Bioinformatic approaches to regulatory genomics and epigenomics

376-1347-00L | week 03

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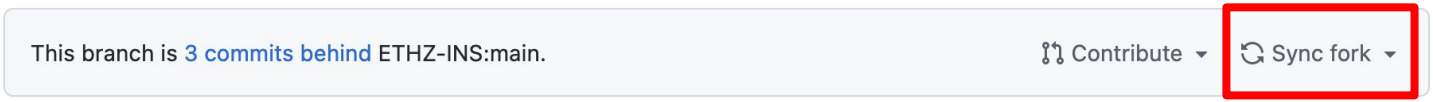
Plan for today

- Debriefing on the assignments
- Overview of NGS technologies, ChIP-seq and its analysis
- Practical:
  - primary processing of a ChIP-seq experiment
    (to be continued next week)

Debriefing on the assignments

- Handing in the exercises etc.:
  - Handing in the exercises: Please name the exercises files just **`assignment.html`** !!!
  - Sync fork before committing:



  - Please join the help channel for hints & questions concerning the exercises, git, package installation etc.
  - Use titles and subtitles with **`#/##`** for the separate questions. Makes the documents structured and easier to read

```
# 1. AnnotationHub

## a) Mouse EnsDb, v102, GRCm38
```

Debriefing on the assignments

- Exercise 2
  - ***Gene ids*** vs ***gene names*** vs ***gene symbols***
    - ***`gene ids:`*** stable ID from Ensembl, truly unique, e.g. "ENSMUSG00000005677"
    - ***`gene symbols:`*** HGNC symbol from the [HUGO Gene Nomenclature Committee]

  - variable naming: `exons_mouse <- width(exsPerTx)`
    - more fitting would be: `exon_widths <- width(exsPerTx)`

  - `genes(ensdb, filter = TxBiotypeFilter("protein_coding"))`
    - here one would use as a filter: `GeneBioTypeFilter("protein_coding")`

Debriefing on the assignments

- Exercise 2
  - If several annotation/sequences are obtained for query one can look at the metadata with:

```
colnames(mcols(q))
```

```
##  [1] "title"              "dataprovider"       "species"
##  [4] "taxonomyid"         "genome"             "description"
##  [7] "coordinate_1_based" "maintainer"         "rdatadateadded"
## [10] "preparerclass"      "tags"               "rdataclass"
## [13] "rdatapath"          "sourceurl"          "sourcetype"
```

```
date_added <- mcols(q)[,c("rdatadateadded", "genome")]
date_added[order(date_added$rdatadateadded),]
```

```
## DataFrame with 19 rows and 2 columns
##         rdatadateadded      genome
##            <character> <character>
## AH49775     2015-12-28      GRCm38
## AH50120     2015-12-29      GRCm38
## AH50611     2016-05-03      GRCm38
## AH51299     2016-08-15      GRCm38
## AH51645     2016-11-03      GRCm38
## ...                ...         ...
## AH70177     2019-04-29   GRCm38.p6
## AH77927     2019-10-29   GRCm38.p6
## AH82549     2020-04-27   GRCm38.p6
## AH84787     2020-10-26   GRCm38.p6
## AH88477     2020-10-27   GRCm38.p6
```

Debriefing on the assignments

- Exercise 2
  - We can get the exons per transcript in the following way:

```
exs <- exonsBy(ensdb,
               column=c("tx_id","tx_biotype"),
               filter=TxBiotypeFilter("protein_coding"))
```

Debriefing on the assignments

- Exercise 2
  - Calculating the number of exons per transcripts

```
# calculating the number of exons per transcript
nbExonsPerPCtx <- lengths(exs)
hist(nbExonsPerPCtx)
```

  - Calculating the lengths of (spliced transcripts) using **`width()`**

```
# with width we can get the lengths of all exons per transcript in a list
ew <- width(exs)

# by summing the exon lengths per transcript we get the spliced transcript lengths
tl <- sum(ew)

# Plot
hist(tl, breaks=100)
```

Debriefing on the assignments

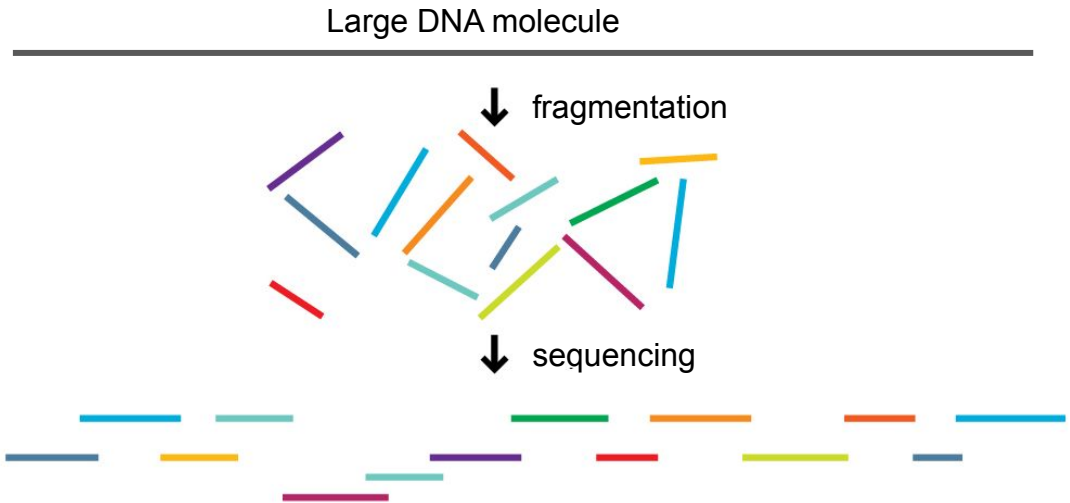
- Exercise 2
  - Alternatively: Calculating the lengths of (spliced transcripts) using **`lengths()`**

```
# with width we can get the lengths of all exons per transcript in a list
ew <- lapply(exs, lengths)

# by summing the exon lengths per transcript we get the spliced transcript lengths
tl <- lapply(ew, sum)
length(tl)
```

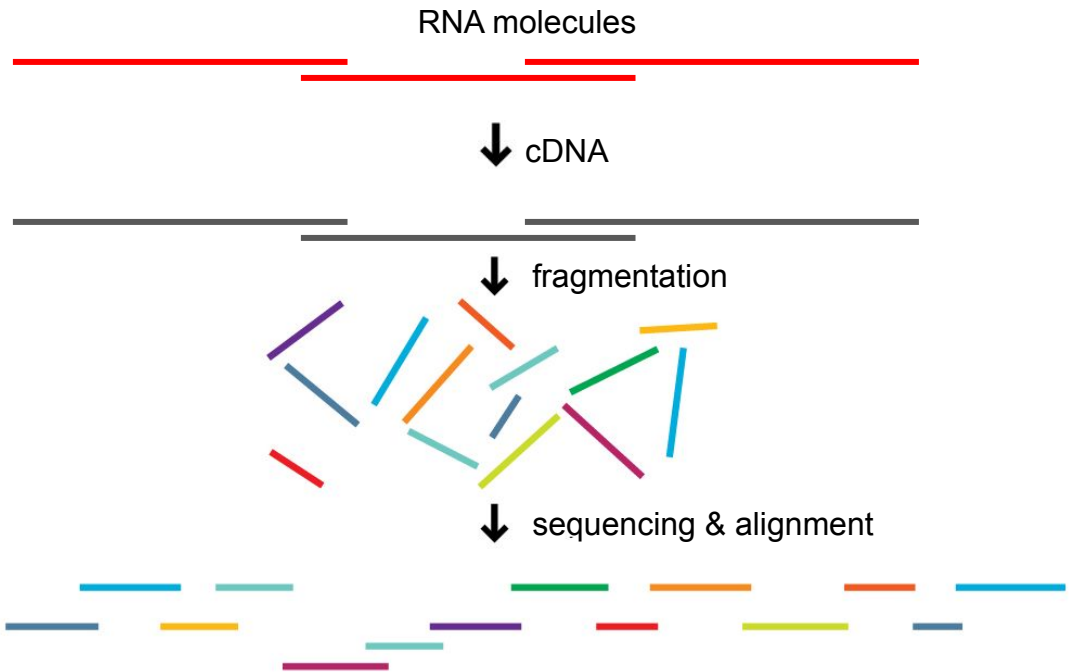
Next Generation Sequencing (NGS)

Shotgun sequencing:



Next Generation Sequencing (NGS)

RNA sequencing:



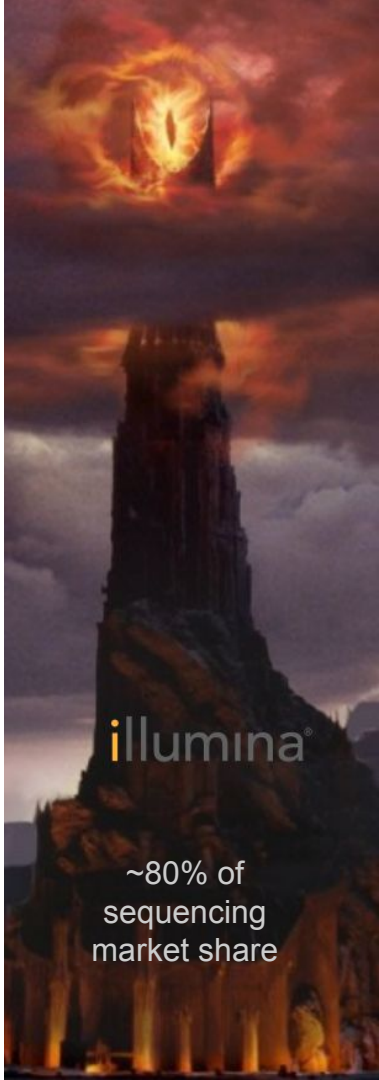
Next Generation Sequencing:
one technology to rule them all

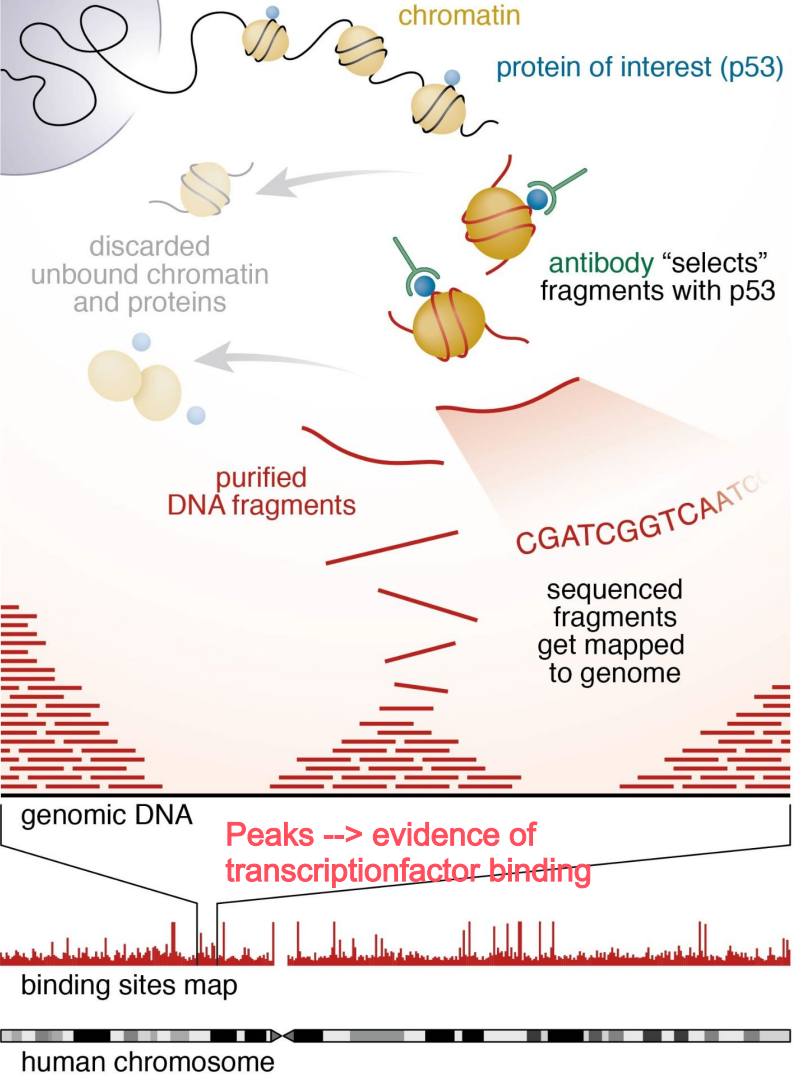
Genomics & meta-genomics

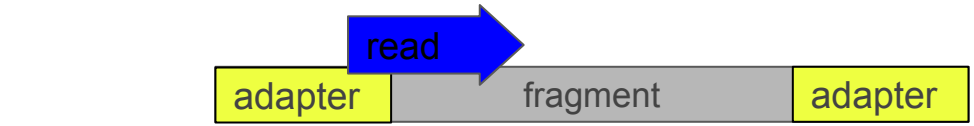
Epigenomics & chromatin conformation

Transcriptomic, translatomics, epi-transcriptomics

A lot of convergence in terms of analysis tools and techniques





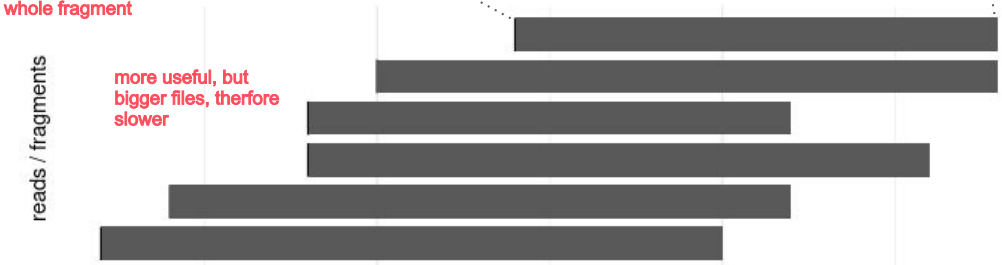


Single-end sequencing:

Paired-end sequencing:

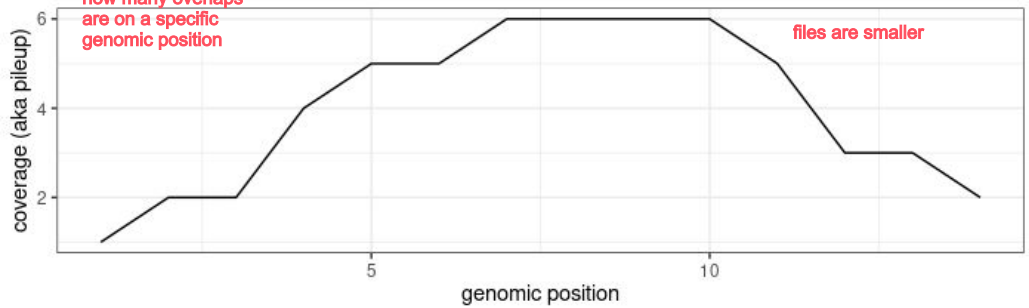
sequencing a Little bit of adaptor and a part of fragment, but not whole fragment

more useful, but bigger files, therfore slower

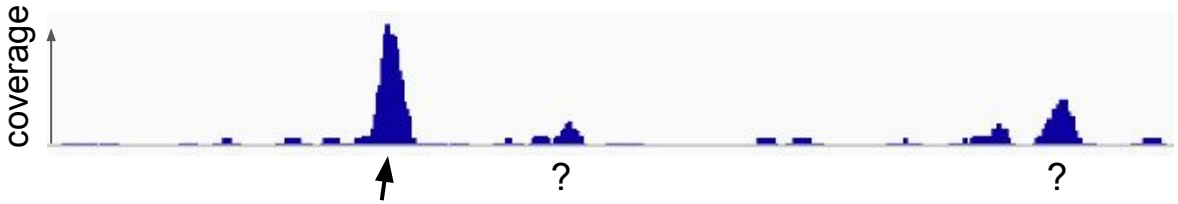


how many overlaps are on a specific genomic position

files are smaller



Peak calling

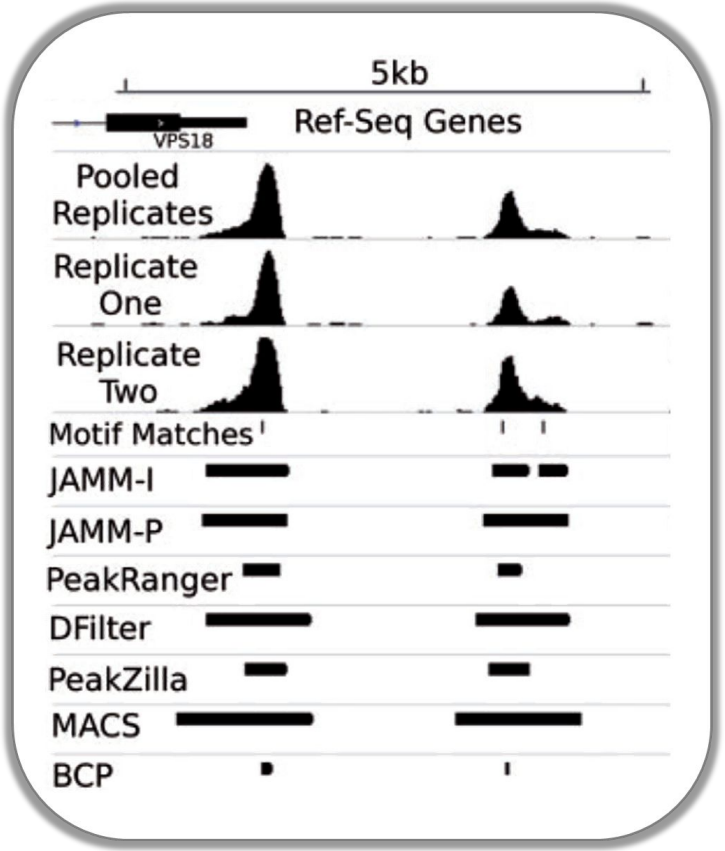


TF binding site

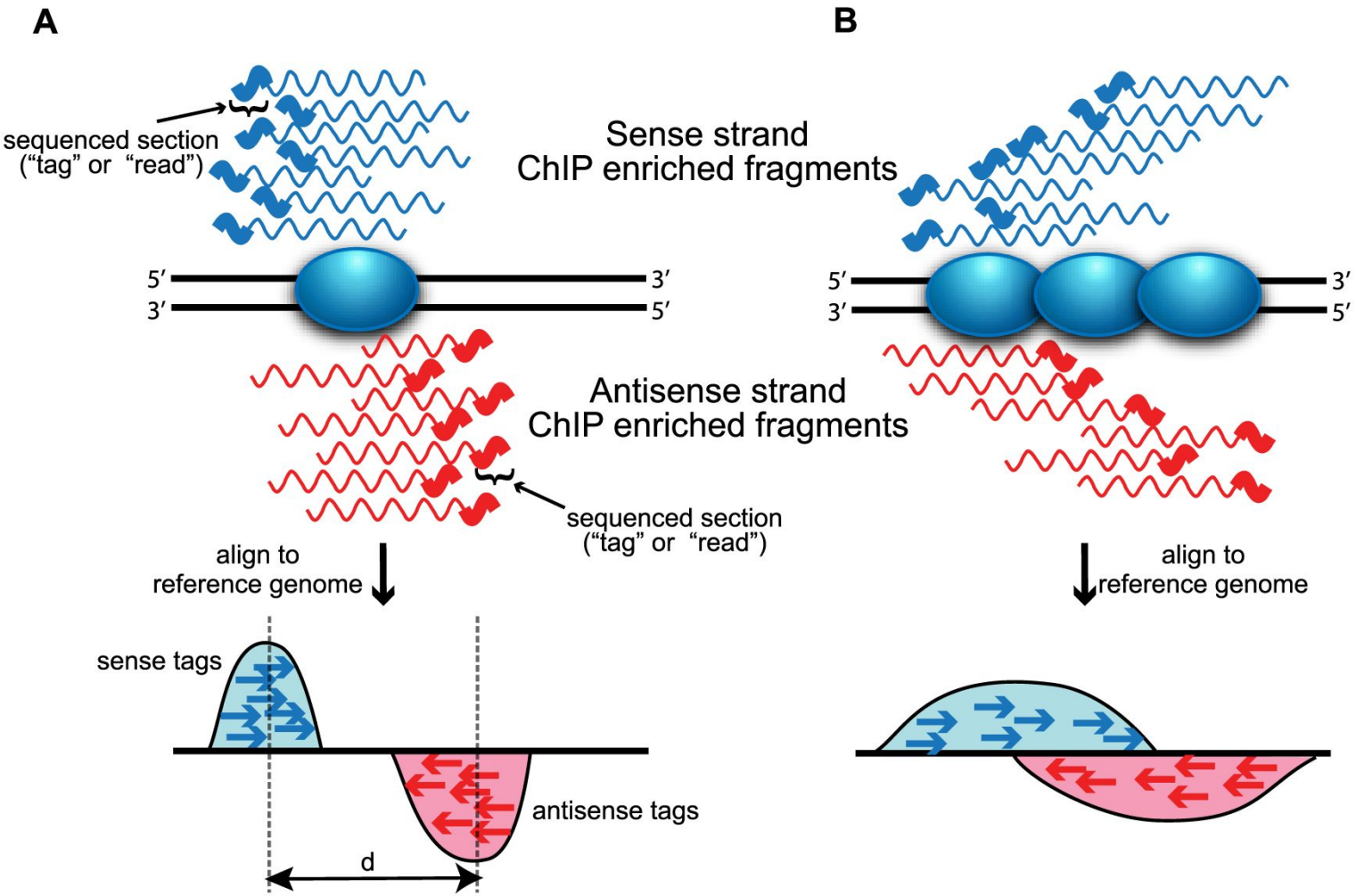
another Experiment where you use an antibody that shouldn't bind to anything --> compare to the first Experiment and see if the Peak is countabe

Enrichment over input?

MACS is most widley uses to call peaks

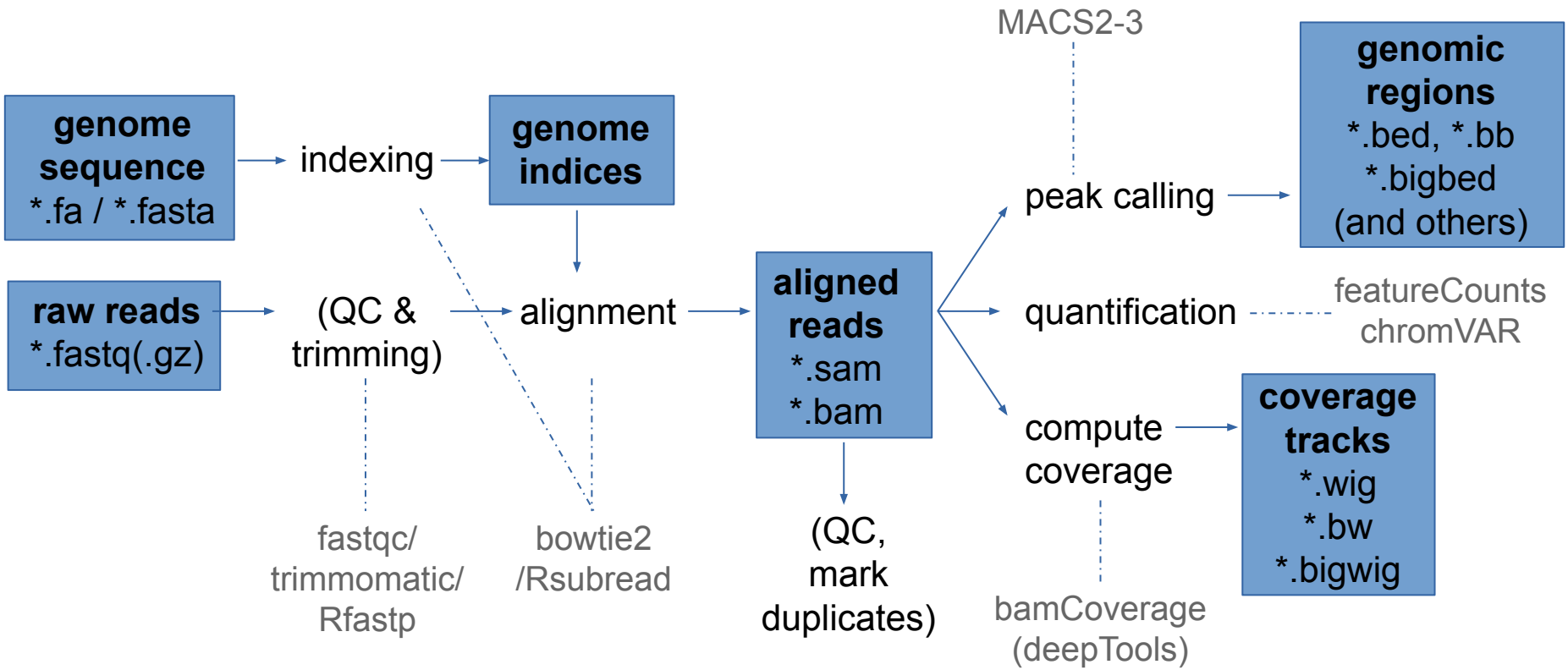


(Ibrahim et al., NAR 2014)



(Wilbanks et al., PLoS One 2010)

Overview of a primary analysis pipeline (ChIP-seq and the likes)



Alternative toolsets for (DNA) primary analysis

- The most standard one:
  - fastqc
  - trimmomatic
  - bowtie2
  - picard
  - deeptools

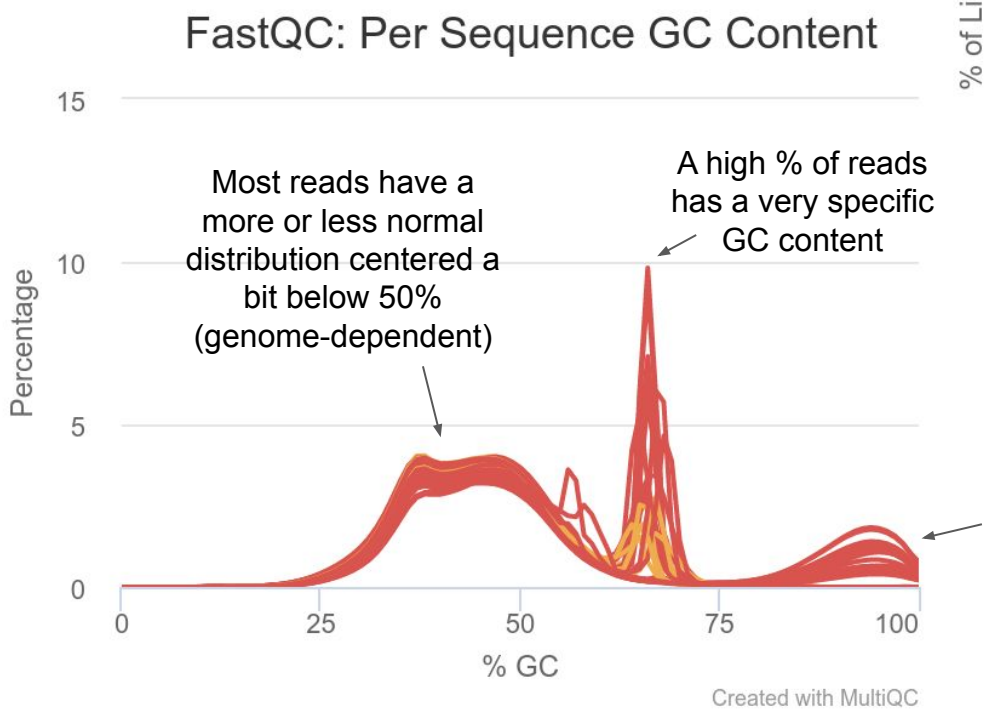
- Pure R-based
  - rfastp
  - Rsubread

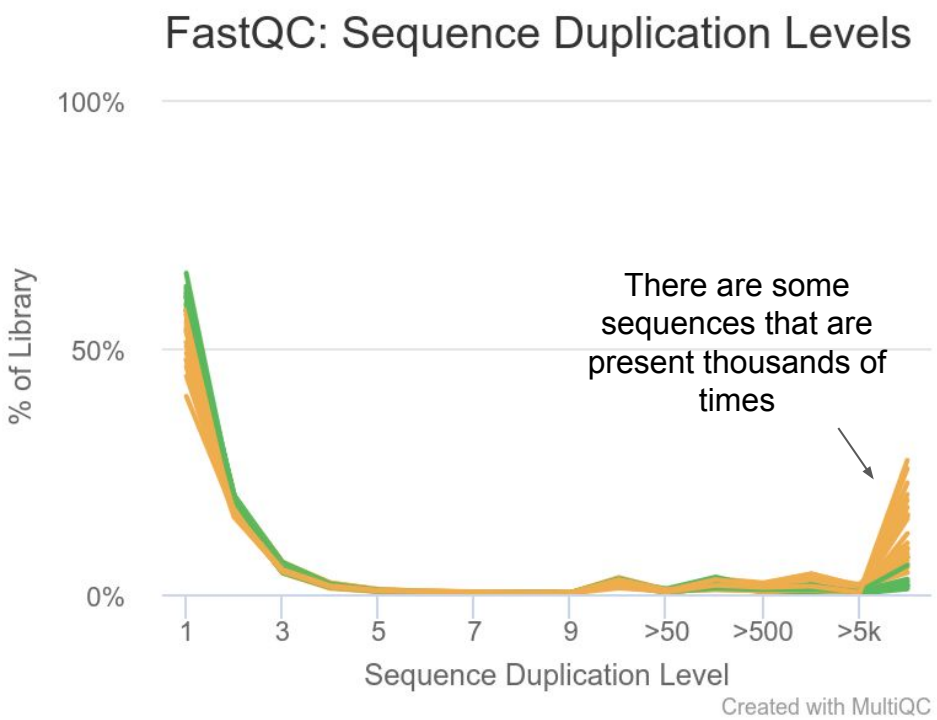
QuasR

Downstream analysis (R)

- epiwraps
- ChIPseeker
- etc…

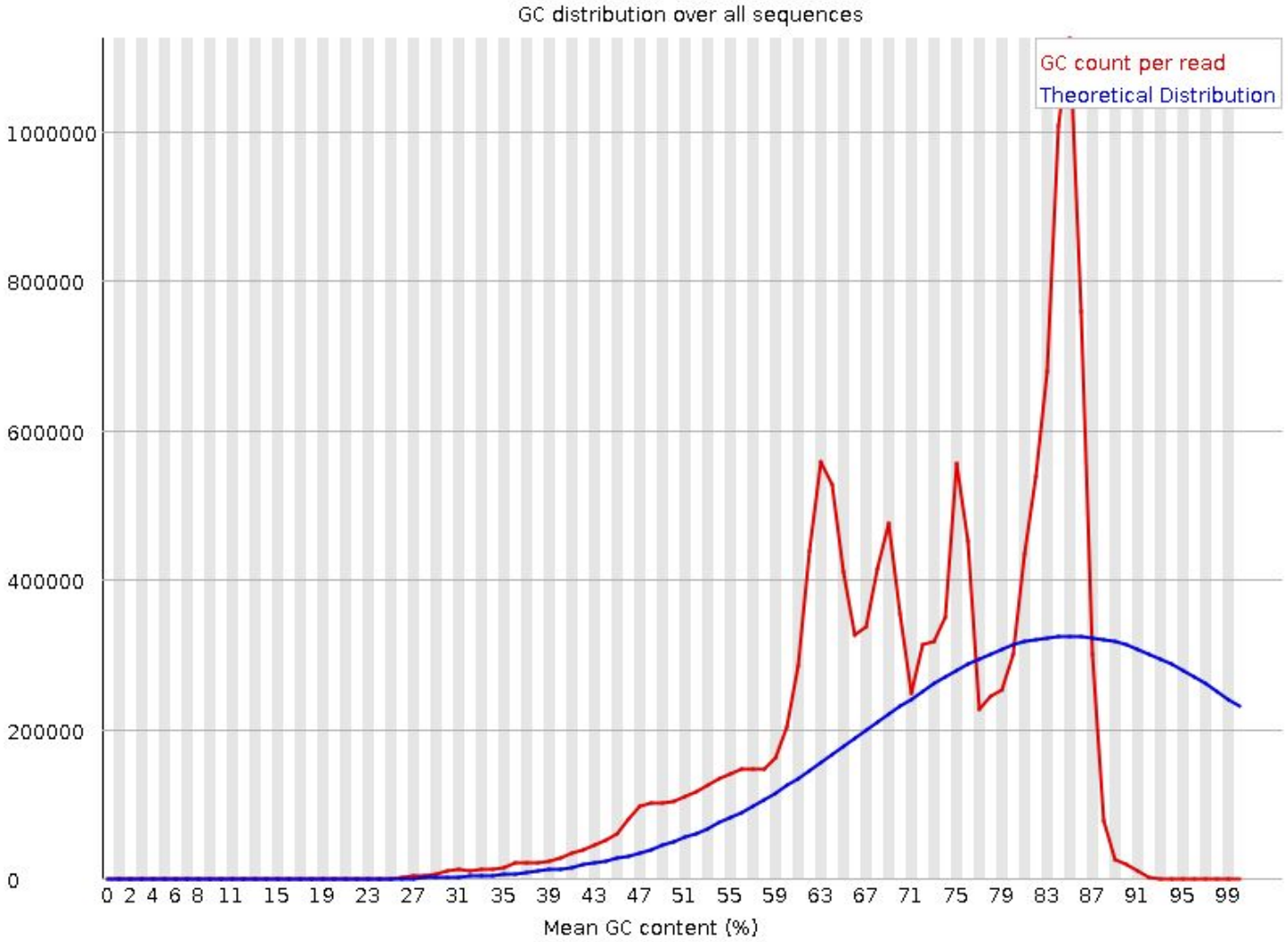
Example (rather extreme) QC problems





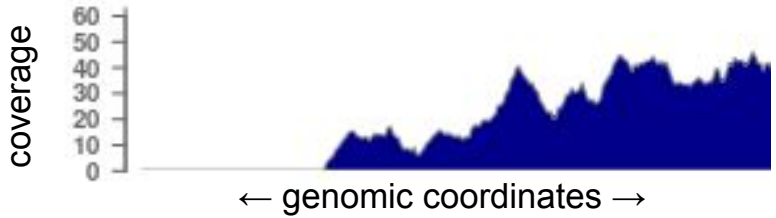
Example (rather extreme)
QC problems:

Bias from overamplification



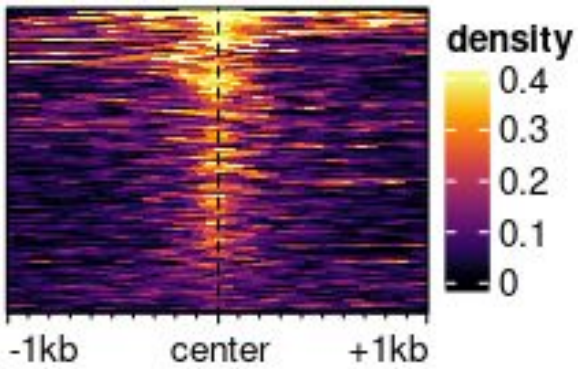
Visualizations available in *epiwraps*

- Signal across one genomic region: `plotSignalTracks`



(Based on the *Gviz* R package)

- Signal across several genomic regions: `signal2Matrix` → `plotEnrichedHeatmaps`



(Mainly based on the EnrichedHeatmap R package, itself based on ComplexHeatmap)

Assignment

- Download the following Drosophila ChIP-seq for the protein CTCF:
  - IP: https://www.encodeproject.org/files/ENCFF127RRR/@@download/ENCFF127RRR.fastq.gz

    (no input control for the purpose of this exercise)
- Process it from the raw data, obtaining:
  - bam file
  - peaks
- Report:
  - how many reads (and what percentage) were mapped
  - how many peaks were found
- Plot the signal around one of the peaks

- Please make sure that you name your final file **`assignment.html`** !!