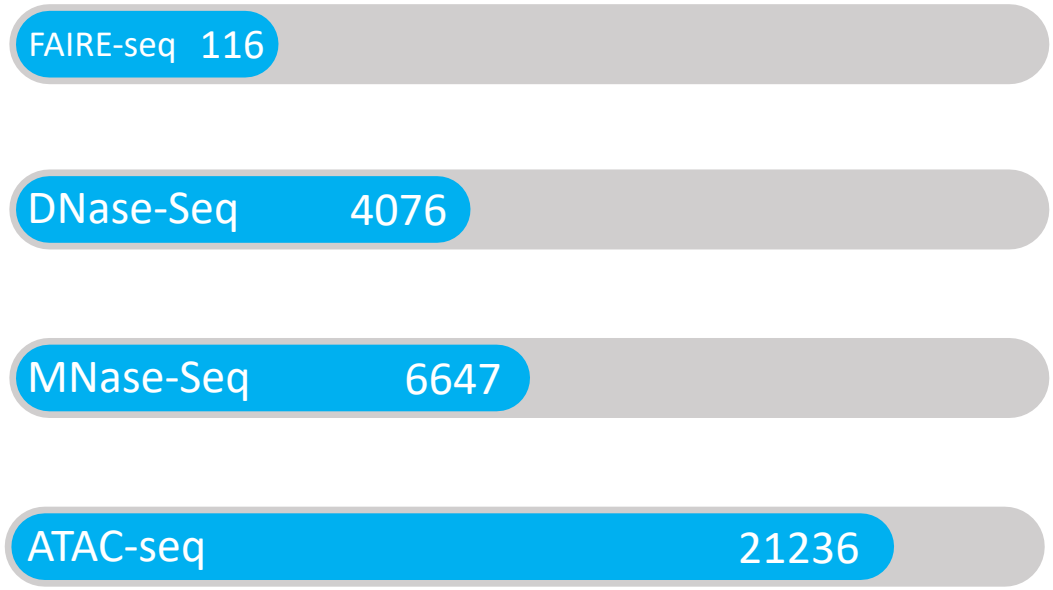


The ENCODE Project Consortium (2011) A User's Guide to the Encyclopedia of DNA Elements (ENCODE). PLOS Biology 9(4): e1001046. https://doi.org/10.1371/journal.pbio.1001046
https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001046

Publish time



The ATAC-seq datasets submitted to GEO



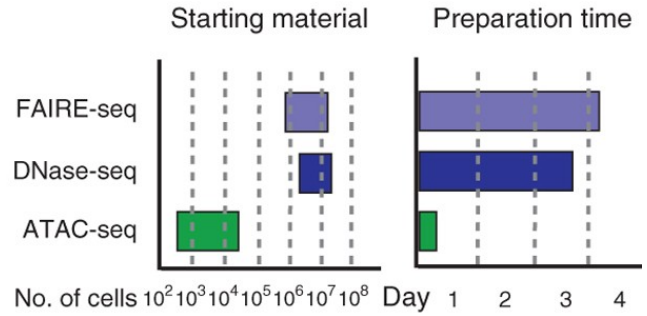

```
library(SRAdb)
sqlfile <- getSRAdbFile()
sra_con <- dbConnect(SQLite(),sqlfile)
(sra_tables <- dbListTables(sra_con))
dbListFields(sra_con,"sra")
library_strategy <-
  dbGetQuery(sra_con,
             'SELECT library_strategy,
             COUNT(*) AS count
             FROM sra
             GROUP BY library_strategy')
subs <- library_strategy[
  library_strategy$library_strategy %in%
    c("ATAC-seq", "FAIRE-seq",
      "MNase-Seq", "DNase-Hypersensitivity"), ]
barplot(subs$count)
```

Methods for profiling

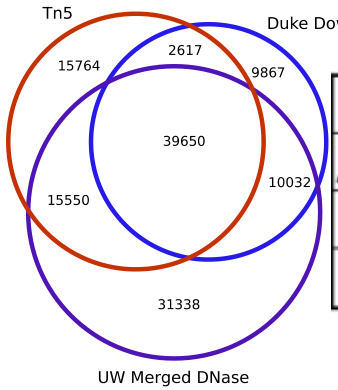
CHROMATIN ACCESSIBILITY

Why ATAC-seq

- rapid and sensitive

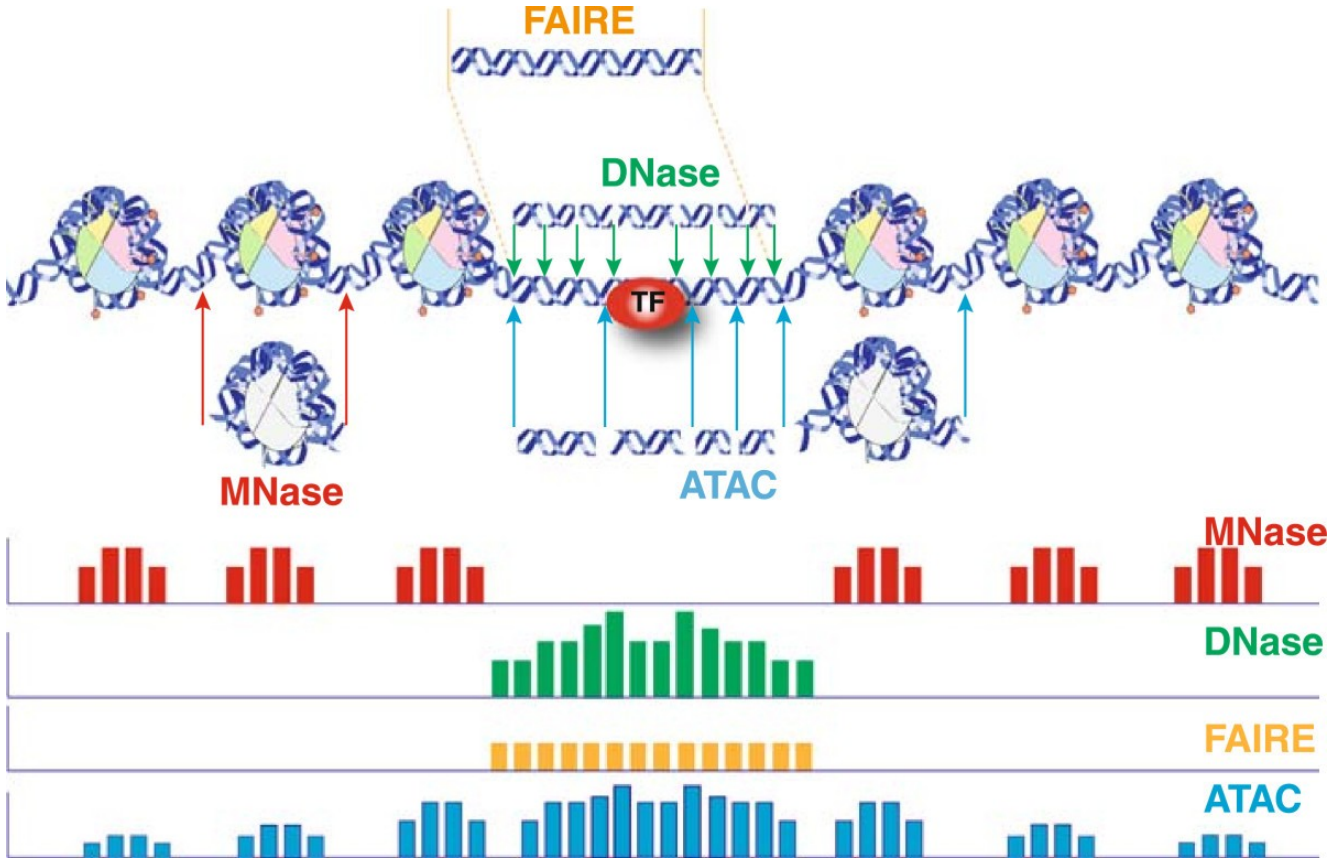


- comprehensive profile

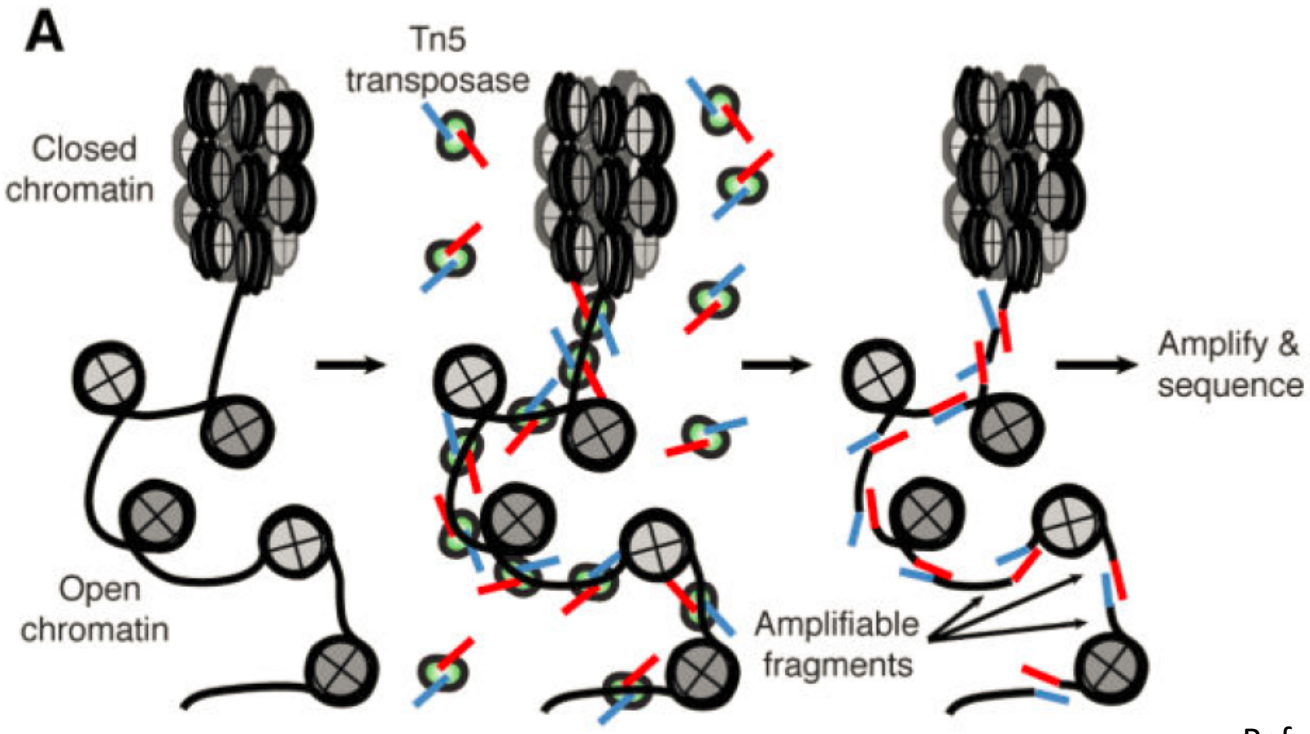


	Intersect
ATAC-Seq	73.80%
UW DNase	77.62%
Duke DNase	65.46%

Ref: Buenrostro et.al., 2013. doi: 10.1038/nmeth.2688



Ref: Tsompana and Buck. 2014. doi: 10.1186/1756-8935-7-33



Ref: Buenrostro et.al., 2015. doi: 10.1002/0471142727.mb2129s109

ATAC-SEQ IS A SIMPLE TWO-STEP PROTOCOL

1. Insertion of Tn5 transposase with adaptors

2. PCR amplification

Question to be answered in QC

Q: Does the sequencing succeed?

S: fastQC

K: sequencing score (or mapping score)

library complexity (duplicates rate)

Q: Does the library provide information of open chromatin, TF and nucleosome occupancy?

S: ATACseqQC

K: periodicity fragment size distribution

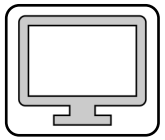
TSSs enrichment for nucleosome-free fragments

detectability of genome-wide TF occupancy

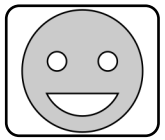
Why ATACseqQC

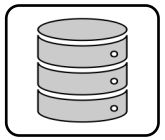

Well documented


Well maintained


cross platform


Bioconductor support site


Easy to use


Provide files for downstream analysis

Access & Citations

10k	16	17
Article Accesses	Web of Science	CrossRef

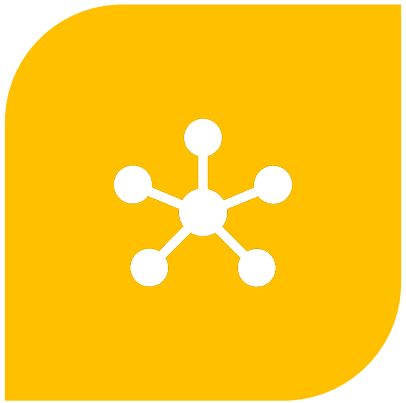
Online attention

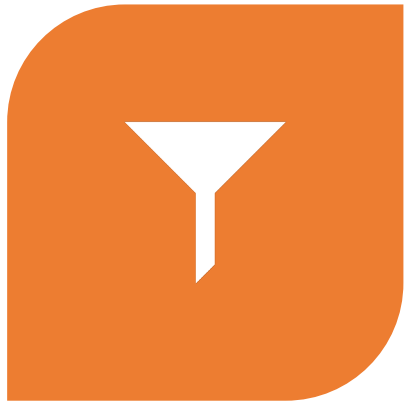
41 tweeters
1 Google+ users
131 Mendeley

This article is in the 88th percentile (ranked 31,409th) of the 275,422 tracked articles of a similar age in all journals and the 91st percentile (ranked 1st) of the 12 tracked articles of a similar age in *BMC Genomics*

Prepare sample data set

Sample data is downloaded from https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1155959





TRIM → ALIGN → FILTER

Ref: Buenrostro et.al., 2013. doi: 10.1038/nmeth.2688

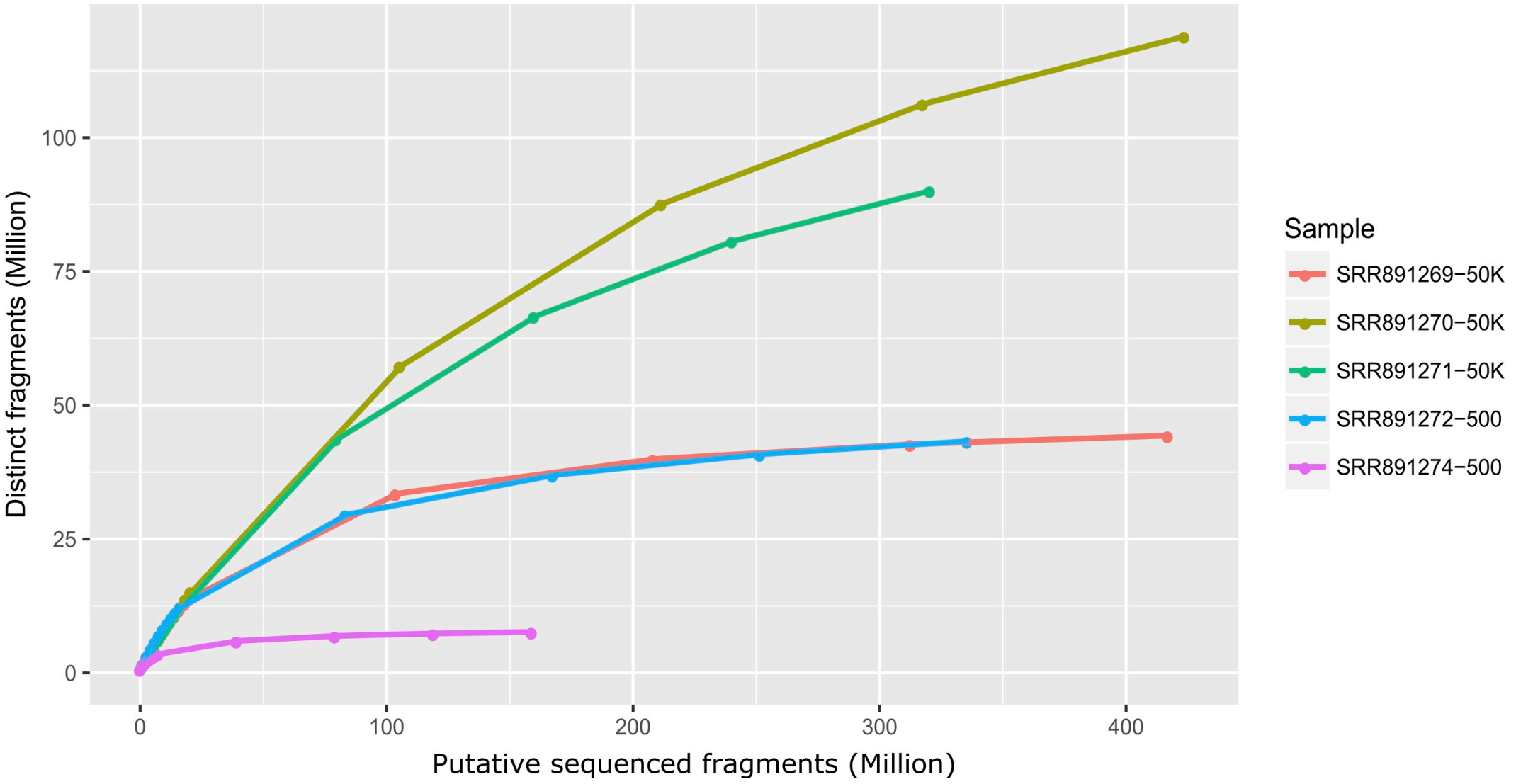
Prepare for inputs

```
## set the working directory,
## replace "~/Downloads/ATACseqQCworkshop" by your path
wd <- "~/Downloads/ATACseqQCworkshop"
dir.create(wd)
setwd(wd)
library(BiocManager)
install("jianhong/workshop2020")
## input the sample bamFile from the installed packaged
## the data is pre-processed chr1 data of GSM1155959
extfilePath <- system.file("extdata", "ATACseqQC",
                           package="workshop2020", mustWork = TRUE)
dir(extfilePath)## see what we have in extdata/ATACseqQC directory
```

```
## [1] "GL3.chr1.bam"          "GL3.chr1.bam.bai"
## [3] "GL3.chr1.rmdup.bam"  "GL3.chr1.rmdup.bam.bai"
```

https://github.com/jianhong/workshop2020
https://bioconductor.org/packages/ATACseqQC

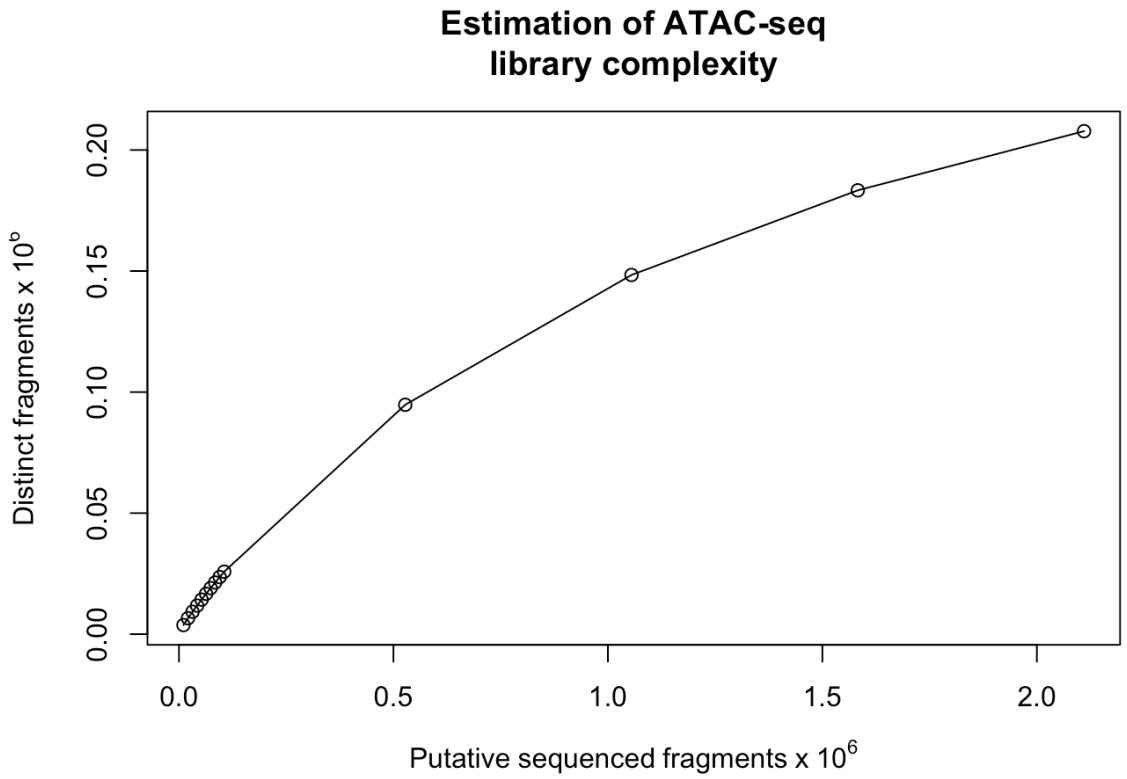
Library complexity

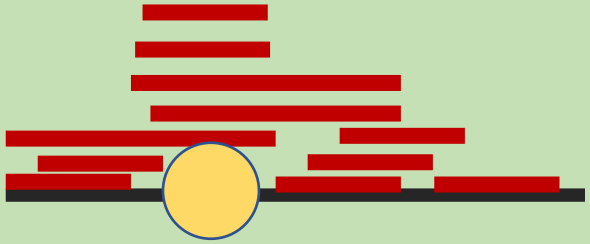


Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

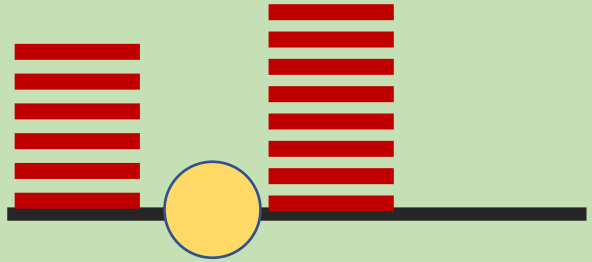
Estimate the library complexity

```
## load the library
library(ATACseqQC)
## mapping status, such as mapping rate,
## duplicate rate, genome-wide distribution,
## mapping quality, and contamination.
## NOTE: requires sorted BAM files with duplicate reads marked as input.
## library complexity
estimateLibComplexity(readsDupFreq(file.path(extfilePath, "GL3.chr1.bam")))
```



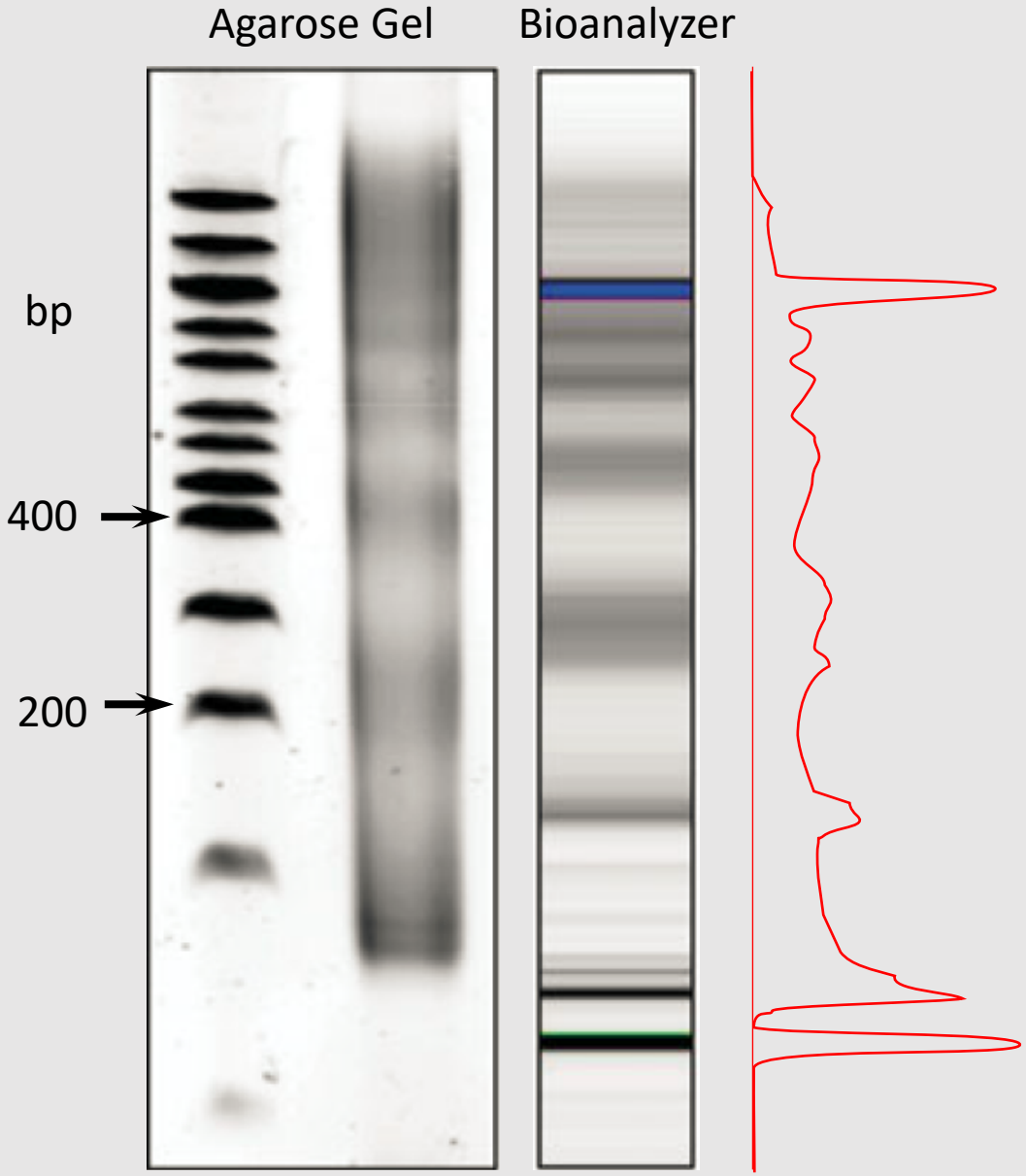


Typical ATAC-seq peak



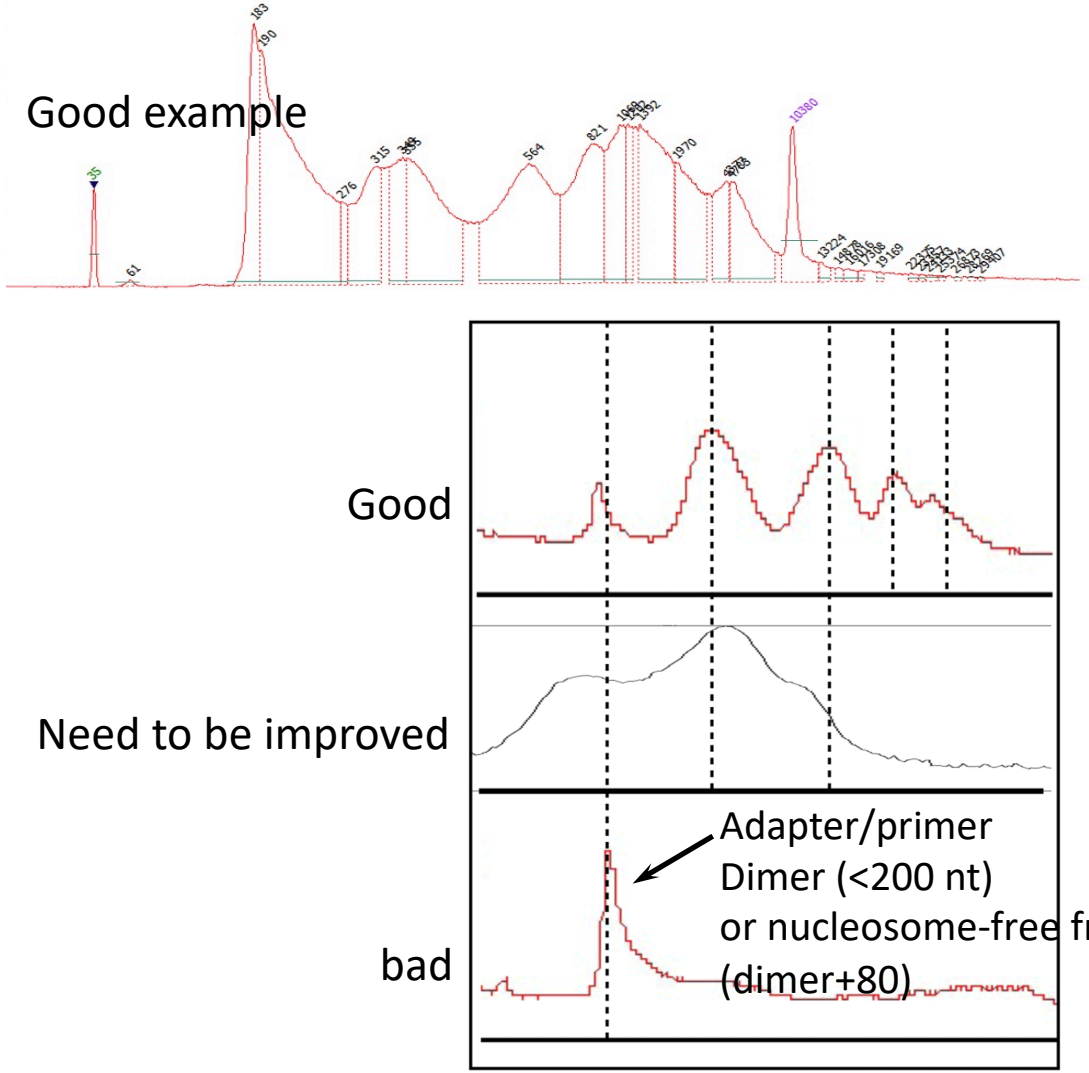
Low-complexity ATAC-seq peak

Library fragment size



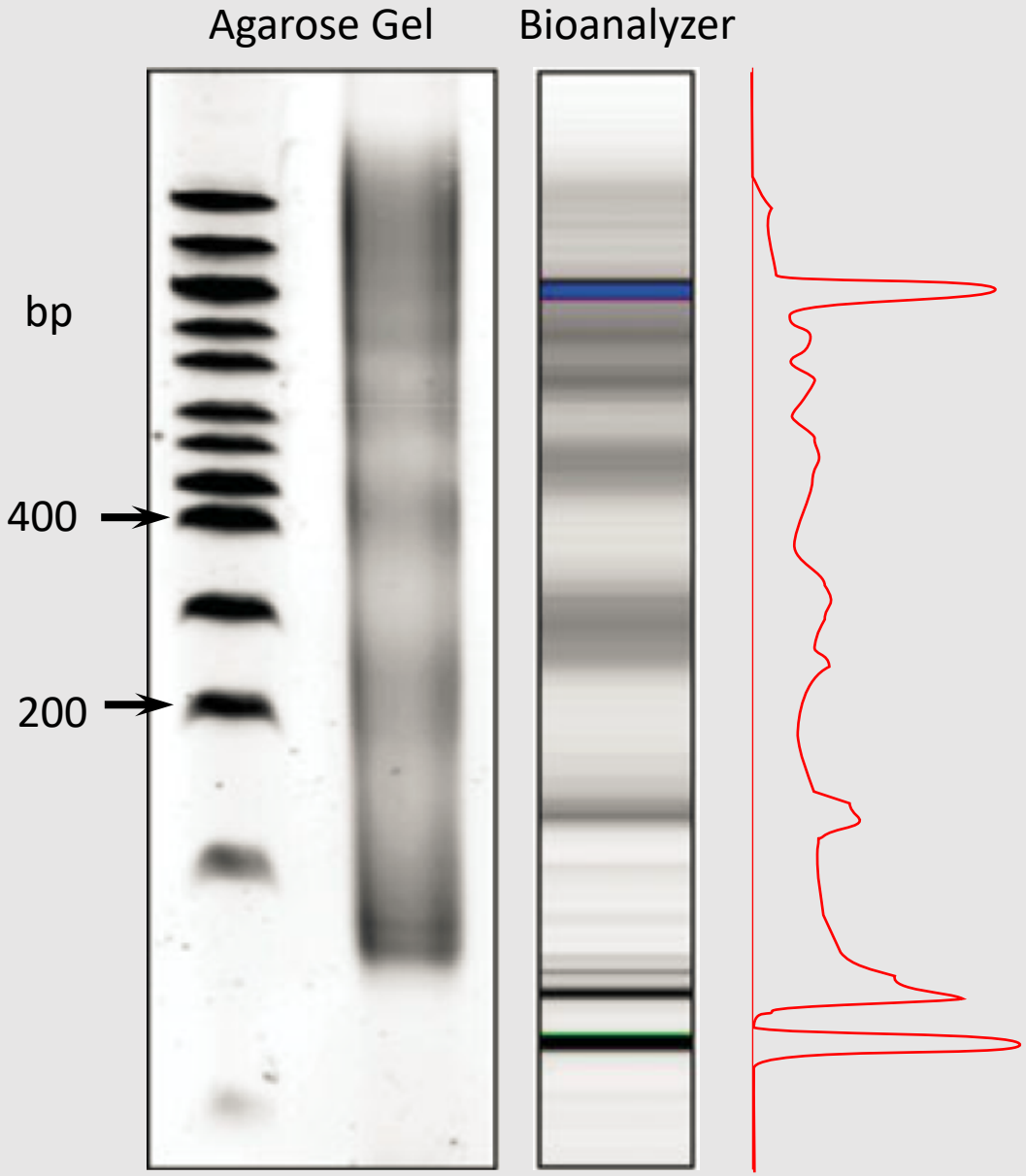
Ref: Buenrostro et.al., 2015. doi: 10.1002/0471142727.mb2129s109

ATAC-SEQ LIBRARY FRAGMENTATION PROFILE



Ref: Augusto et.al., 2020. doi: 10.12688/wellcomeopenres.15552.1

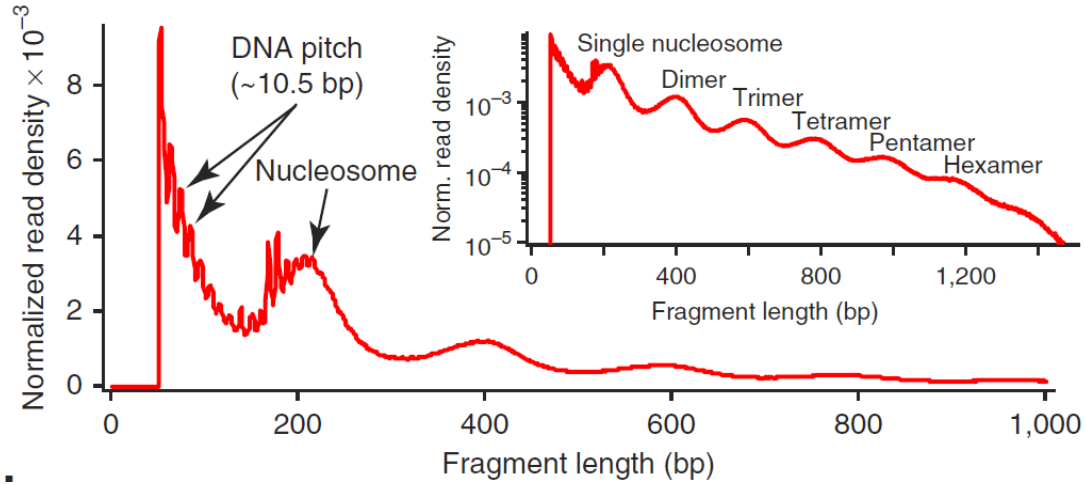
Library fragment size



Ref: Buenrostro et.al., 2015. doi: 10.1002/0471142727.mb2129s109

ATAC-seq library Insertion profile

Library insert size (=size in gel – adapter/primer size)

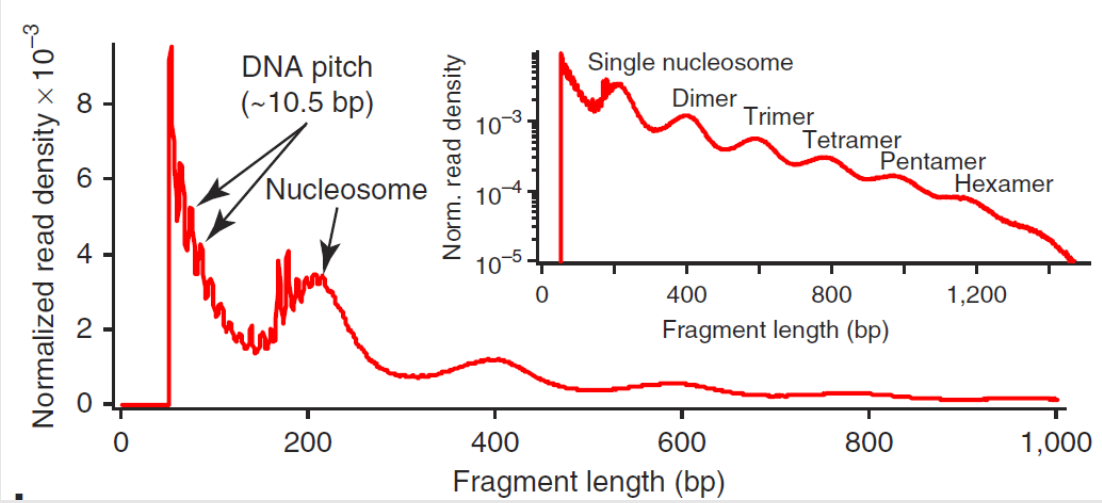


Ref: Buenrostro et.al., 2013. doi: 10.1038/nmeth.2688

Must be Paired End reads

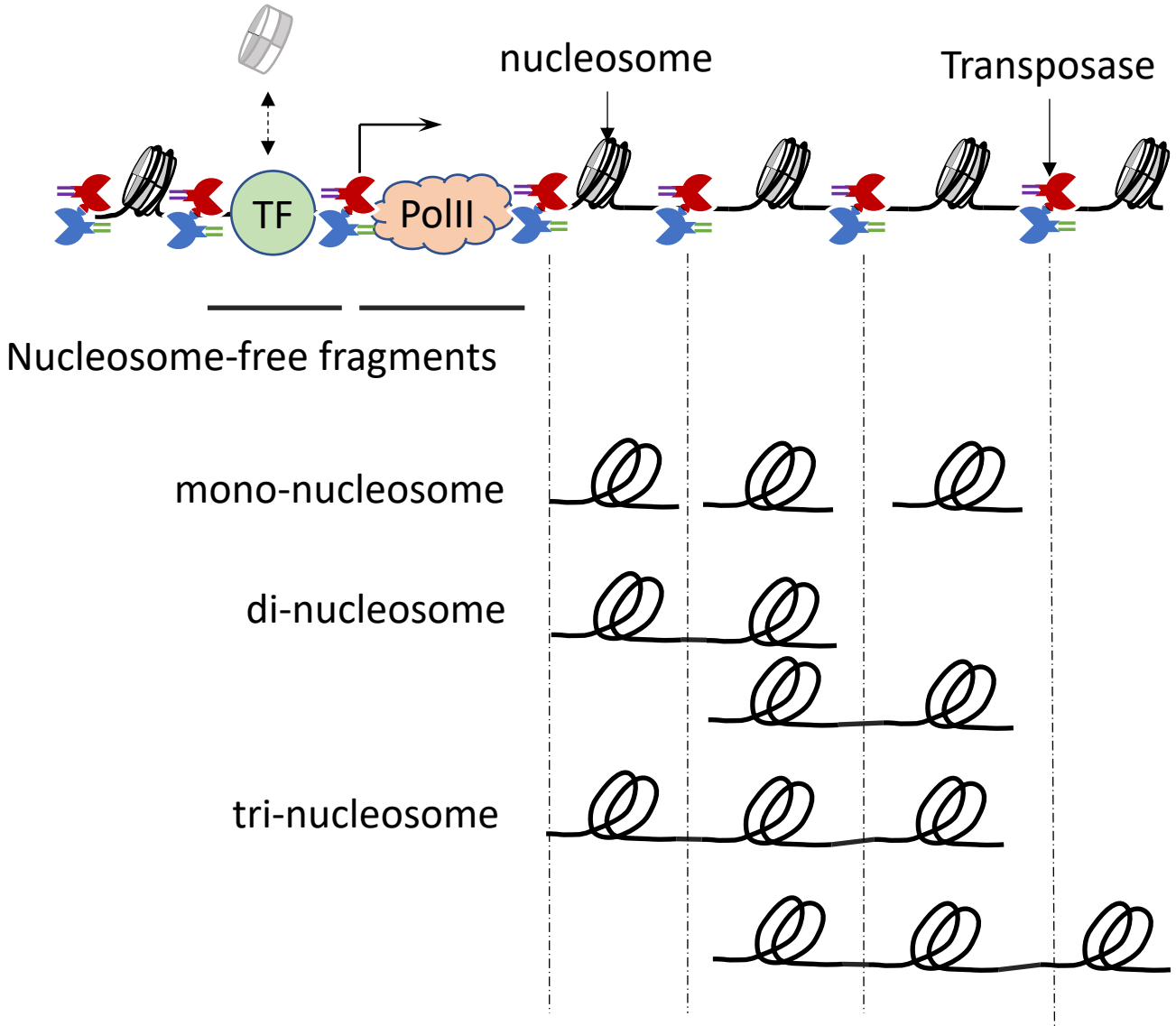
```
fragSizeDist(bamfile, bamfile.labels)
```

Function in ATACseqQC



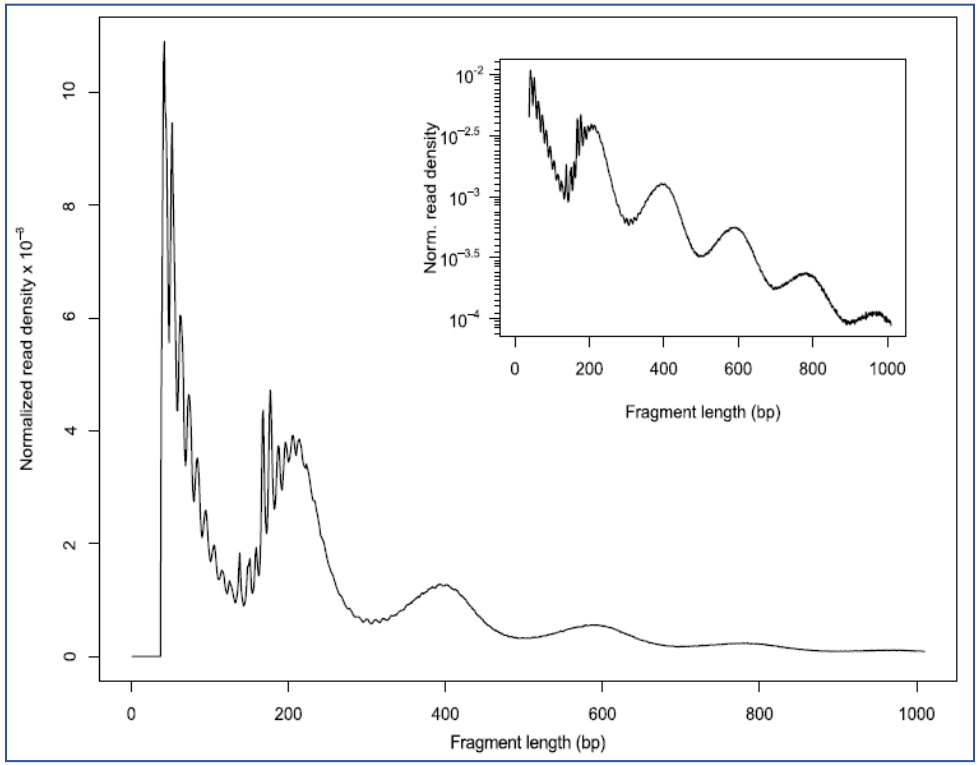
Ref: Buenrostro et.al., 2015. doi: 10.1002/0471142727.mb2129s109

ATAC-SEQ LIBRARY FRAGMENTATION PROFILE

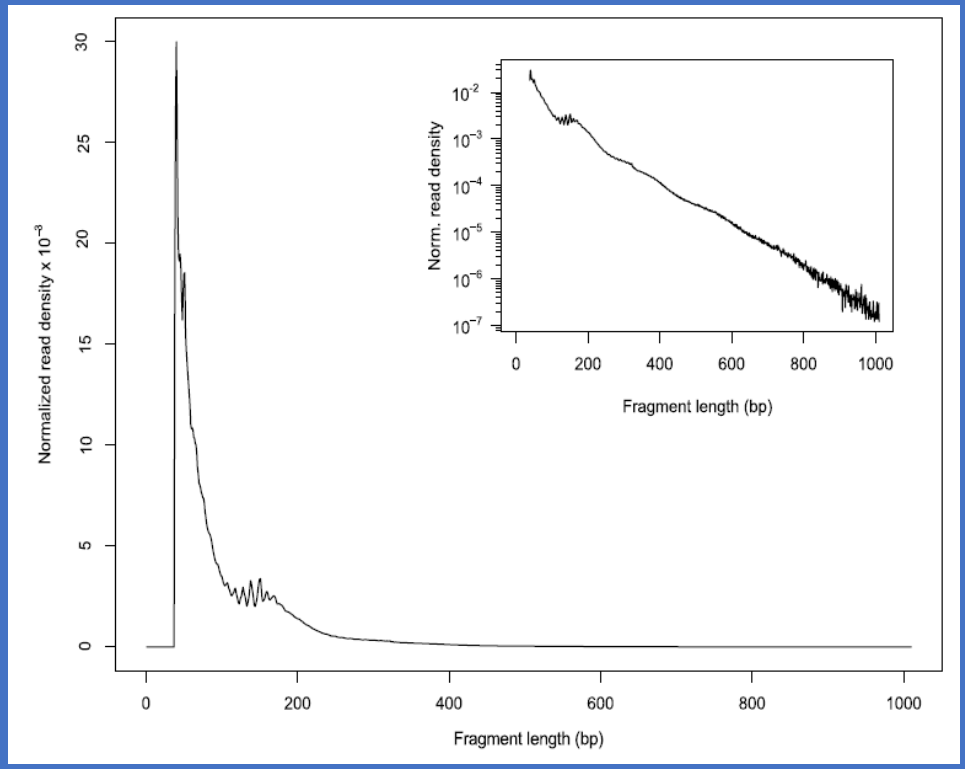


Assessing insert size distribution

A (High quality)



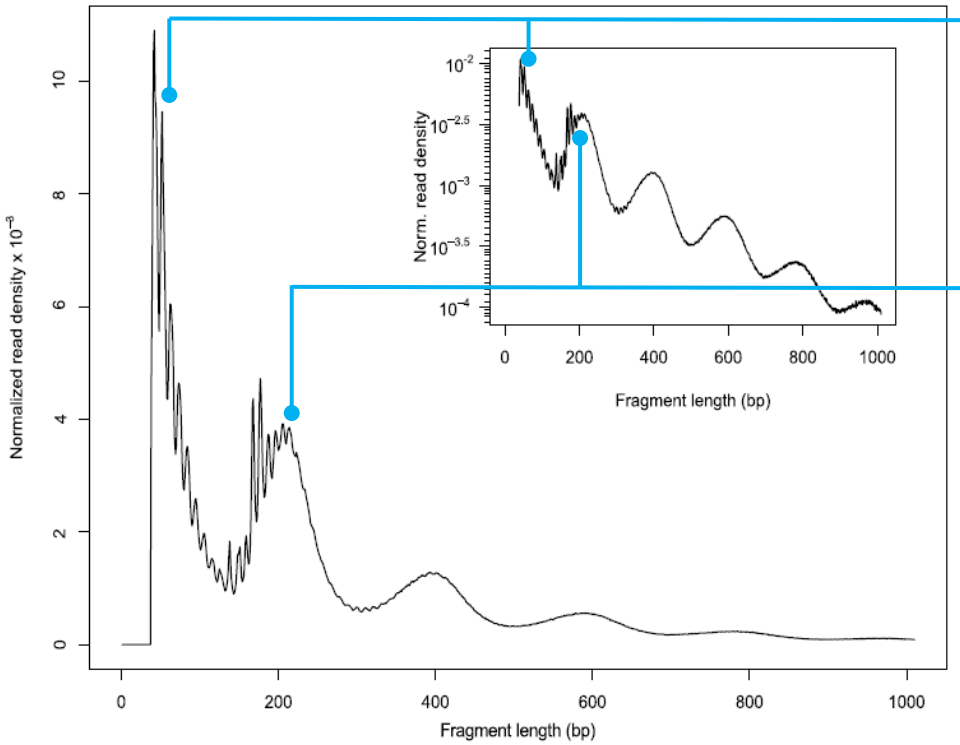
B (Poor quality)



Ref: Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

Minimal criteria
for insert size distribution

You **must see** in the distribution of insert size:

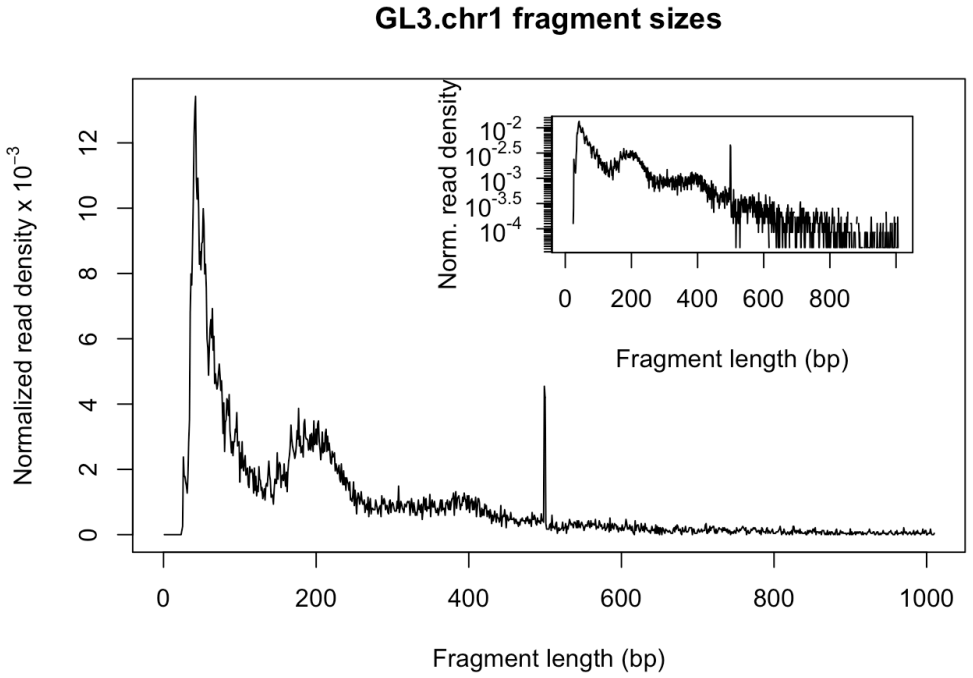


- A **nucleosome-free region (NFR)** peak
- A **mono-nucleosome** region peak (147~294 bp).

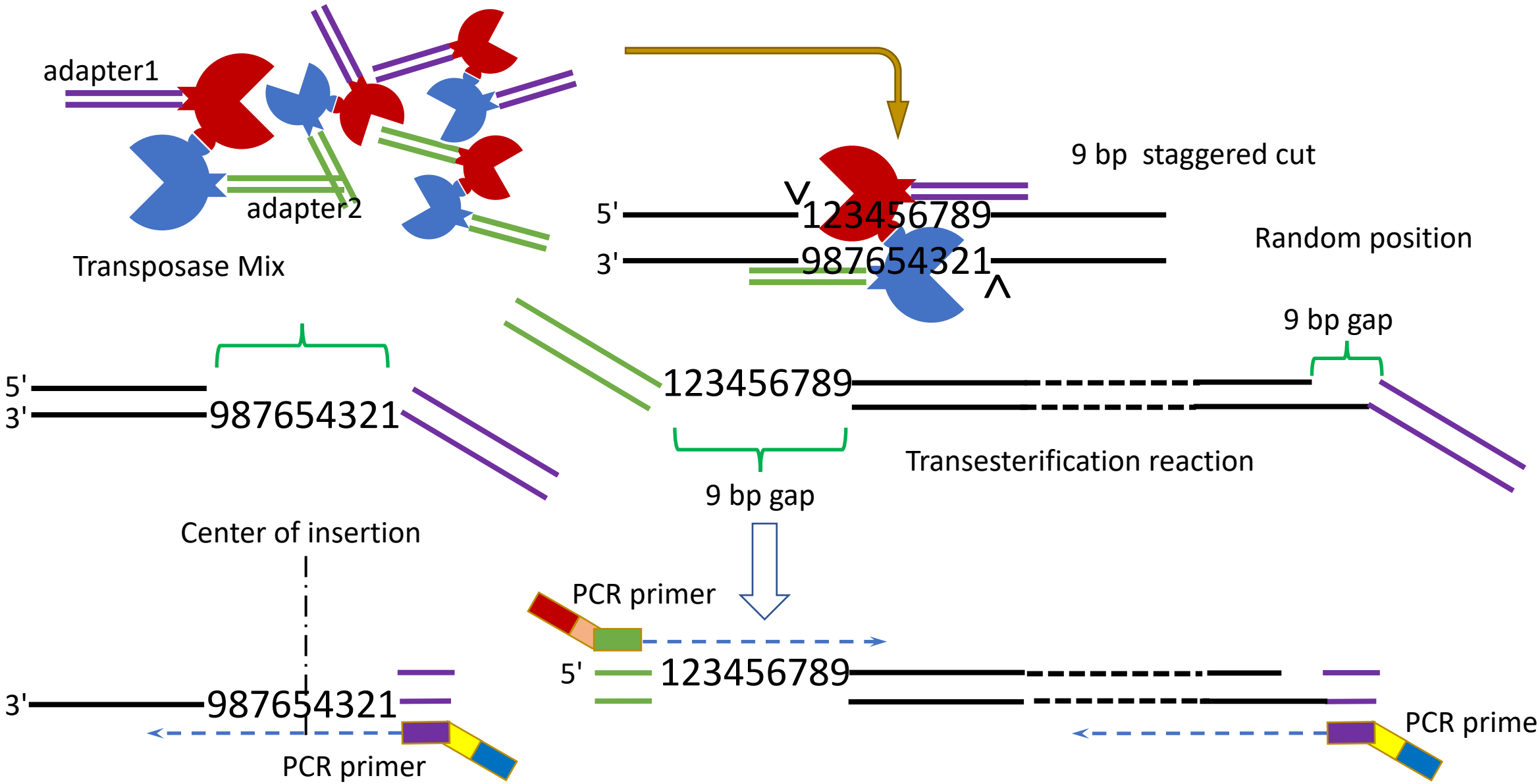
Assessing insert size distribution

First, there should be a large proportion of reads with less than 100 bp, which represents the nucleosome-free region. Second, the fragment size distribution should have a clear periodicity, which is evident in the inset figure, indicative of nucleosome occupancy (present in integer multiples).

```
## set bam file name, replace file.path(extfilePath,
"GL3.chr1.rmdup.bam")
## by your bam file name
bamfile <- file.path(extfilePath, "GL3.chr1.rmdup.bam" )
(bamfile.labels <- gsub(".rmdup.bam", "", basename(bamfile)))
## [1] "GL3.chr1"
## generate fragment size distribution
fragSize <- fragSizeDist(bamfile, bamfile.labels)
```



SHIFTING ALIGNED READS



Shifting aligned reads

```
## bamfile tags to be read in
possibleTag <- combn(LETTERS, 2)
possibleTag <- c(paste0(possibleTag[1, ], possibleTag[2, ]),
                 paste0(possibleTag[2, ], possibleTag[1, ]))
library(Rsamtools)
bamTop100 <- scanBam(BamFile(bamfile, yieldSize = 100), ## use top 100 reads to test possible tags
                     param = ScanBamParam(tag=possibleTag))[[1]]$tag
tags <- names(bamTop100)[lengths(bamTop100)==100]
tags
## [1] "AS" "MD" "PG" "XG" "NM" "XM" "XN" "XO" "XS" "YS" "YT”
## files will be output into outPath
outPath <- "splited"
dir.create(outPath)
## shift the coordinates of 5'ends of alignments in the bam file
library(BSgenome.Hsapiens.UCSC.hg38)
seqlev <- "chr1”
## subsample data for quick run
which <- as(seqinfo(Hsapiens)[seqlev], "GRanges")
gal <- readBamFile(bamfile, tag=tags, which=which, asMates=TRUE, bigFile=TRUE)
shiftedBamfile <- file.path(outPath, "shifted.bam")
gal1 <- shiftGAlignmentsList(gal, outbam=shiftedBamfile)
```

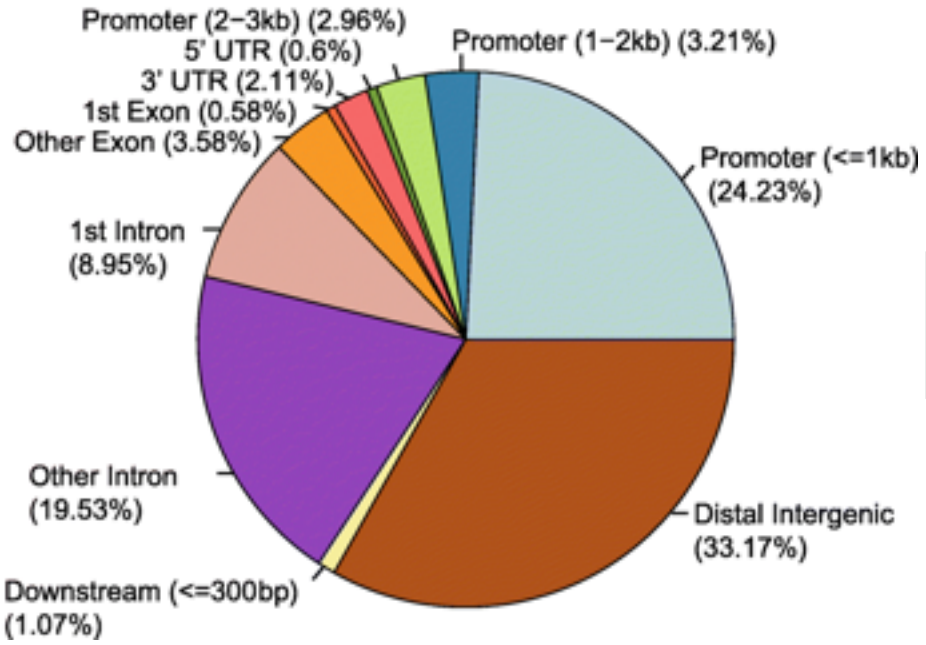
Errors when Shifting aligned reads

```
>  gal1 <- shiftGAlignmentsList(gal, outbam=shiftedBamfile)
[E::sam_parse1] unrecognized type N
[W::sam_read1] Parse error at line 26
```

```
> gal1 <- shiftGAlignmentsList(gal, outbam=shiftedBamfile.outPath)
[bam_translate] PG tag "MarkDuplicates" on read "M01263:122:000000000-CWJNL:1:2106:23406:17450"
encountered with no corresponding entry in header, tag lost. Unknown tags are only reported once per input file
for each tag ID.
[bam_translate] PG tag "MarkDuplicates" on read "M01263:122:000000000-CWJNL:1:2104:12761:8947"
encountered with no corresponding entry in header, tag lost. Unknown tags are only reported once per input file
for each tag ID.
```

```
tags <- tags[tags != "PG"]
```

Enrichment in promoters



Typical peak annotation pie chart

Ref: Yan et.al.,2020. doi: 10.1186/s13059-020-1929-3

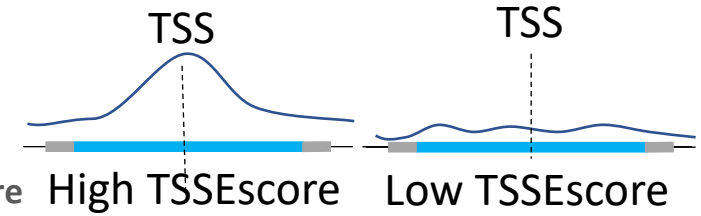
PTscore

Promoter/Transcript body score

PT score is calculated as the coverage of promoter divided by the coverage of its transcript body. PT score will show if the signal is enriched in promoters.

TSSEscore

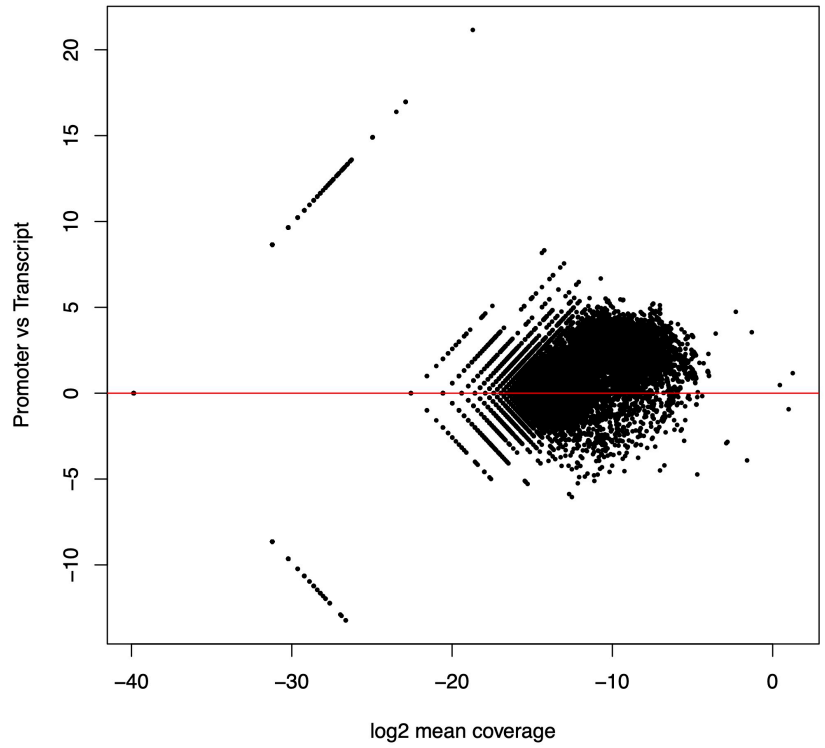
Transcription Start Site (TSS) Enrichment Score



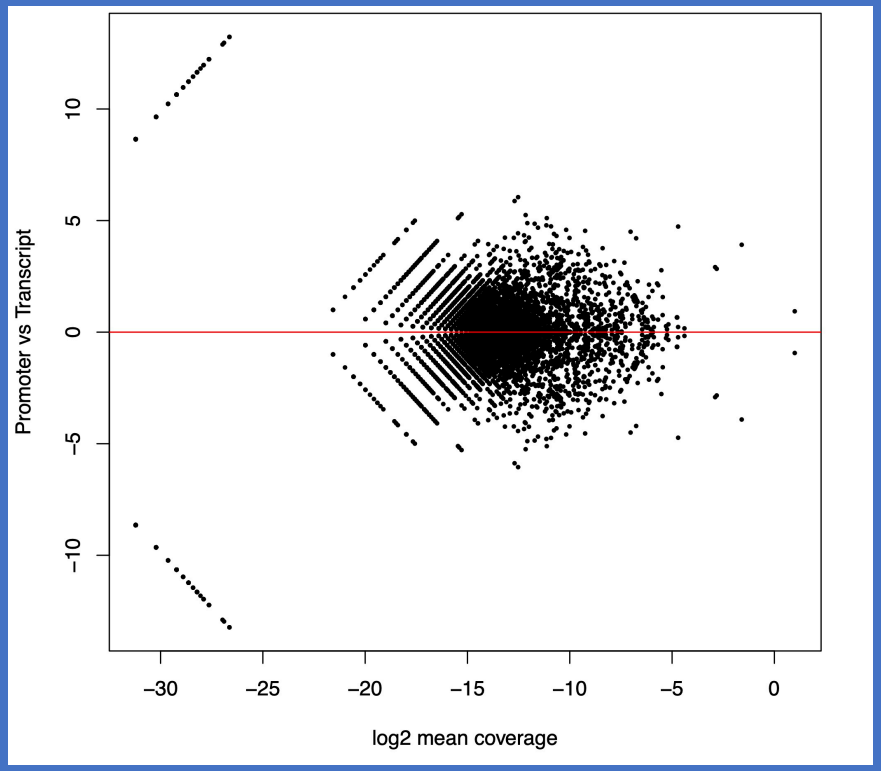
TSS enrichment score is a ratio between aggregate distribution of reads centered on TSSs and that flanking the corresponding TSSs. TSS score = the depth of TSS (1000 bp each side) / the depth of end flanks (100bp each end).

PT SCORE

A (High quality)



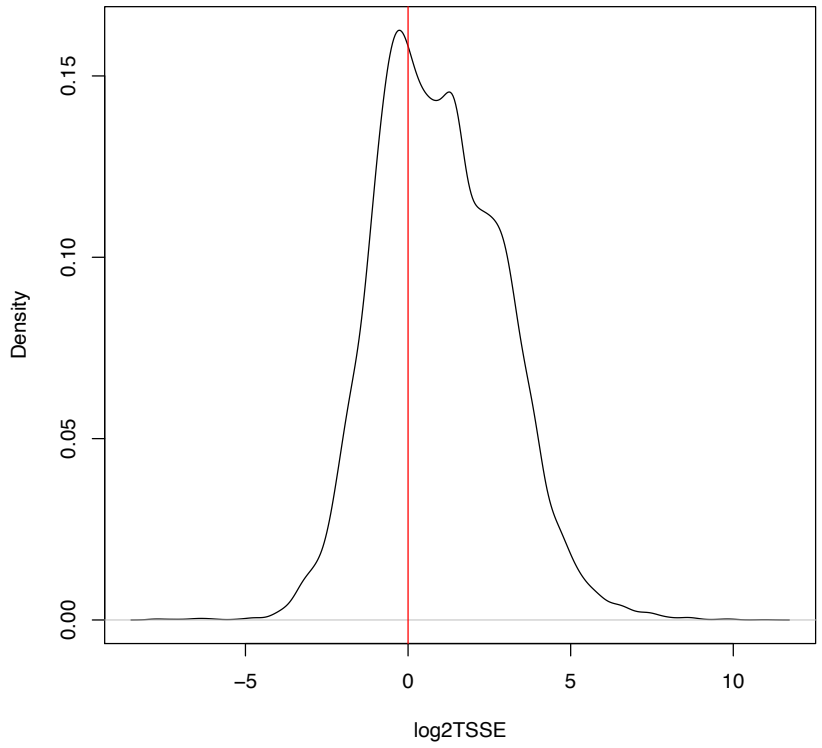
B (Poor quality)



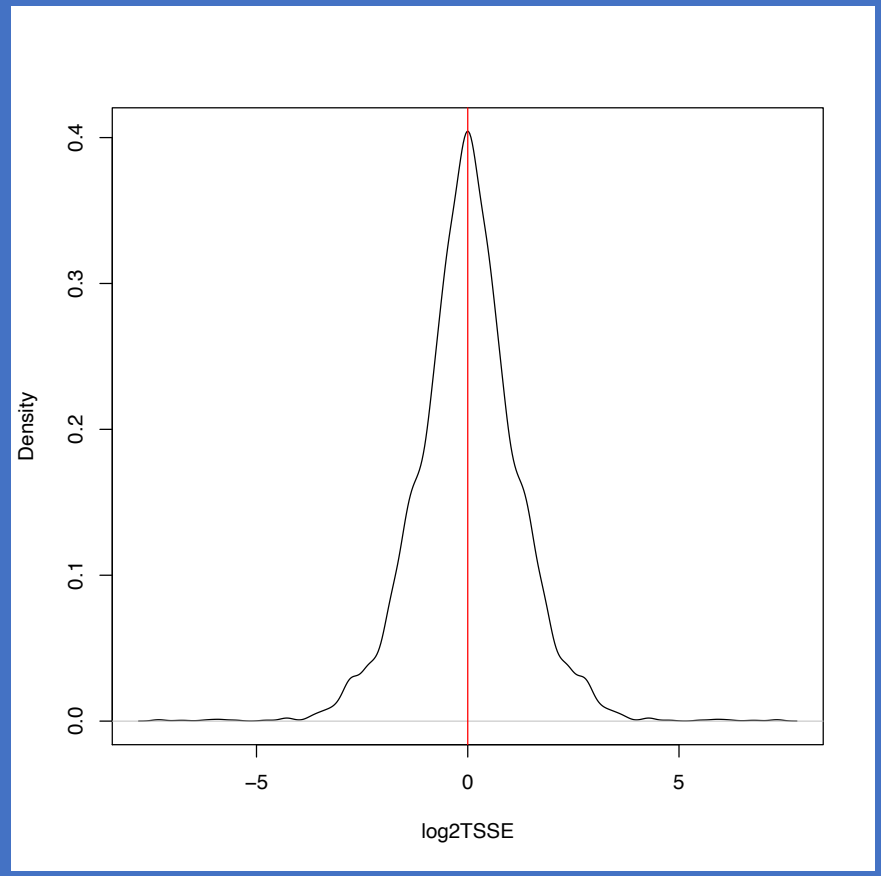
Ref: Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

TSSE SCORE

A (High quality)



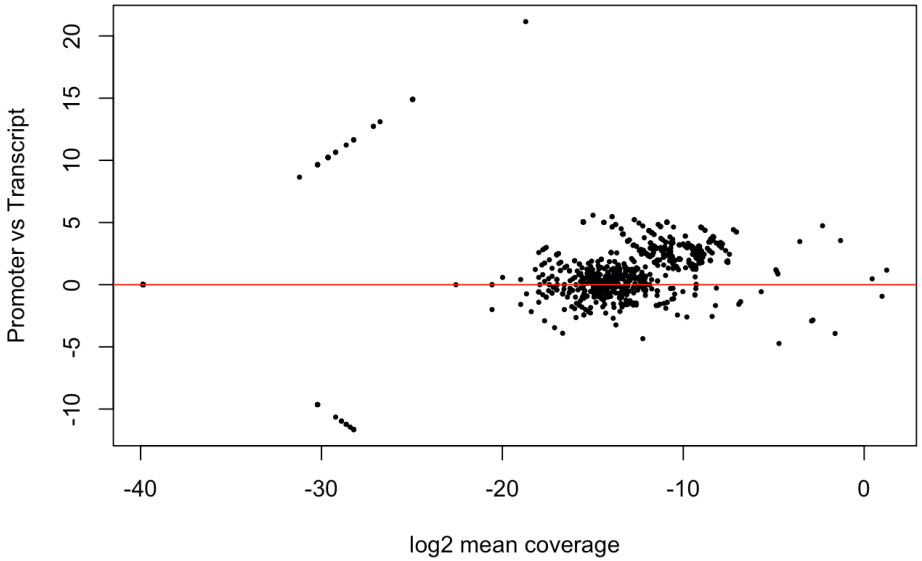
B (Poor quality)



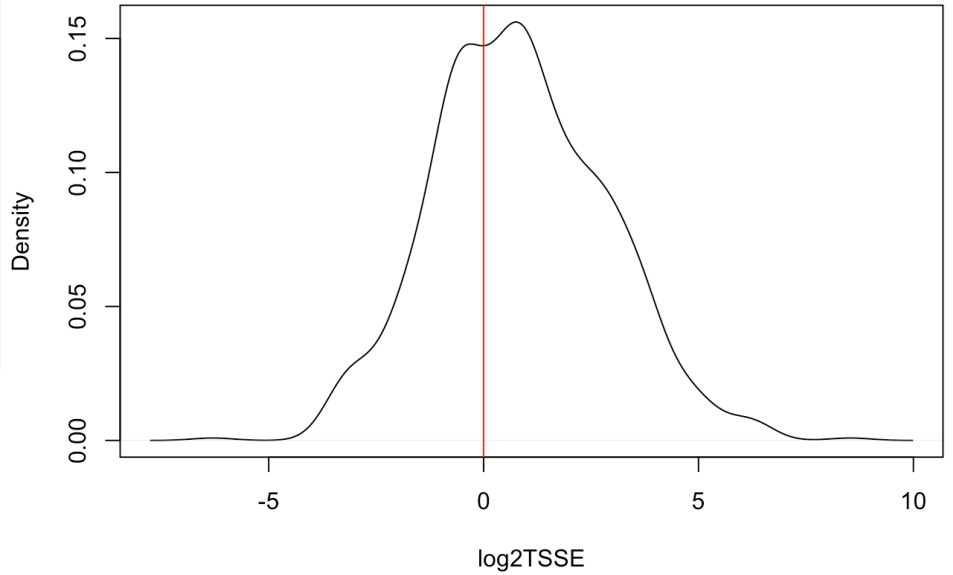
Ref: Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

Enrichment in promoters

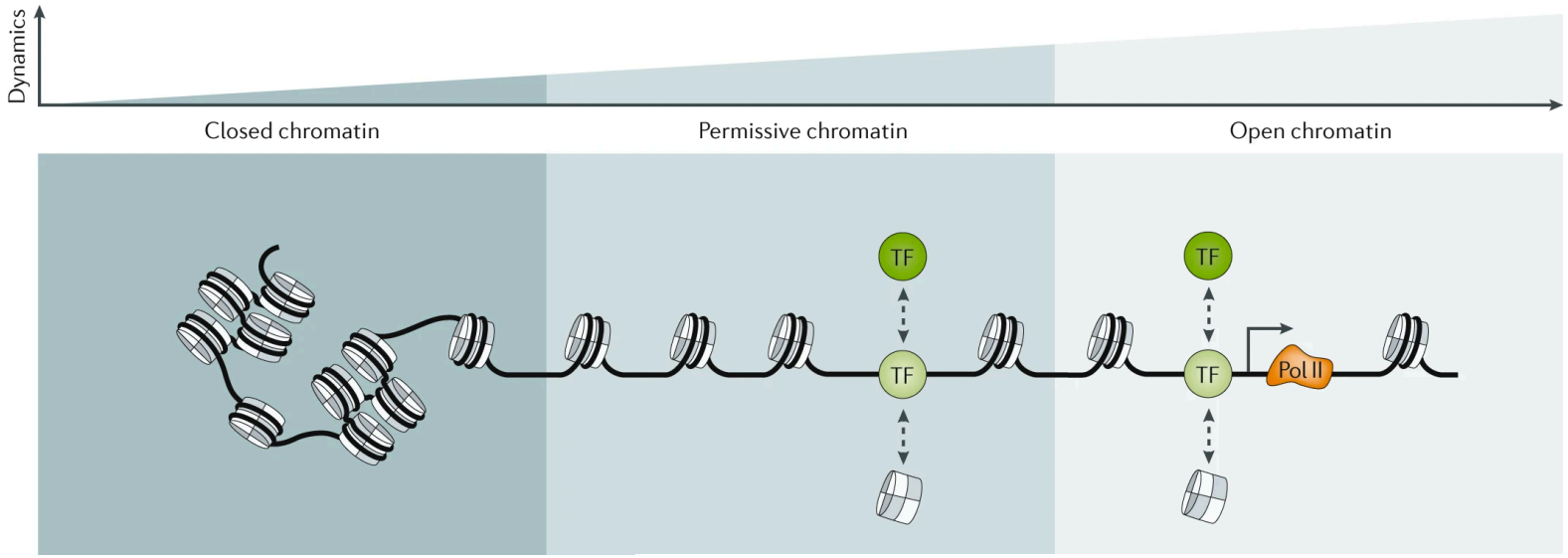
```
library(TxDb.Hsapiens.UCSC.hg38.knownGene)
txs <- transcripts(TxDb.Hsapiens.UCSC.hg38.knownGene)
pt <- PTscore(gal1, txs)
plot(pt$log2meanCoverage, pt$PT_score, pch=16, cex = .5,
      xlab="log2 mean coverage", ylab="Promoter vs Transcript")
abline(h=0, col="red")
```



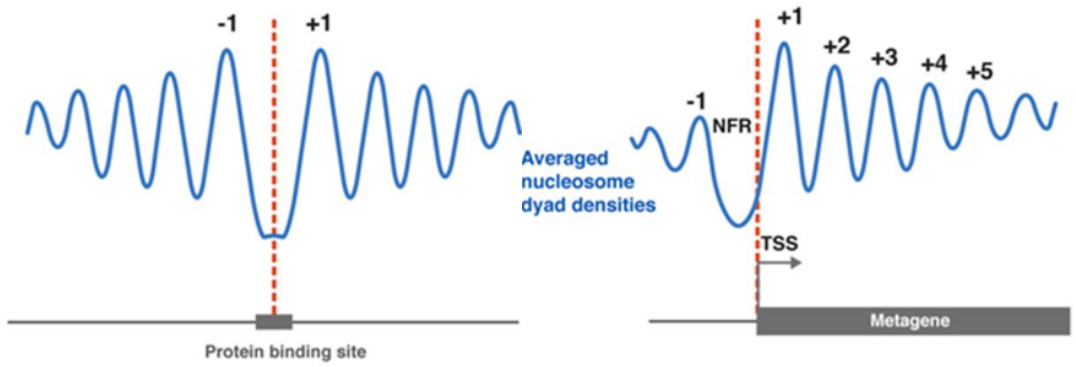

```
tsse <- TSSEscore(gal1, txs)
log2TSSE <- log2(tsse$TSS.enrichment.score)
log2TSSE <- log2TSSE[!is.na(log2TSSE)]
plot(density(log2TSSE), xlab = "log2TSSE", main = "")
abline(v=0, col="red")
```



Nucleosome positioning around TSSs and TF-bound sites



Ref: Klemm et.al., 2019. doi: 10.1038/s41576-018-0089-8

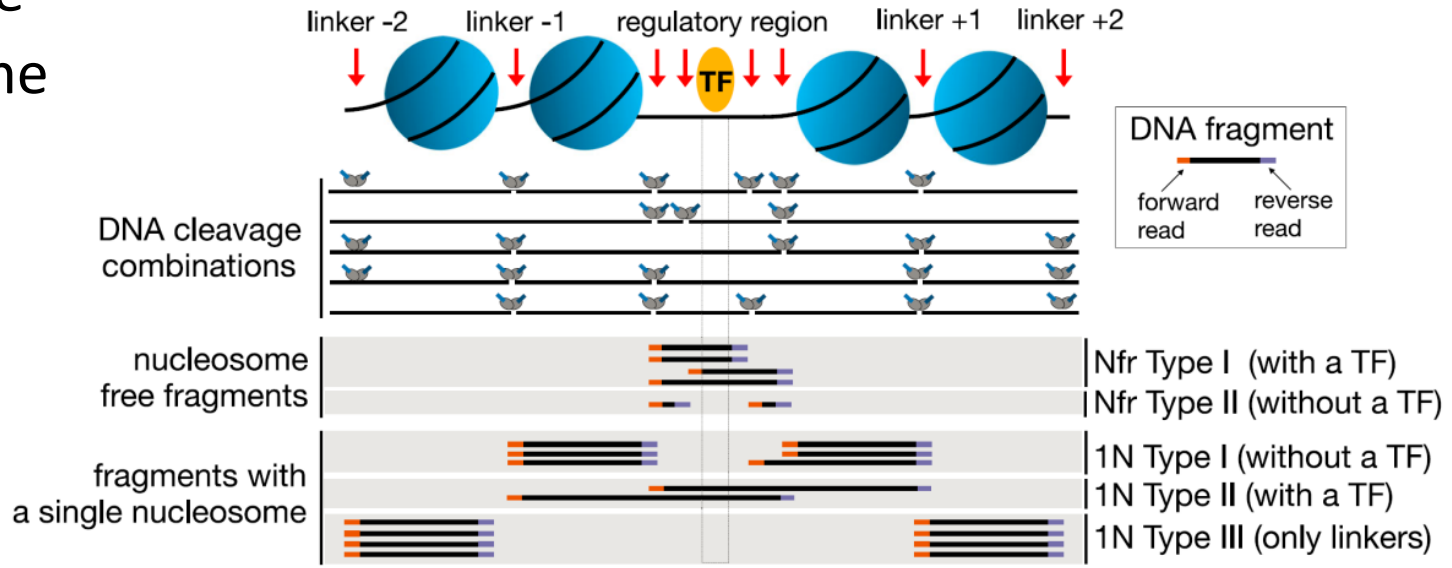


Ref: Baldi. 2018 doi: 10.1042/EBC20180058

Splitting BAM files

split aligned reads into different bins:

- Nucleosome-free
- Mononucleosome
- Dinucleosome
- Trinucleosome



Li et al. 2019. doi: 10.1186/s13059-019-1642-2

```
objs <- splitGAlignmentsByCut(gal1, txs=txs,
                              genome=genome, outPath = outPath)
```

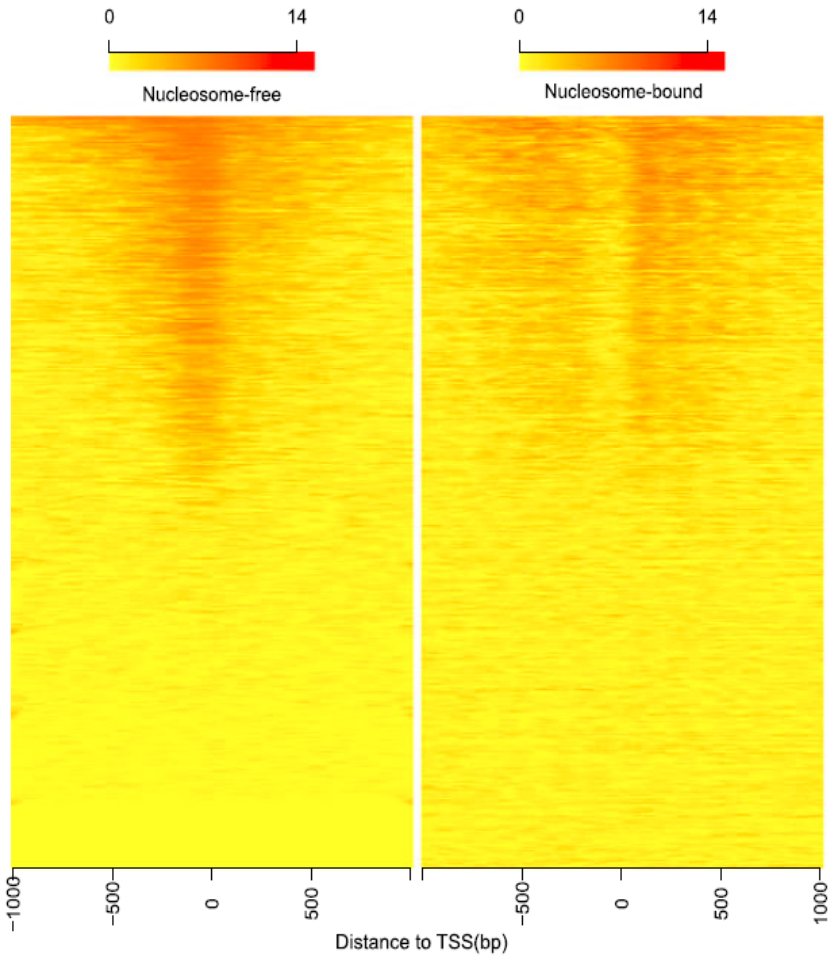
Split reads

```
## run program for chromosome 1 only
txs <- txs[seqnames(txs) %in% "chr1"]
genome <- Hsapiens
## split the reads into NucleosomeFree, mononucleosome,
## dinucleosome and trinucleosome.
## and save the binned alignments into bam files.
objs <- splitGAlignmentsByCut(gal1, txs=txs, genome=genome, outPath = outPath)
## list the files generated by splitGAlignmentsByCut.
dir(outPath)
```

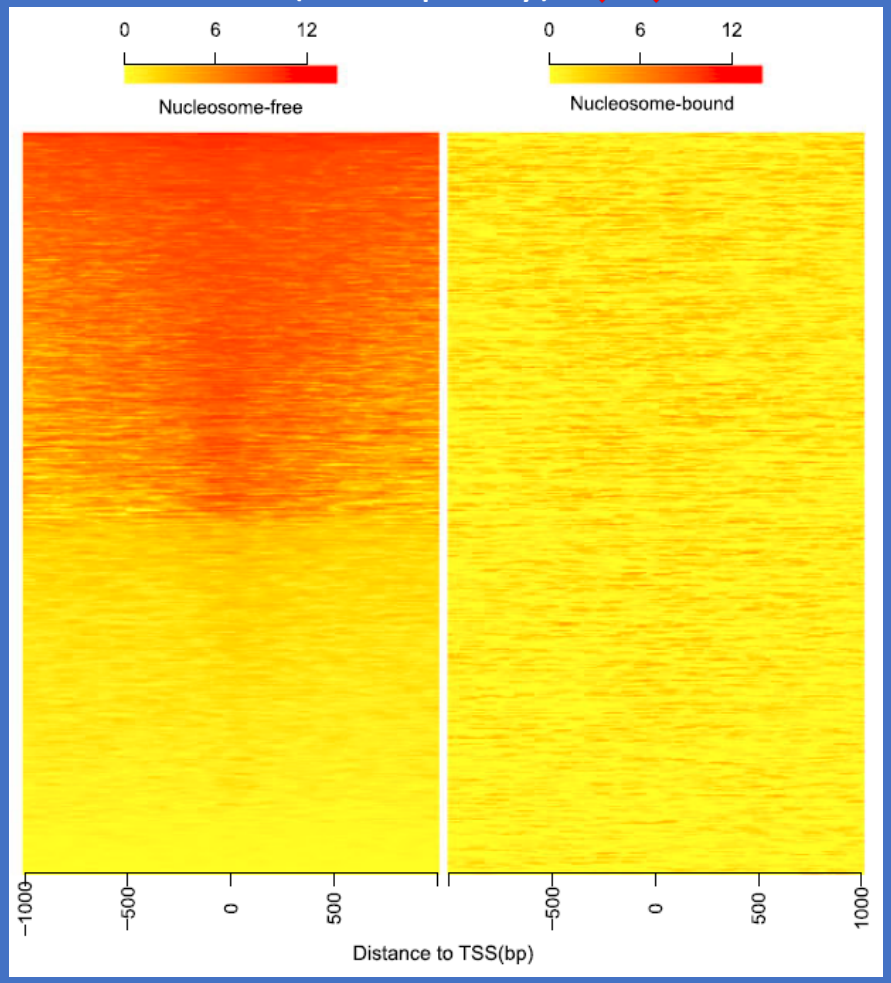
```
## [1] "dinucleosome.bam"                "dinucleosome.bam.bai"    "inter1.bam"
## [4] "inter1.bam.bai"                  "inter2.bam"              "inter2.bam.bai"
## [7] "inter3.bam"                      "inter3.bam.bai"          "mononucleosome.bam"
## [10] "mononucleosome.bam.bai"         "NucleosomeFree.bam"      "NucleosomeFree.bam.bai"
## [13] "others.bam"                     "others.bam.bai"          "shifted.bam"
## [16] "shifted.bam.bai"                "trinucleosome.bam"       "trinucleosome.bam.bai"
```

Heatmaps showing distribution of reads from different bins around TSSs

A (High quality)



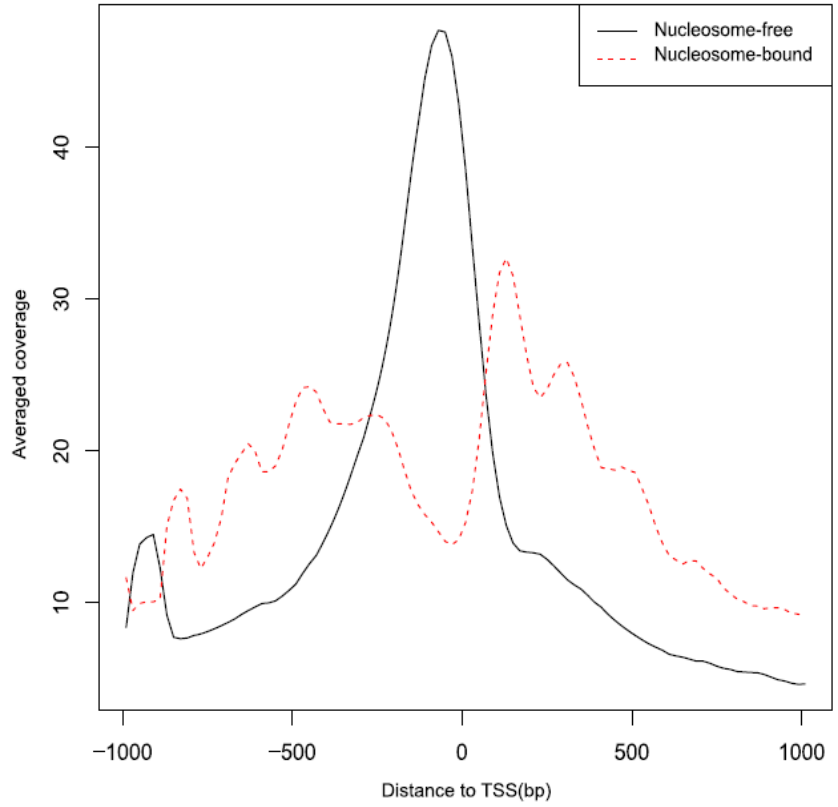
B (Poor quality)



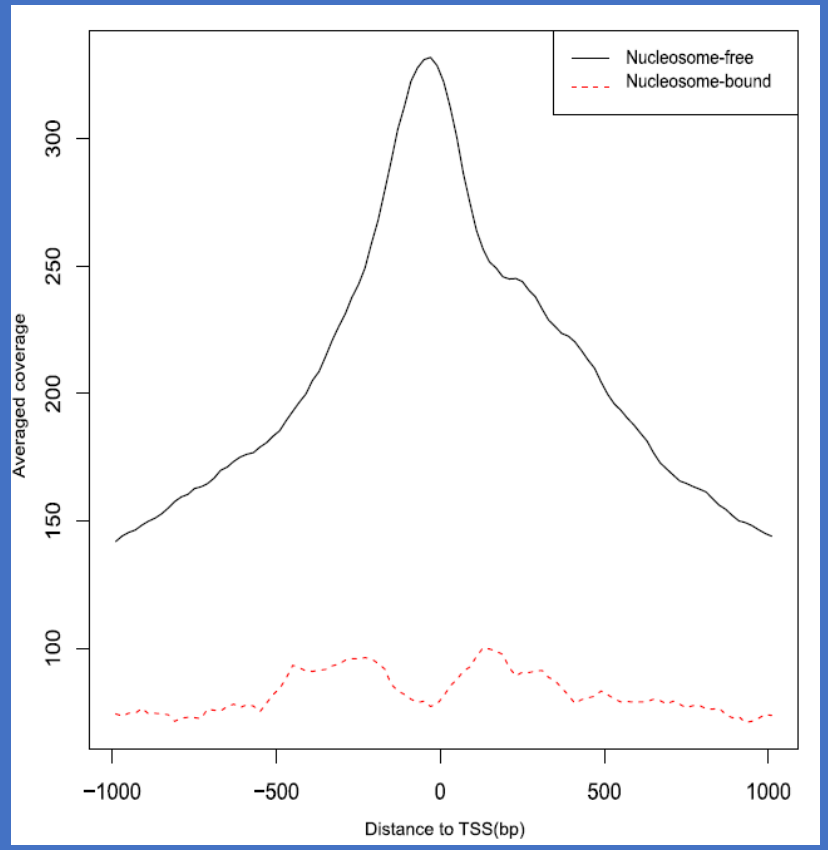
Ref: Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

Density plots showing distribution of reads from different bins around TSSs

A (High quality) ✓



B (Poor quality) ✗



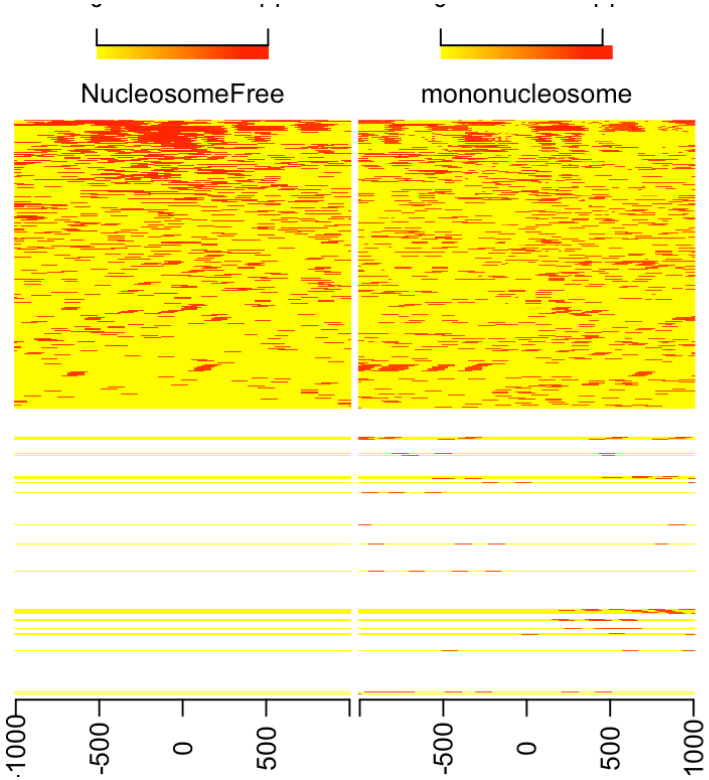
Ref: Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

Heatmap for nucleosome positions

```
library(ChIPpeakAnno)
bamfiles <- file.path(outPath,
                      c("NucleosomeFree.bam", "mononucleosome.bam",
                        "dinucleosome.bam", "trinucleosome.bam"))
TSS <- promoters(txs, upstream=0, downstream=1)
TSS <- unique(TSS)
## estimate the library size for normalization
(librarySize <- estLibSize(bamfiles))
```

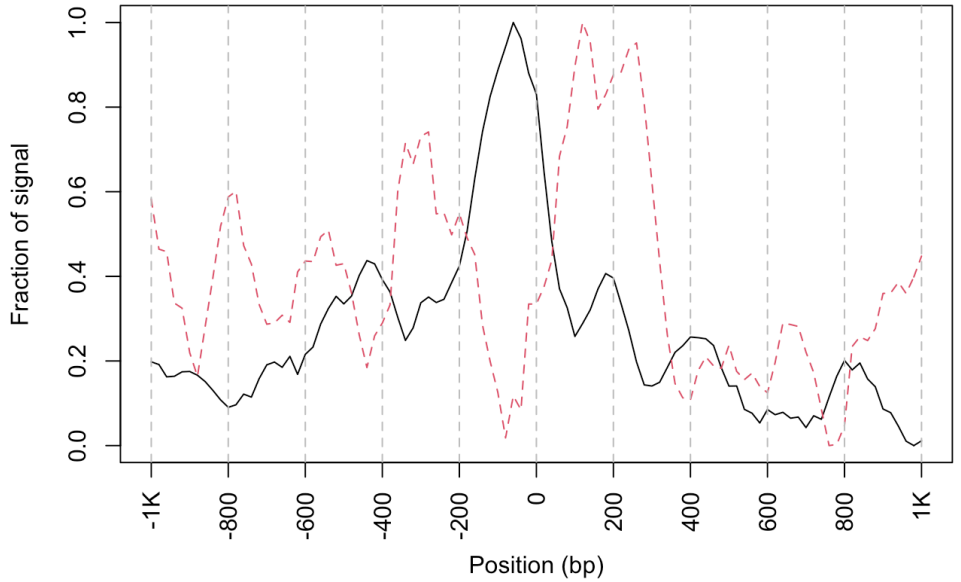
```
## splited/NucleosomeFree.bam      splited/mononucleosome.bam
##                      10140                            3426
##     splited/dinucleosome.bam     splited/trinucleosome.bam
##                       2748                               0
```

```
## calculate the signals around TSSs.
NTILE <- 101
dws <- ups <- 1010
sigs <- enrichedFragments(gal=objs[c("NucleosomeFree", "mononucleosome",
                                     "dinucleosome", "trinucleosome")],
                          TSS=TSS, librarySize=librarySize, seqlev=seqlev,
                          TSS.filter=0.5, n.tile = NTILE,
                          upstream = ups, downstream = dws)
## log2 transformed signals
sigs.log2 <- lapply(sigs, function(.ele) log2(.ele+1))
featureAlignedHeatmap(sigs.log2, reCenterPeaks(TSS, width=ups+dws),
                      zeroAt=.5, n.tile=NTILE) #plot heatmap
```



Coverage curve for nucleosome positions

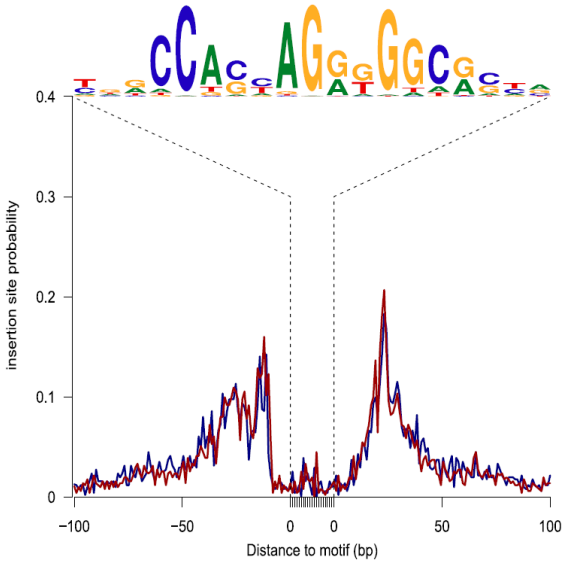
```
## get signals normalized for nucleosome-free and nucleosome-bound regions.
out <- featureAlignedDistribution(sigs, reCenterPeaks(TSS, width=ups+dws),
                                  zeroAt=.5, n.tile=NTILE, type="l", ylab="Averaged coverage")
## rescale the nucleosome-free and nucleosome signals to 0~1
range01 <- function(x){(x-min(x))/(max(x)-min(x))}
out <- apply(out, 2, range01)
matplot(out, type="l", xaxt="n", xlab="Position (bp)", ylab="Fraction of signal")
        axis(1, at=seq(0, 100, by=10)+1, labels=c("-1K", seq(-800, 800, by=200), "1K"), las=2)
abline(v=seq(0, 100, by=10)+1, lty=2, col="gray")
```



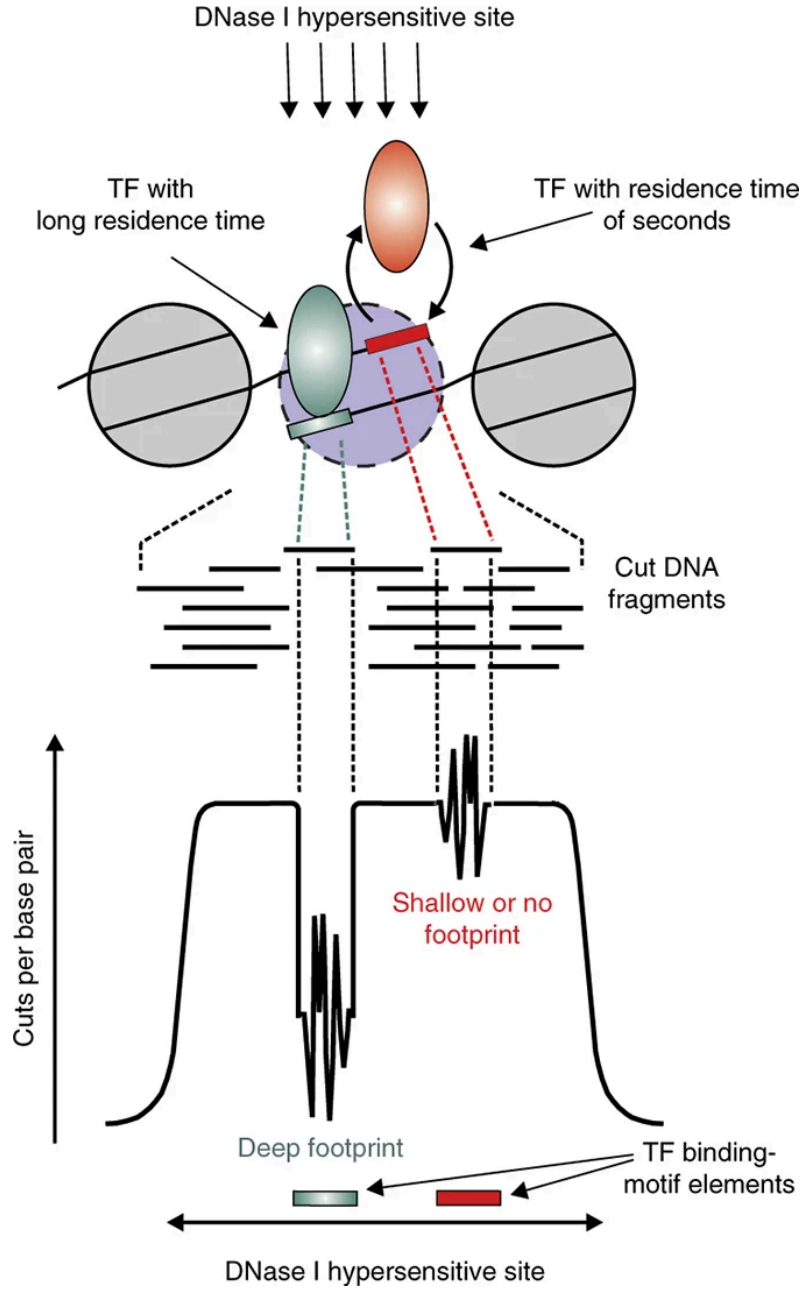
Assessing footprints of DNA-binding factors

```
## get PWM of TFs
CTCF <- query(MotifDb, c("CTCF"))
CTCF <- as.list(CTCF)

sigs <- factorFootprints(shiftedBamFile, pfm=CTCF[[1]],
        genome=genome, min.score="90%", seqlev=seqlev,
        upstream=100, downstream=100)
```



Ref: Ou et.al., 2018. doi: 10.1186/s12864-018-4559-3

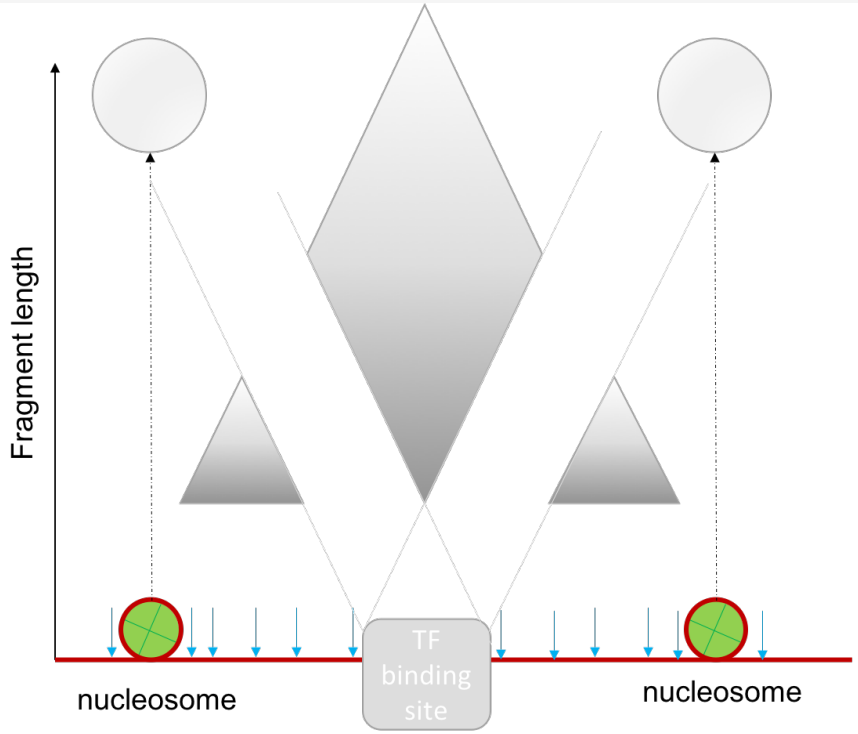


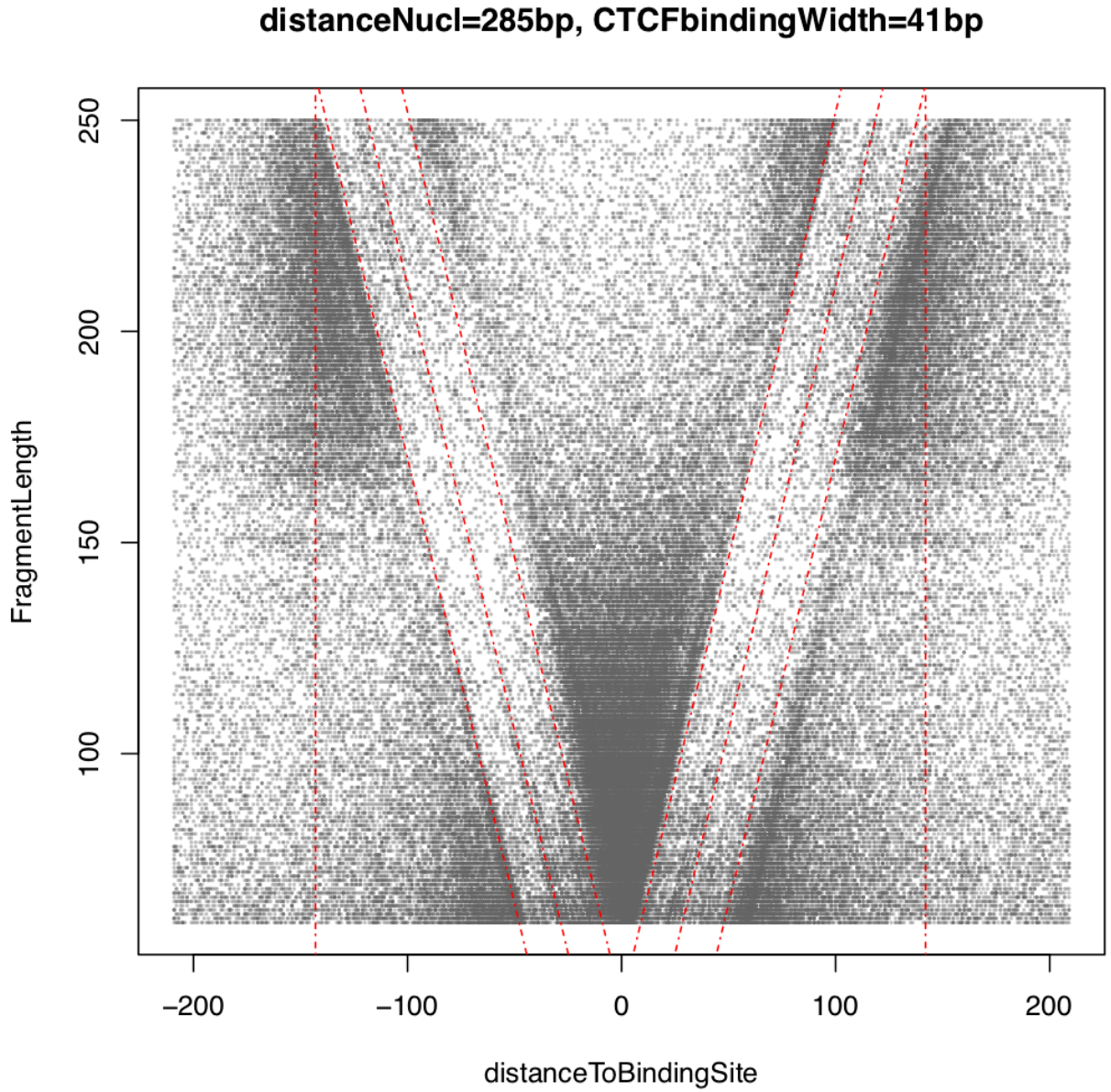
Ref: Sung et.al., 2016. doi: 10.1038/nmeth.3766

vPlot

V-plot is a plot to visualize fragment midpoint vs length for a given transcription factors.

```
vp <- vPlot(shiftedBamfile, pfm=CTCF[[1]],
        genome=genome, min.score="90%",
        seqlev=seqlev,
        upstream=200, downstream=200,
        ylim=c(30, 250), bandwidth=c(2, 1))
```

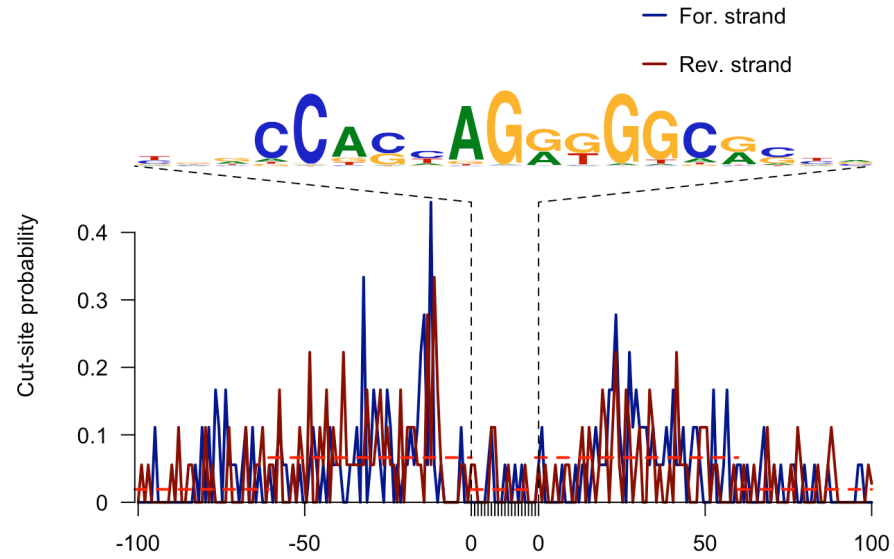




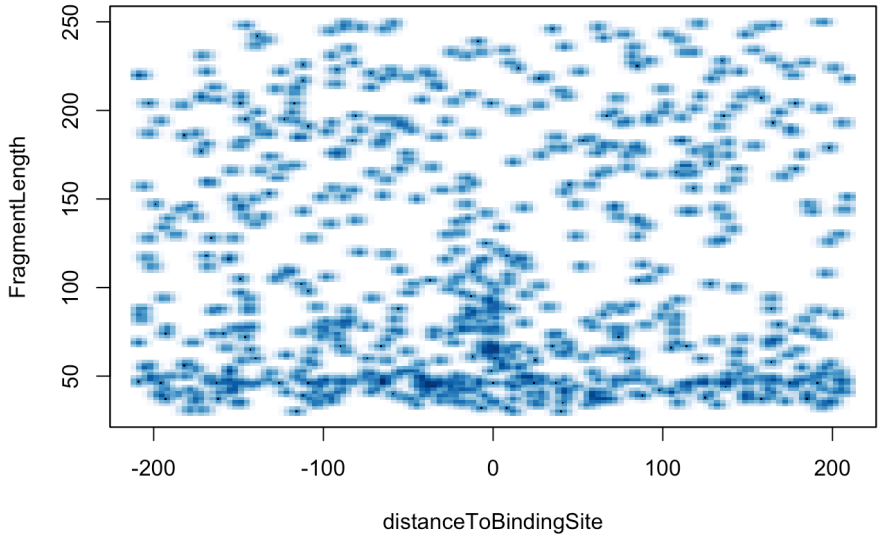
Ref: Henikoff et.al., 2011. doi: 10.1073/pnas.1110731108

Footprints

```
## foot prints
library(MotifDb)
CTCF <- query(MotifDb, c("CTCF")) CTCF <- as.list(CTCF)
sigs <- factorFootprints(shiftedBamfile, pfm=CTCF[[1]],
                                genome=genome, min.score="90%",
                                seqlev=seqlev,
                                upstream=100, downstream=100)
```




```
vp <- vPlot(shiftedBamfile, pfm=CTCF[[1]],
                genome=genome, min.score="90%", seqlev=seqlev,
                upstream=200, downstream=200,
                ylim=c(30, 250), bandwidth=c(2, 1))
```

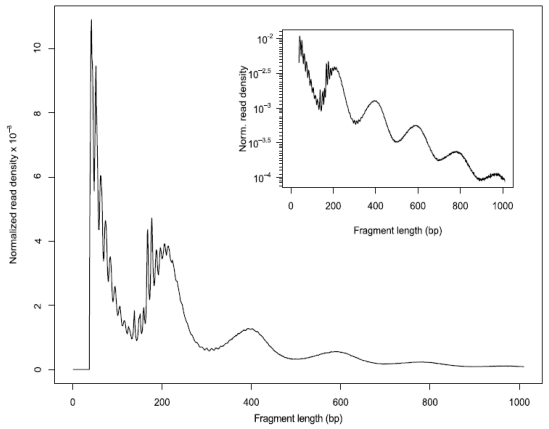


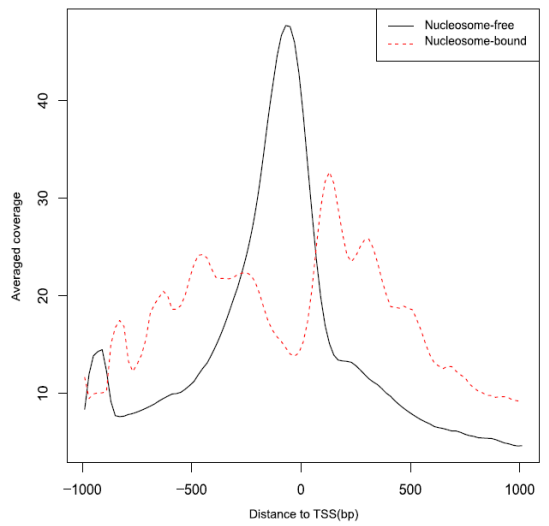
ATACseqQC CAN ...

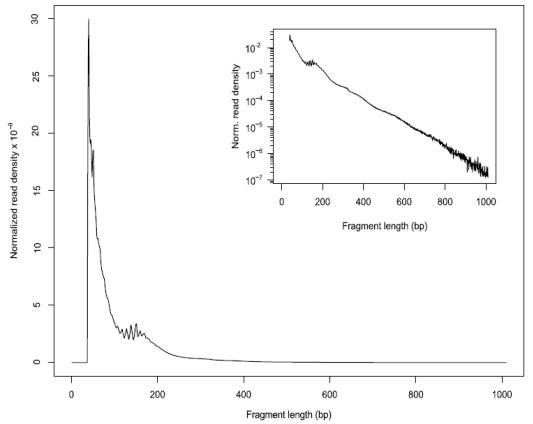
help researcher to assess the quality of the ATAC library preparation by simple plots.

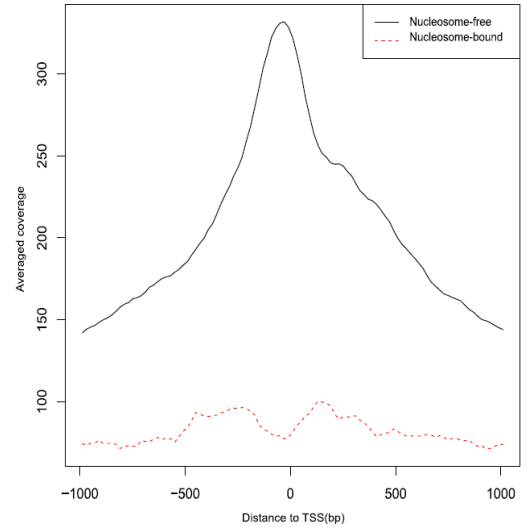
split bam files for downstream analysis and validation.

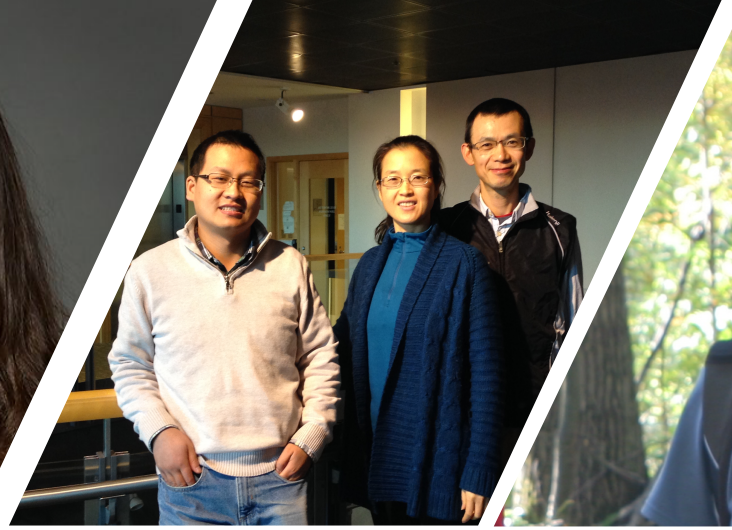
- Shifted aligned reads
- Splitting BAM files

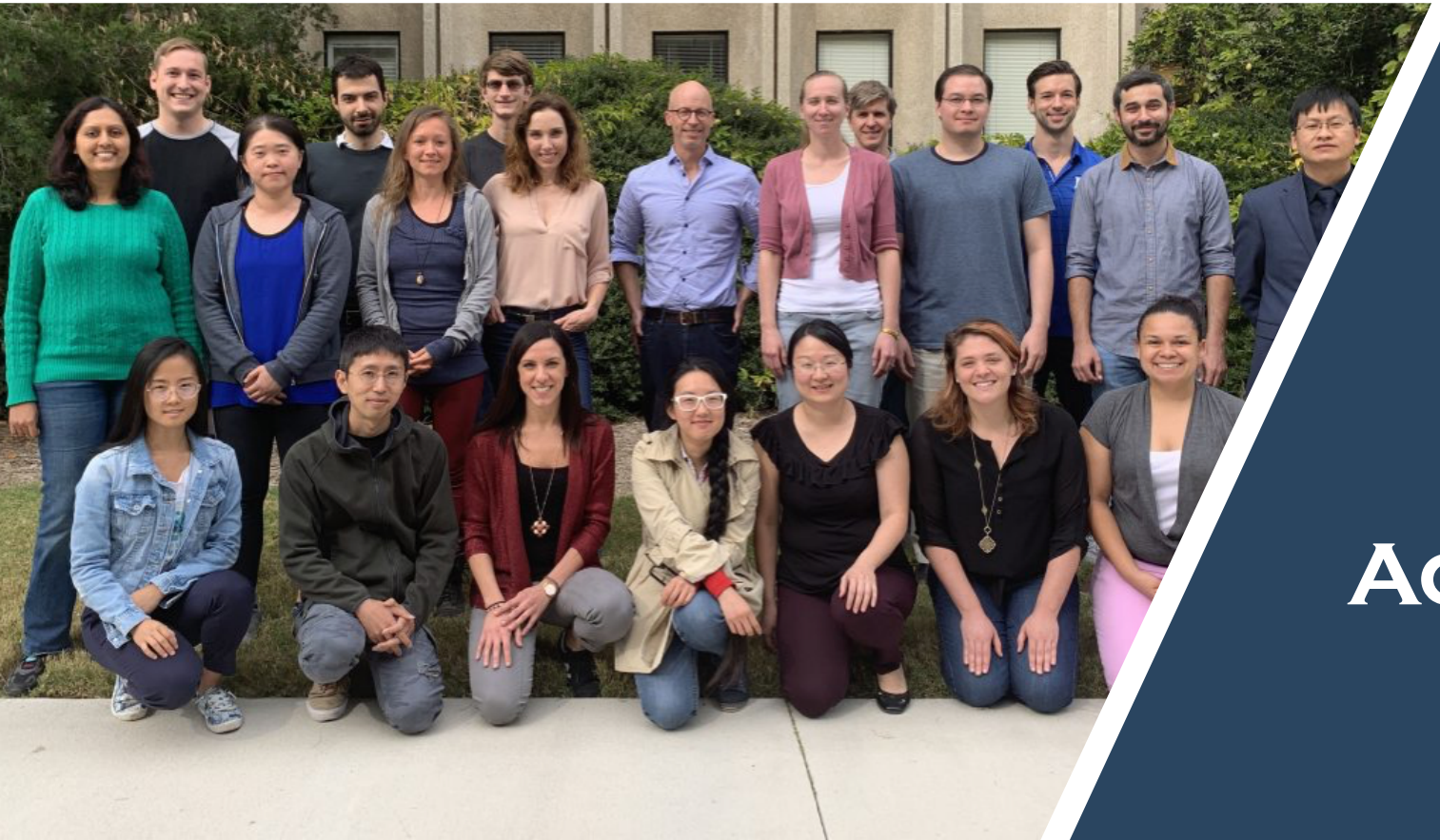








Acknowledgement