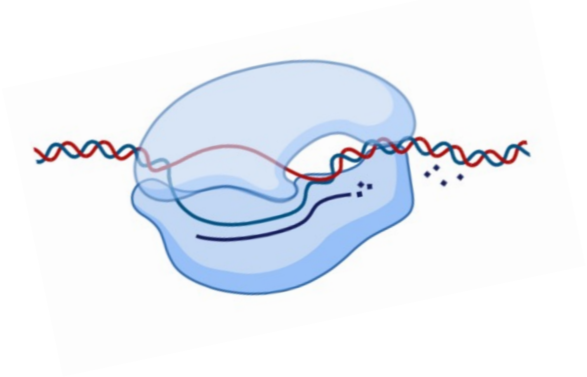
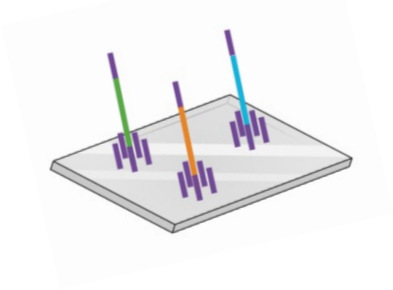
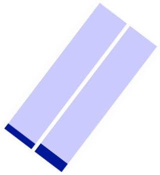




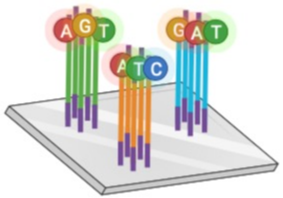


Quality control - ChIP-Seq Data

Junfan Huang

MRC Cancer Unit
University of Cambridge

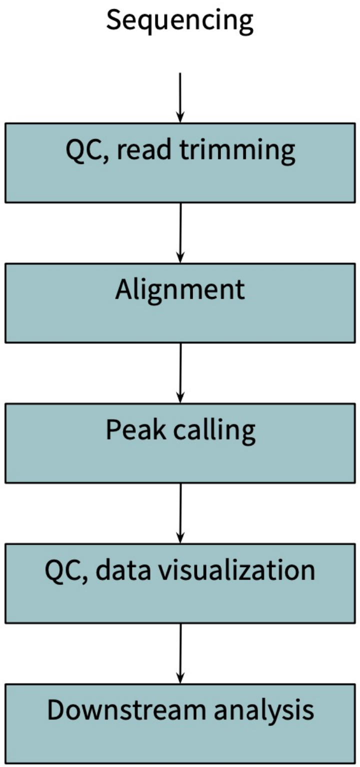
CRUK Bioinformatics Summer School 2021
27th July 2021



Quality control – ChIP-Seq data

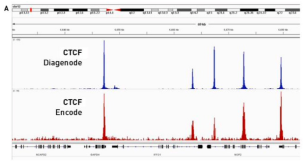
Visually

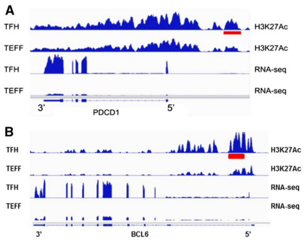
Computationally



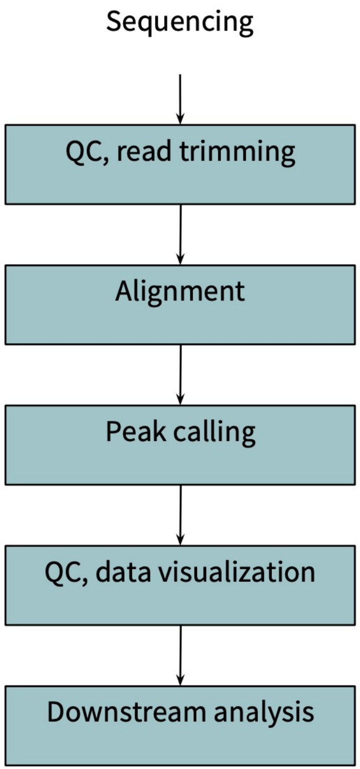
Quality control – ChIP-Seq data

Visually



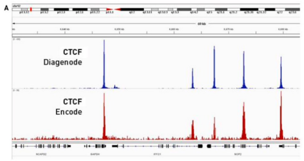


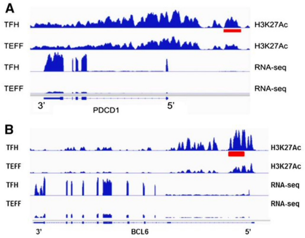
Computationally



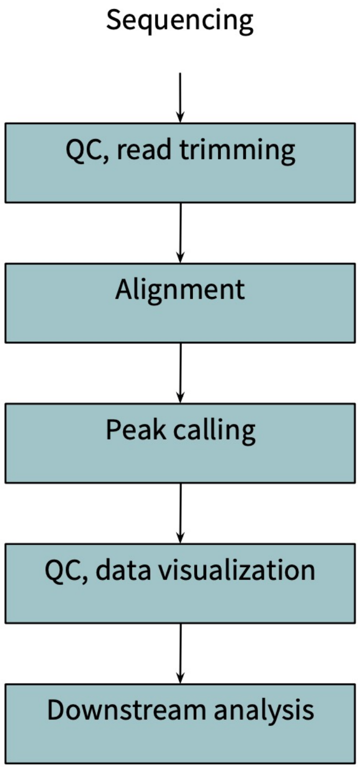
Quality control – ChIP-Seq data

Visually (IGV or USCS genome browser)





Computationally



Sequencing

QC, read trimming

Alignment

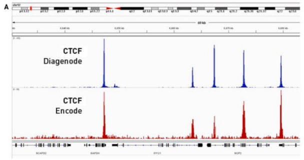
Peak calling

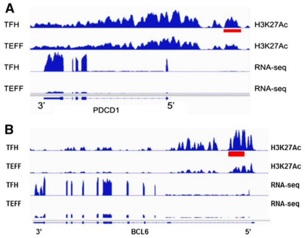
QC, data visualization

Downstream analysis

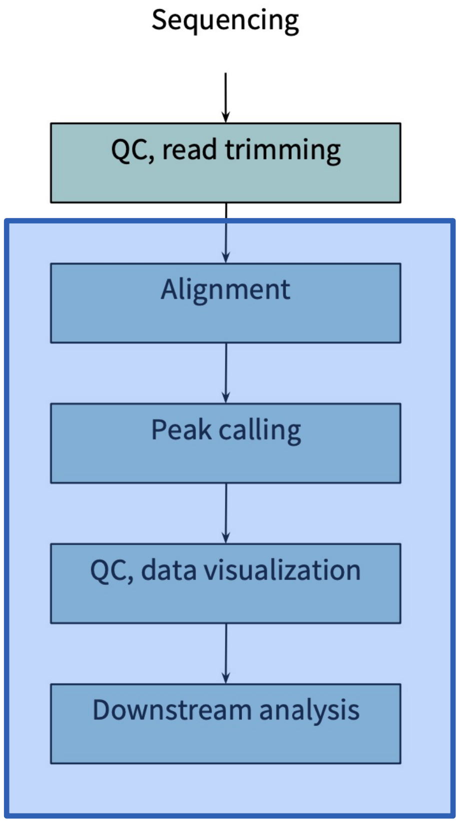
Quality control – ChIP-Seq data

Visually





Computationally



Sequencing

QC, read trimming

Alignment

Peak calling

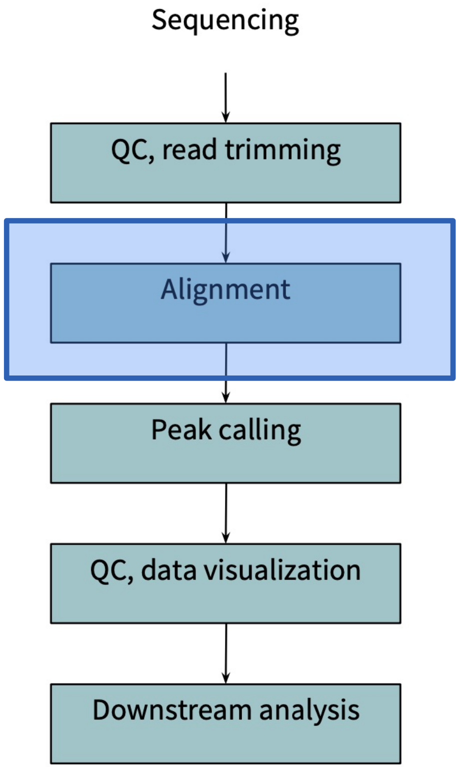
QC, data visualization

Downstream analysis

Quality control – ChIP-seq data

Visually (Alignment)

- Relative Enrichment in genomic intervals (REGI)

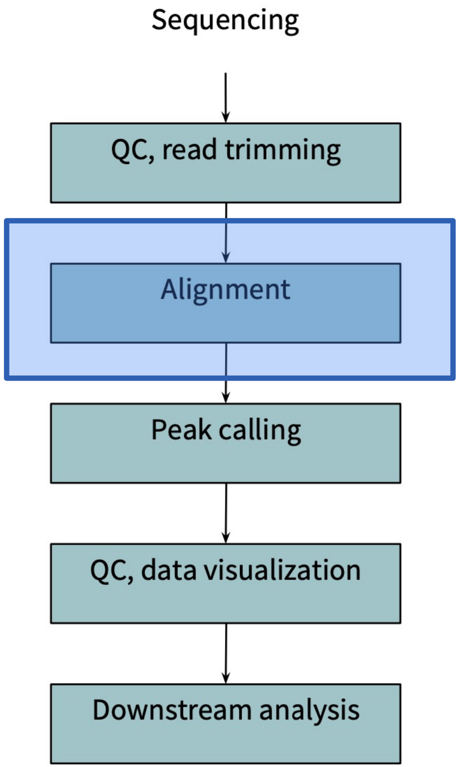


Computationally

Quality control – ChIP-seq data

Visually (Alignment)

- Relative Enrichment in genomic intervals (REGI)
  - Proteins might have a high expected enrichment in certain genomic regions



Adapted from Dora Bihary's slides

Quality control – ChIP-seq data

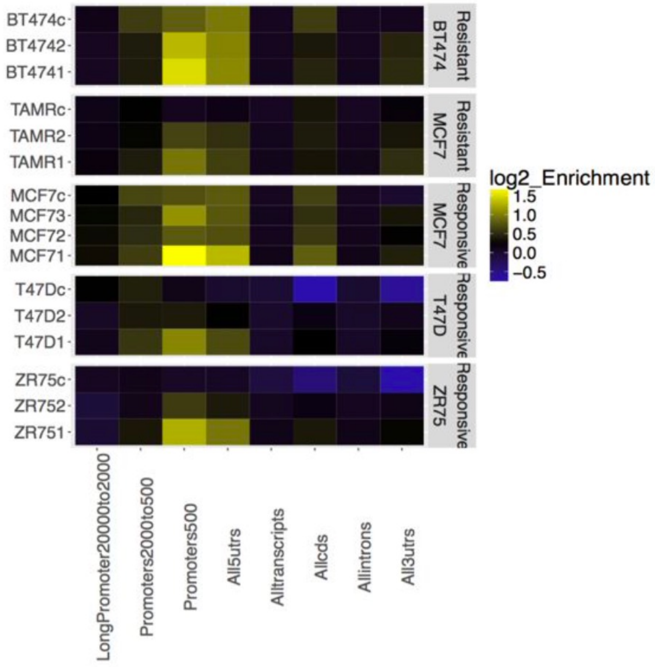
Visually (Alignment)

- Relative Enrichment in genomic intervals (REGI)
  - Proteins might have a high expected enrichment in certain genomic regions

Promoter region

UTRs

introns



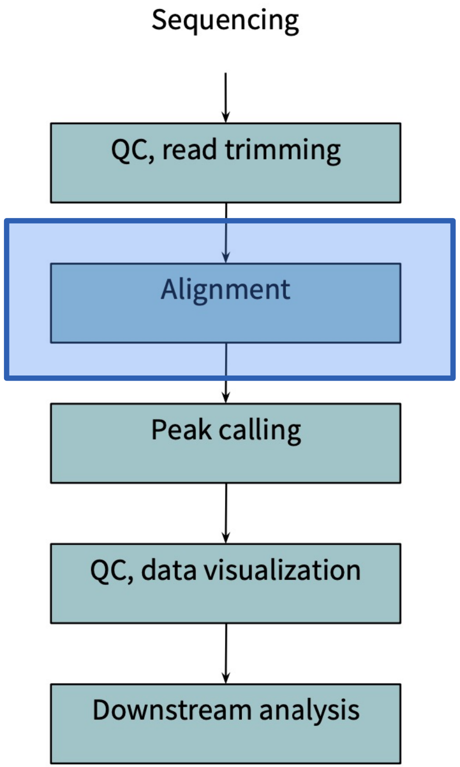
Adapted from Dora Bihary's slides

Quality control – ALL NGS data

Visually

Computationally

- Read Mapping% (Higher the better)

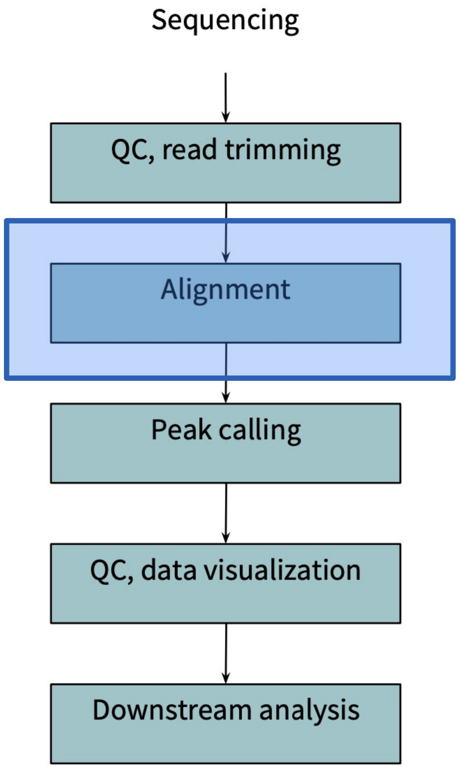


Quality control – ChIP-seq data

Visually

Computationally (Alignment)

- **Remove Blacklisted regions**
- Strand cross-correlation
- PCR Bottleneck coefficient (PBC)

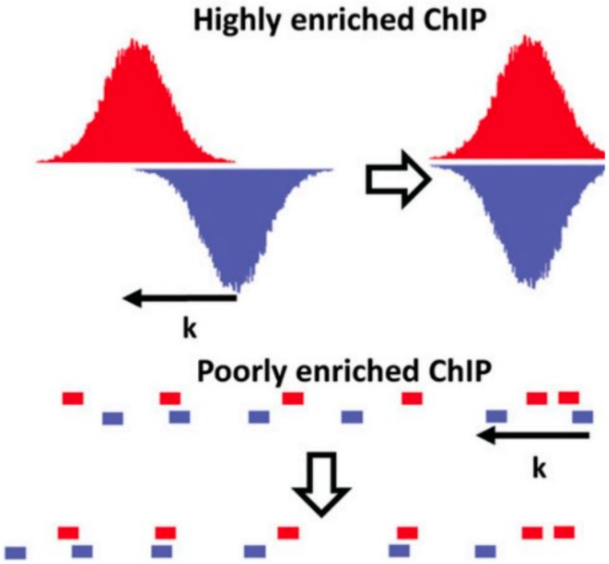


Quality control – ChIP-seq data

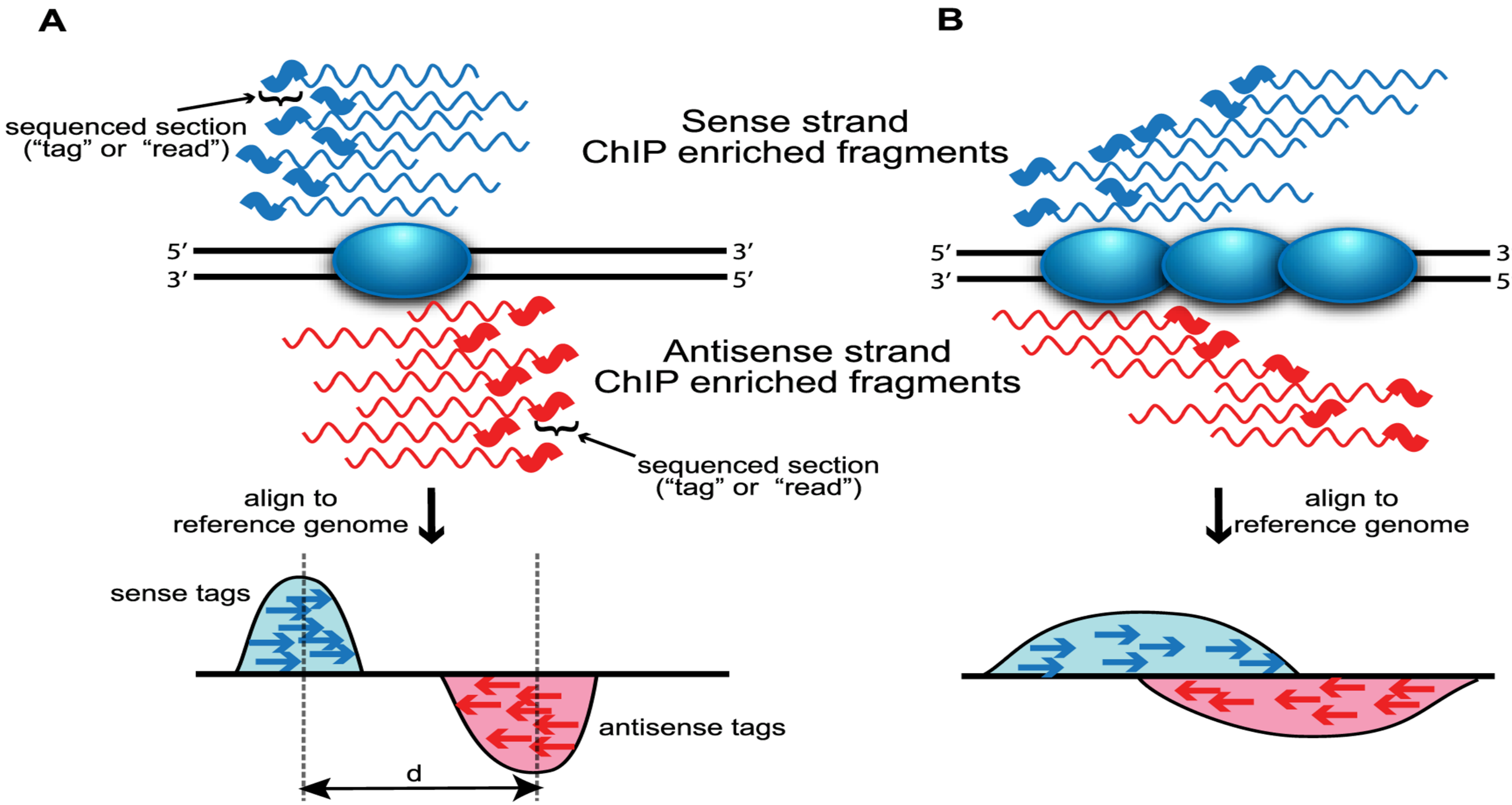
Visually

Computationally (Alignment)

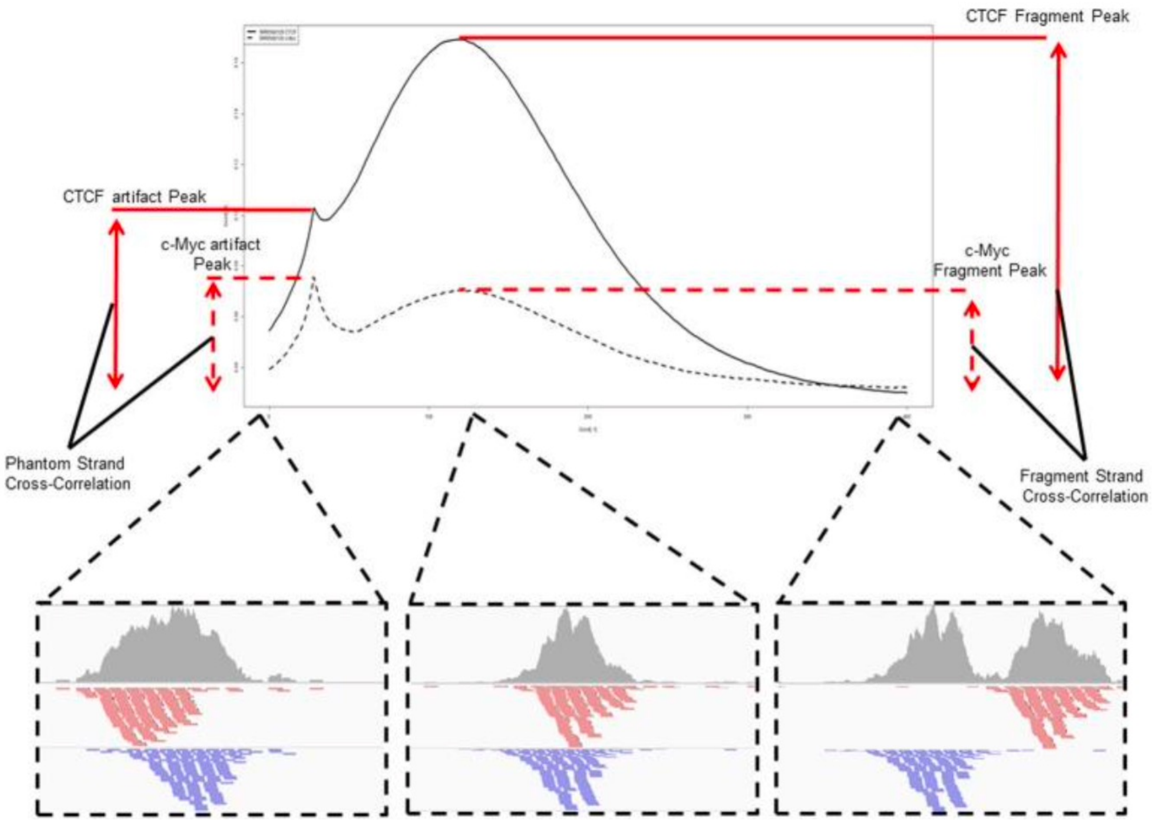
- Remove Blacklisted regions
- **Strand cross-correlation**
- PCR Bottleneck coefficient (PBC)



Strand dependent bimodality



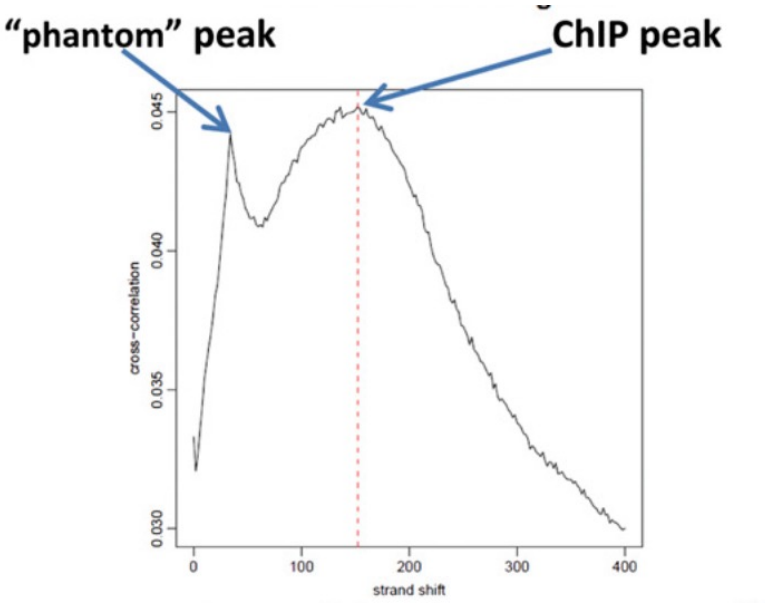
Strand cross-correlation



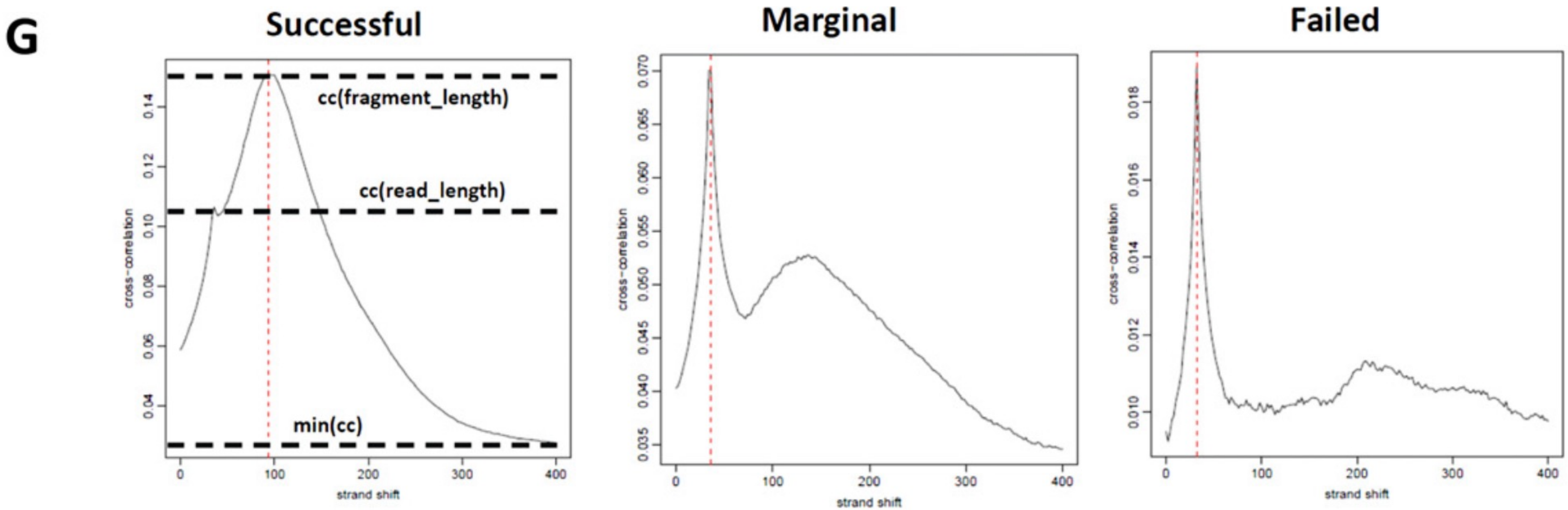
Strand cross-correlation plot

The cross-correlation plot typically produces two peaks:

- ChIP peak – fragment length
- Phantom peak – read length



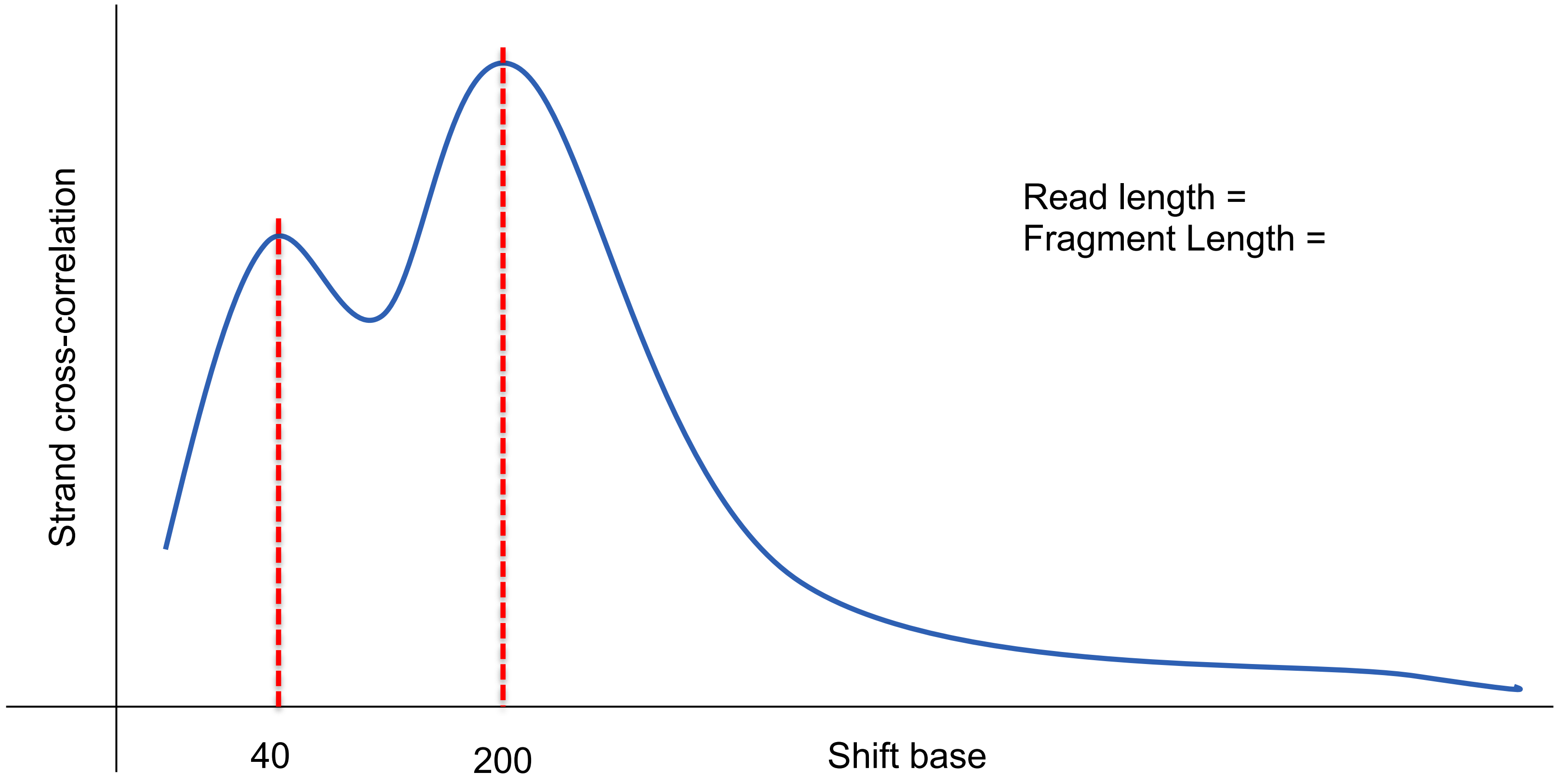
Strand cross-correlation plot



$$NSC = \frac{cc(fragment\ length)}{min(cc)} \qquad RSC = \frac{cc(fragment\ length) - min(cc)}{cc(read\ length) - min(cc)}$$

Very successful ChIP experiments generally have NSC>1.05 and RSC>0.8

Strand cross-correlation plot



Read length =
Fragment Length =

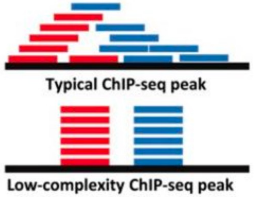
Quality control – ChIP-seq data

Visually

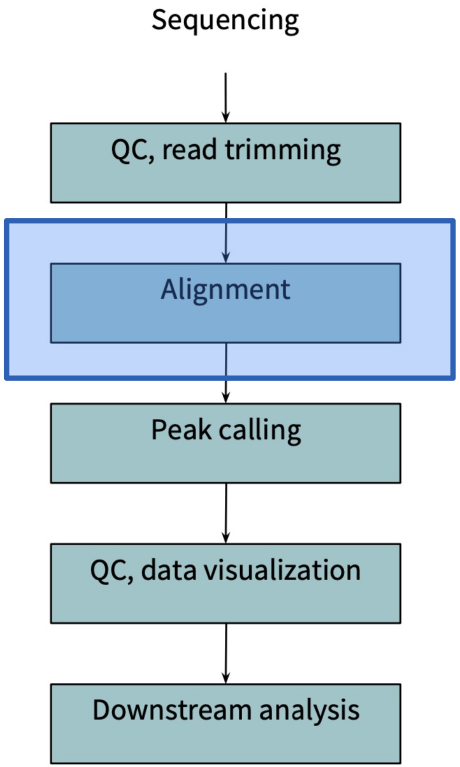
Computationally (Alignment)

- Remove Blacklisted regions
- Strand cross-correlation
- **PCR Bottleneck coefficient (PBC)**

$$PBC = \frac{N_1}{N_2}$$



N1: # genomic positions with one read aligned (higher)
N2: # genomic positions with one or more reads

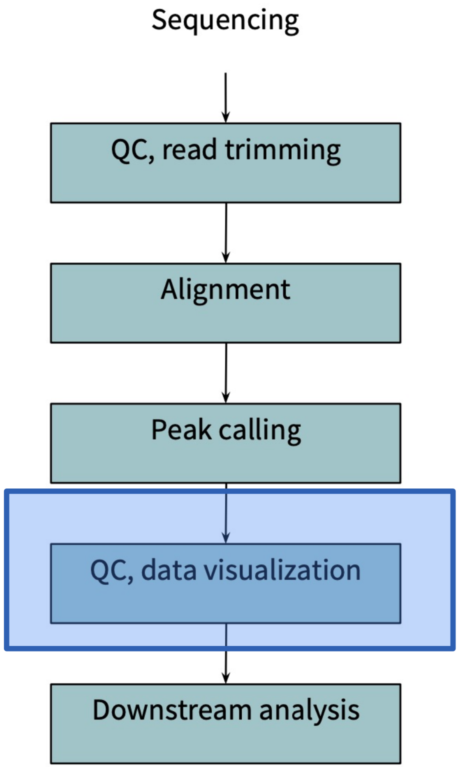


Quality control – ChIP-seq data

Visually

Computationally (peak)

- Num of peaks with good FDR(<0.05)

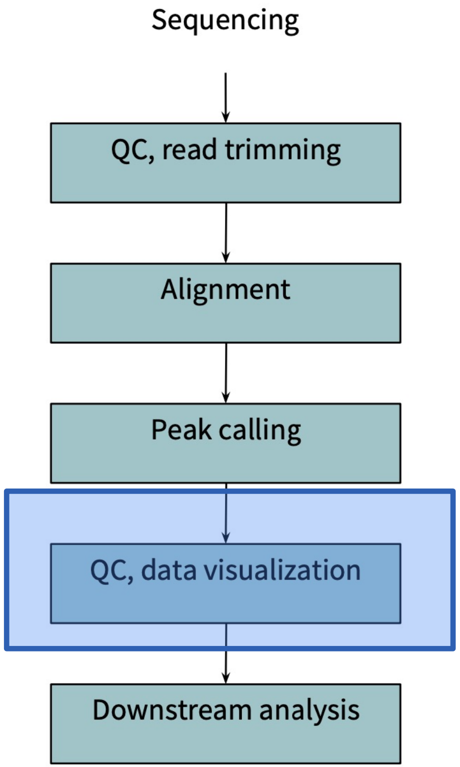


Quality control – ChIP-seq data

Visually

Computationally (peak)

- Num of peaks with good FDR(<0.05)
- **Fold Change**

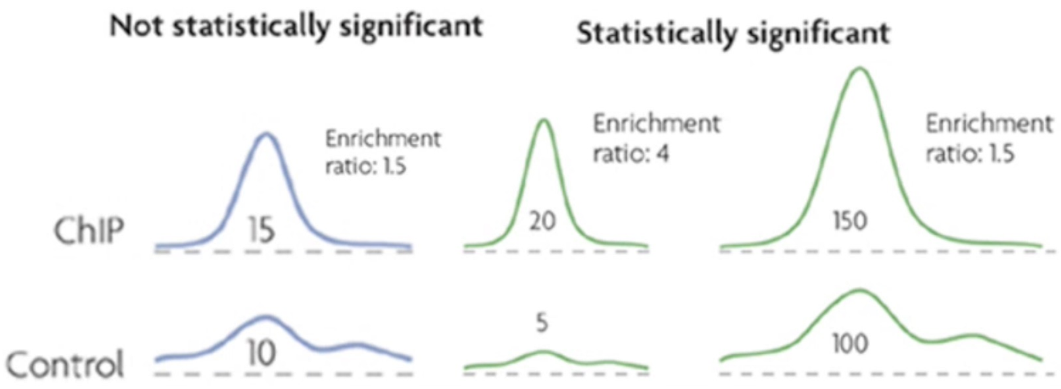


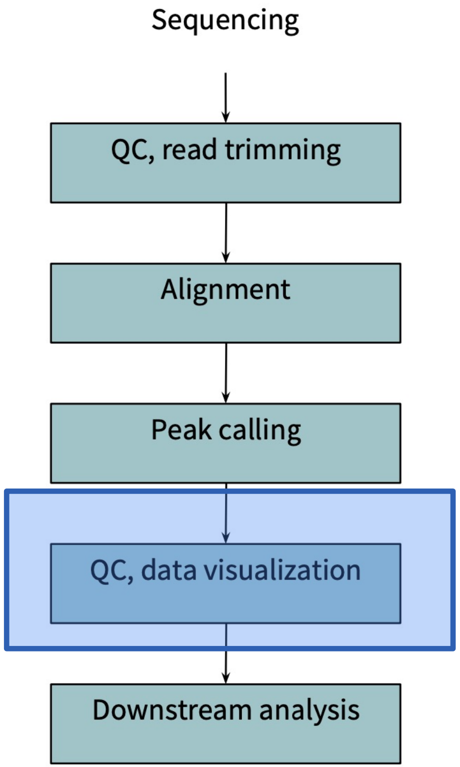
Quality control – ChIP-seq data

Visually

Computationally (peak)

- Num of peaks with good FDR(<0.05)
- **Fold Change (enrichment ratio)**




Sequencing

QC, read trimming

Alignment

Peak calling

QC, data visualization

Downstream analysis

Quality control – ChIP-seq data

Visually

Computationally (peak)

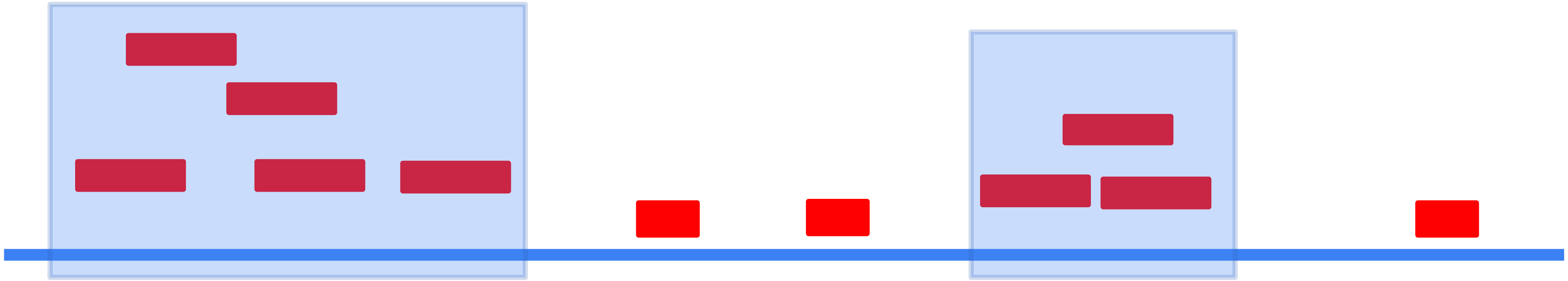
- Num of peaks with good FDR (0.05)
- Fold Change
- **Fraction of Reads in Peaks (FRiP>5%)**

Quality control – ChIP-seq data

Visually

Computationally (peak)

- Num of peaks with good FDR (0.05)
- Fold Change
- **Fraction of Reads in Peaks (FRiP>5%)**



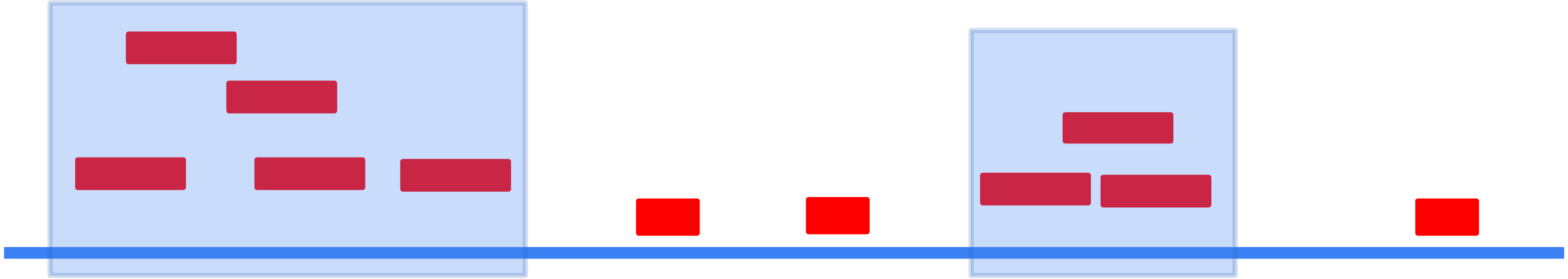
Quality control – ChIP-seq data

Visually

Computationally (peak)

- Num of peaks with good FDR (0.05)
- Fold Change
- **Fraction of Reads in Peaks (FRiP>5%)**

$$\text{FRiP} = \frac{\text{reads} \in \text{peaks}}{\text{total reads}}$$



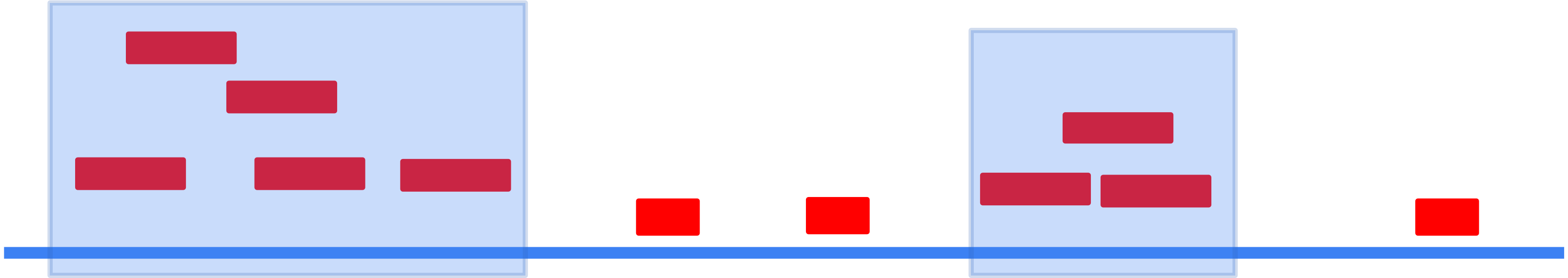
Quality control – ChIP-seq data

Visually

Computationally (peak)

- Num of peaks with good FDR (0.05)
- Fold Change
- **Fraction of Reads in Peaks (FRiP>5%)**

$$\text{FRiP} = \frac{\text{reads} \in \text{peaks}}{\text{total reads}}$$



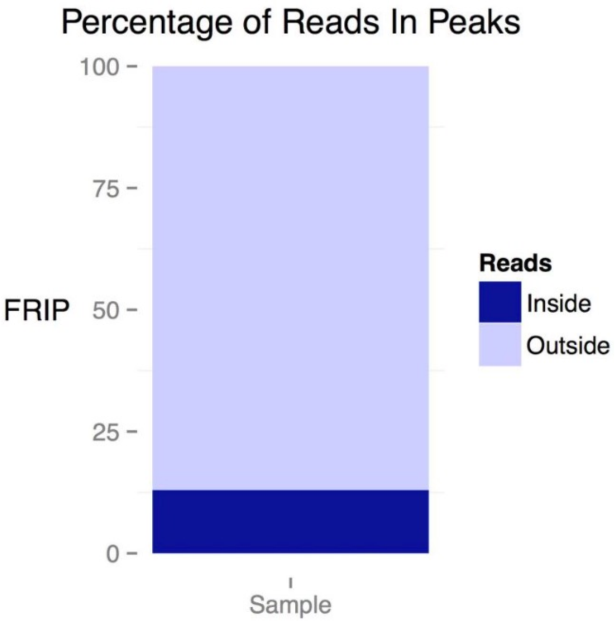
N.B. FRiP is sensitive to the specifics of peak calling method, antibody & target factor pair, so FRiP < 1% does not automatically mean failure

Quality control – ChIP-seq data

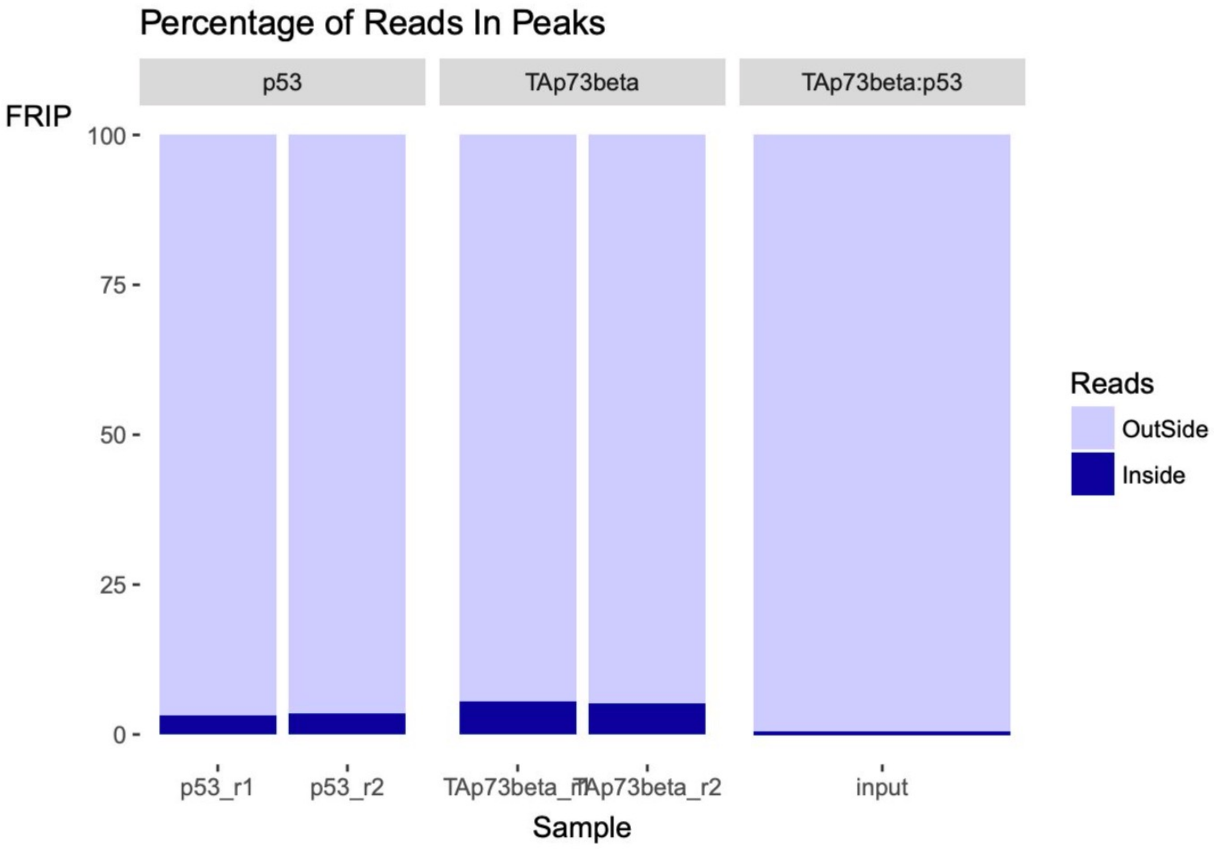
Visually

Computationally (peak)

- Num of peaks with good FDR (0.05)
- Fold Change
- **Fraction of Reads in Peaks** (FRiP>5%)



What do you see here?



Adapted from Dora Bihary's slides

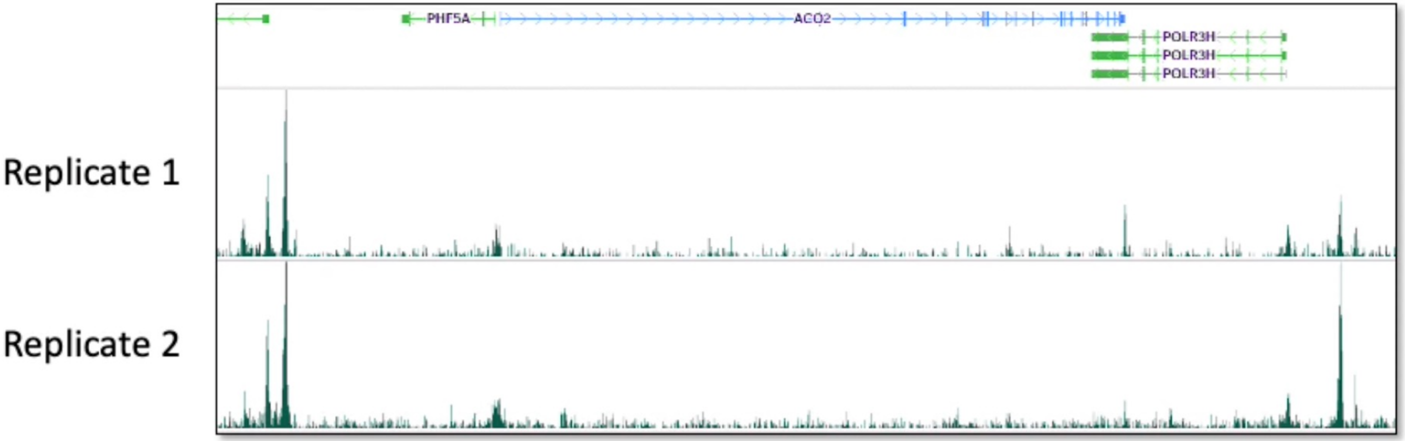
Quality control – ChIP-seq data

Visually

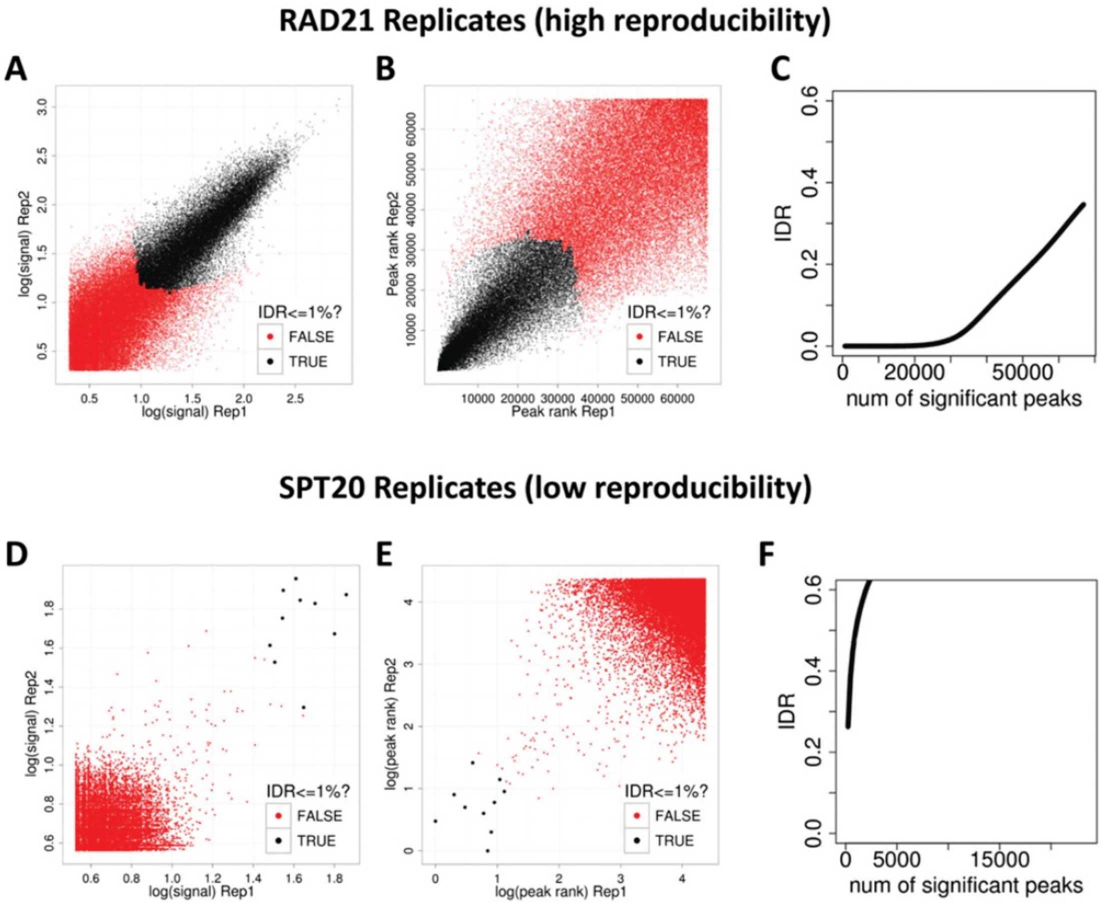
Computationally (peak)

- Num of peaks with good FDR (0.05)
- Fold Change
- Fraction of Reads in Peaks (FRiP>5%)
- **Irreproducible discovery rate (IDR - replicates)**

Irreproducible discovery rate (IDR)



IDR



RAD21 Replicates (high reproducibility)

SPT20 Replicates (low reproducibility)

Quality control – ChIP-seq data

Visually

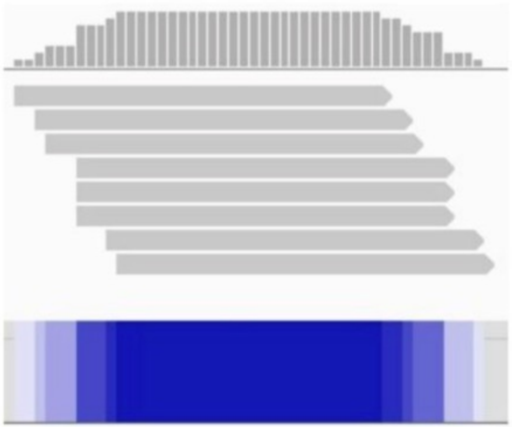
Computationally (peak)

- Num of peaks with good FDR (0.05)
- Fold Change
- Fraction of Reads in Peaks (FRiP>5%)
- Irreproducible discovery rate (IDR - replicates)
- **Standardised standard deviation (SSD)**

Dispersion of coverage

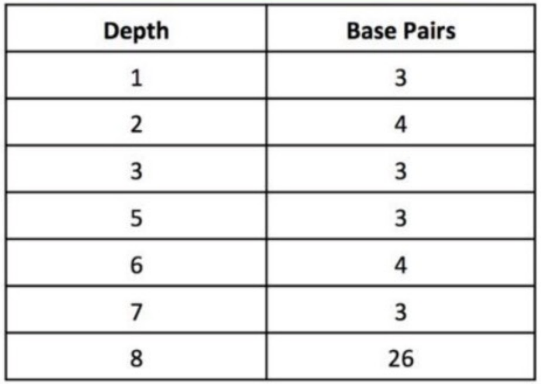
The depth of coverage:

- The number of fragments at a specific genomic region



Expectation:

The depth to have large diversity in an enriched ChIP dataset !



Depth	Base Pairs
1	3
2	4
3	3
5	3
6	4
7	3
8	26

Measure the dispersion of coverage

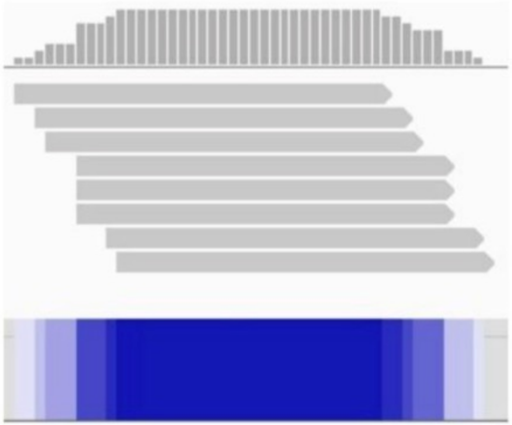
- Based on whole genome pile-up signal

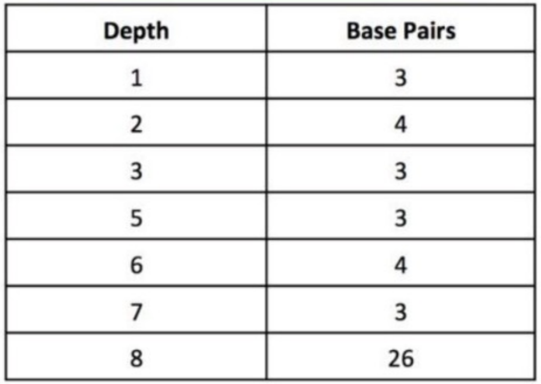
$$SSD = \frac{SD}{\sqrt{n}}$$

An enriched sample: significant pile-up

SSD (higher the better)

- High for samples with enriched regions
- Low for controls with uniform coverage





Depth	Base Pairs
1	3
2	4
3	3
5	3
6	4
7	3
8	26

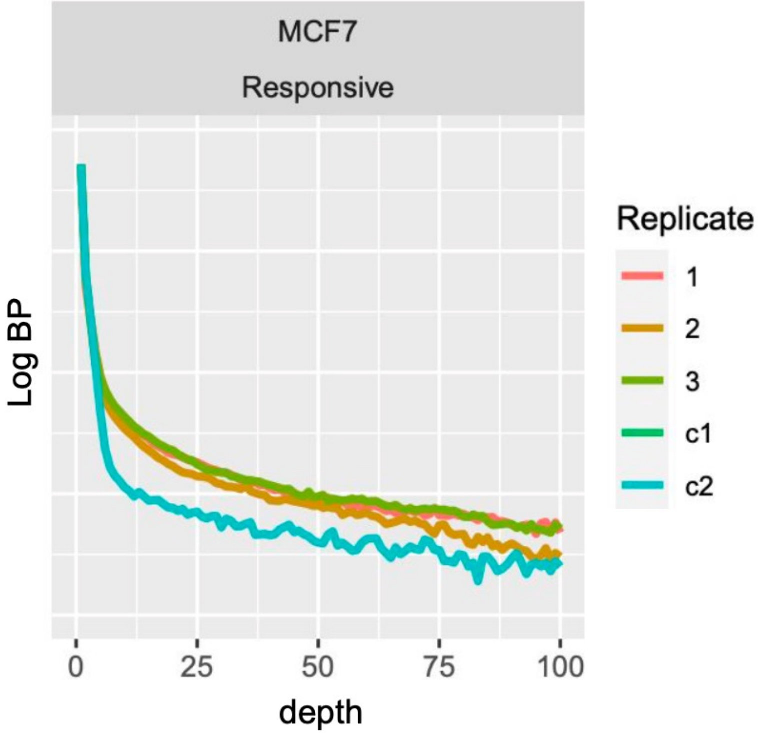
Visualisation

Coverage histogram

X: depth – read pileup height at a bas
pair position

Y: logBP - the number of positions that have this pileup height in log scale

- Good enrichment (1,2,3)
- Input (c1, c2)

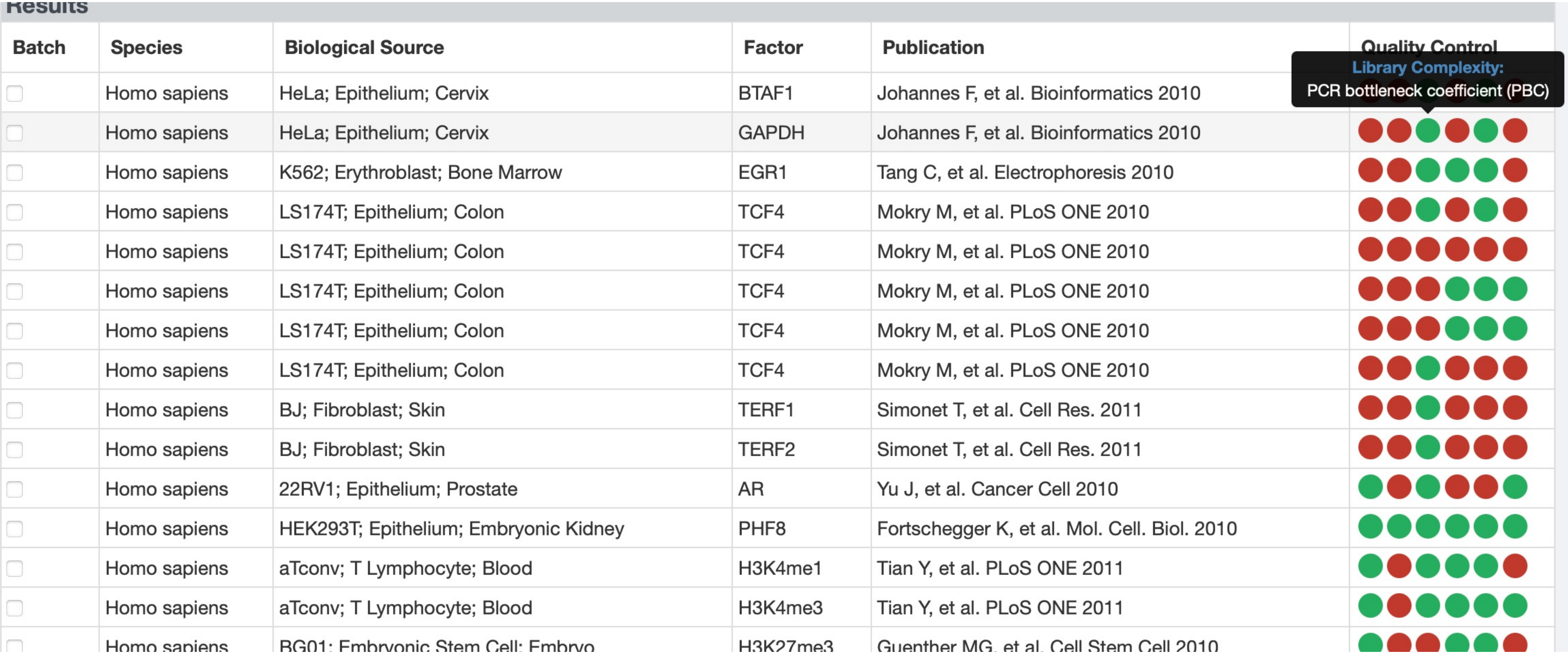


Documentation from bioconductor ChIPQC (https://bioconductor.riken.jp/packages/3.4/bioc/html/ChIPQC.html)
Carroll and Stark

Adapted from Shoko Hirosue

Database

- http://cistrome.org/db



Results

Batch	Species	Biological Source	Factor	Publication	Quality Control
□	Homo sapiens	HeLa; Epithelium; Cervix	BTAF1	Johannes F, et al. Bioinformatics 2010	🔴🔴🟢🔴🟢🔴
□	Homo sapiens	HeLa; Epithelium; Cervix	GAPDH	Johannes F, et al. Bioinformatics 2010	🔴🔴🟢🔴🟢🔴
□	Homo sapiens	K562; Erythroblast; Bone Marrow	EGR1	Tang C, et al. Electrophoresis 2010	🔴🔴🟢🟢🟢🔴
□	Homo sapiens	LS174T; Epithelium; Colon	TCF4	Mokry M, et al. PLoS ONE 2010	🔴🔴🟢🔴🟢🔴
□	Homo sapiens	LS174T; Epithelium; Colon	TCF4	Mokry M, et al. PLoS ONE 2010	🔴🔴🔴🔴🔴🔴
□	Homo sapiens	LS174T; Epithelium; Colon	TCF4	Mokry M, et al. PLoS ONE 2010	🔴🔴🔴🟢🟢🟢
□	Homo sapiens	LS174T; Epithelium; Colon	TCF4	Mokry M, et al. PLoS ONE 2010	🔴🔴🔴🟢🟢🟢
□	Homo sapiens	LS174T; Epithelium; Colon	TCF4	Mokry M, et al. PLoS ONE 2010	🔴🔴🟢🔴🔴🔴
□	Homo sapiens	BJ; Fibroblast; Skin	TERF1	Simonet T, et al. Cell Res. 2011	🔴🔴🟢🔴🔴🔴
□	Homo sapiens	BJ; Fibroblast; Skin	TERF2	Simonet T, et al. Cell Res. 2011	🔴🔴🟢🔴🔴🔴
□	Homo sapiens	22RV1; Epithelium; Prostate	AR	Yu J, et al. Cancer Cell 2010	🟢🔴🟢🔴🔴🟢
□	Homo sapiens	HEK293T; Epithelium; Embryonic Kidney	PHF8	Fortschegger K, et al. Mol. Cell. Biol. 2010	🟢🟢🟢🟢🟢🟢
□	Homo sapiens	aTconv; T Lymphocyte; Blood	H3K4me1	Tian Y, et al. PLoS ONE 2011	🟢🔴🟢🟢🟢🔴
□	Homo sapiens	aTconv; T Lymphocyte; Blood	H3K4me3	Tian Y, et al. PLoS ONE 2011	🟢🔴🟢🟢🟢🟢
□	Homo sapiens	BG01; Embryonic Stem Cell; Embryo	H3K27me3	Guenther MG, et al. Cell Stem Cell 2010	🟢🔴🔴🟢🟢🔴

Library Complexity:
PCR bottleneck coefficient (PBC)

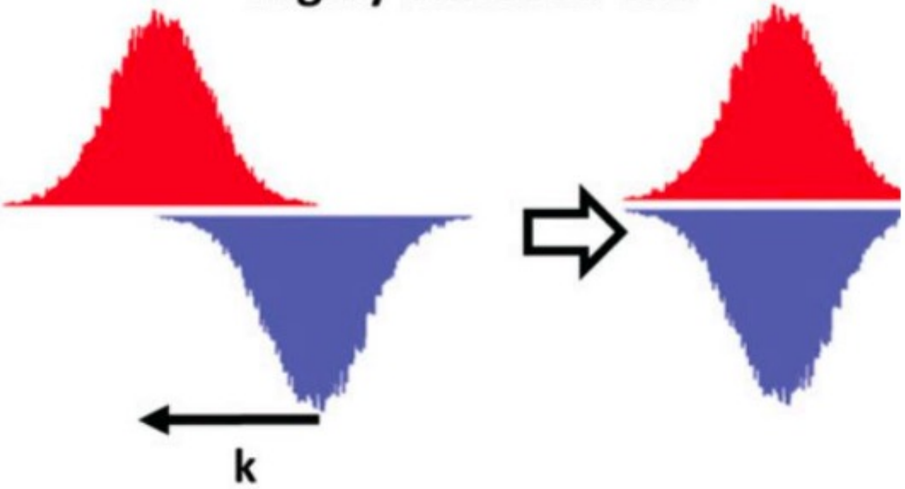
Lunch break before practical

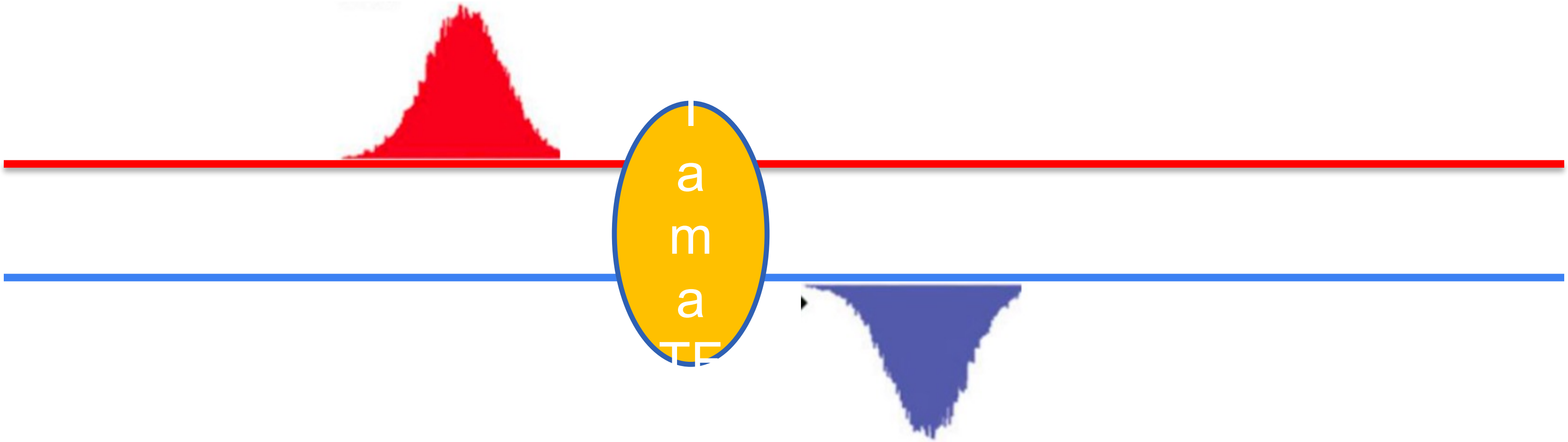
Back at 13.30

Supplementary

- Strand cross-correlation

Highly enriched ChIP

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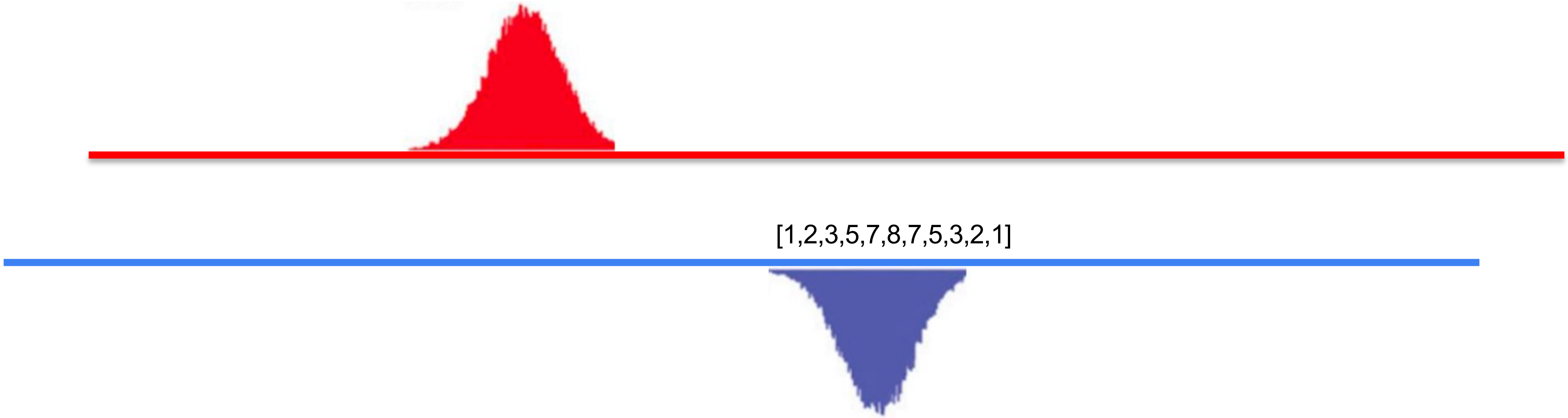
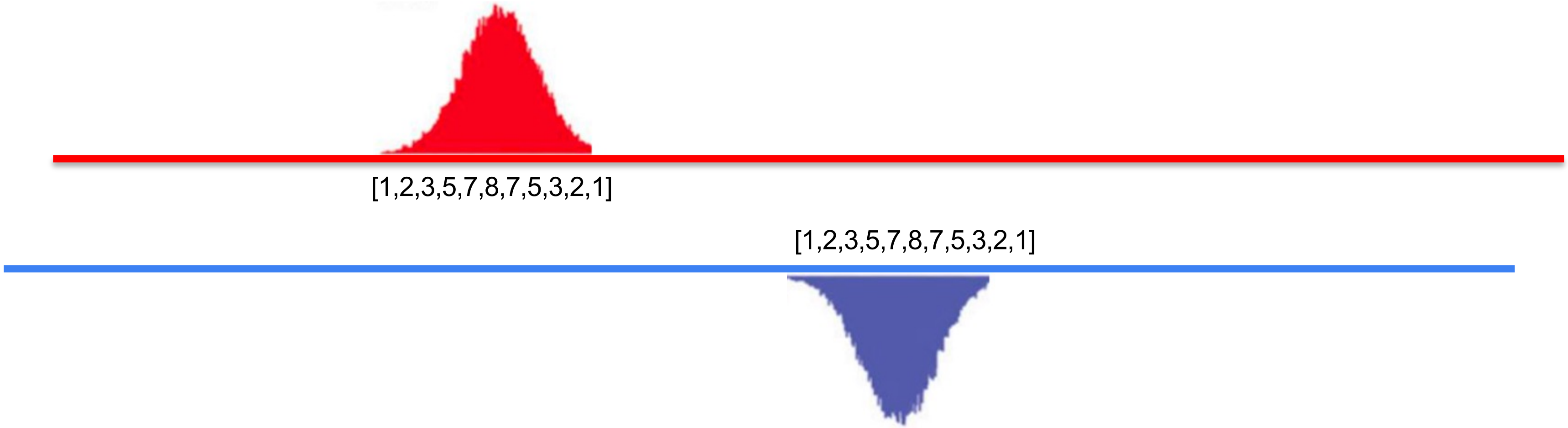
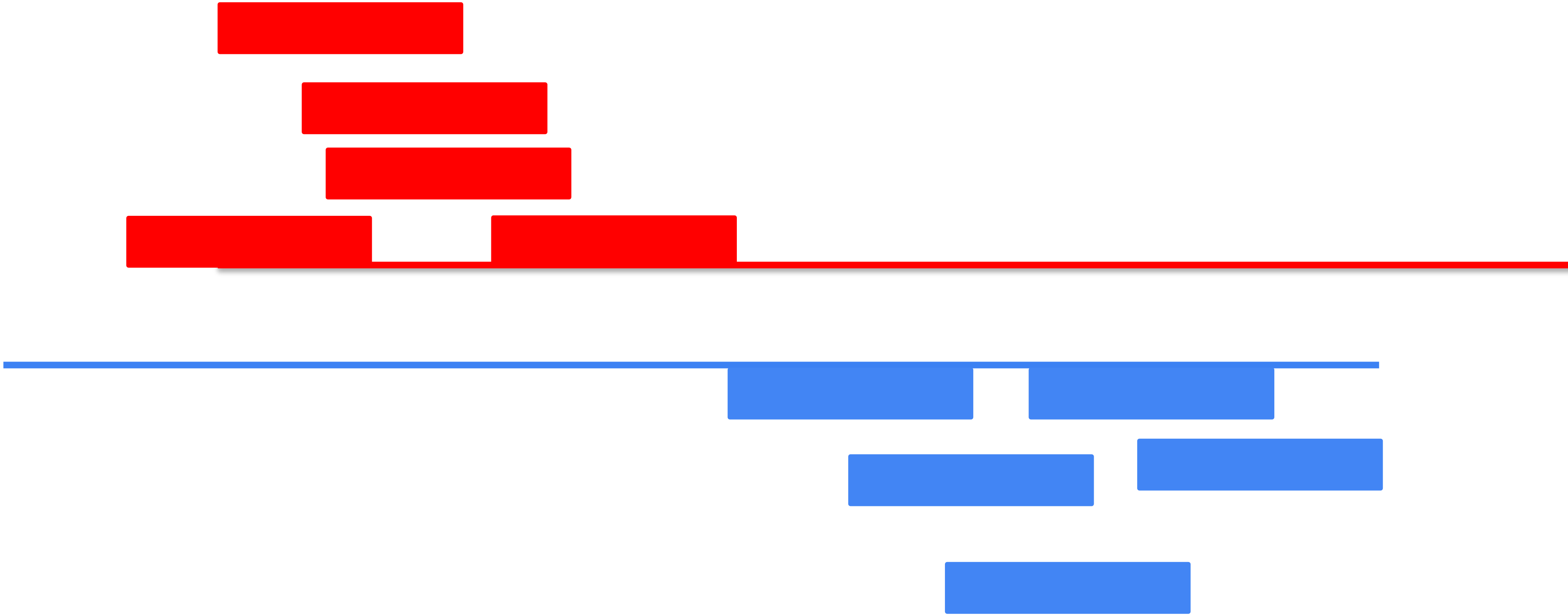


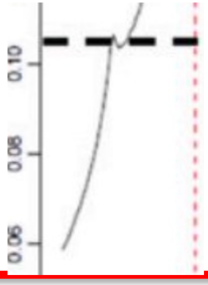
[1,2,3,5,7,8,7,5,3,2,1]

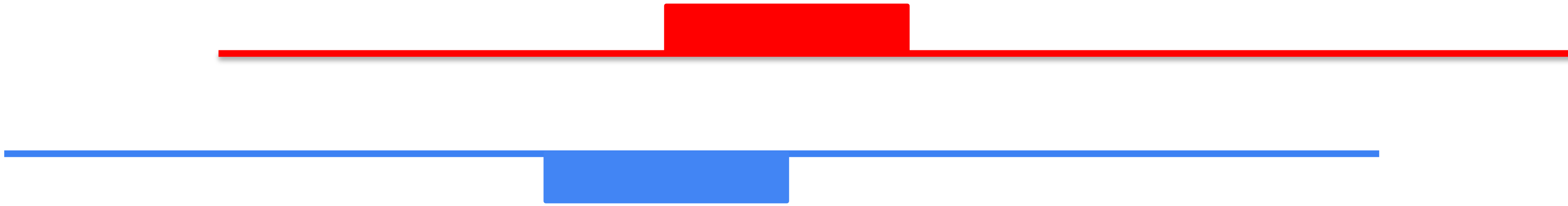


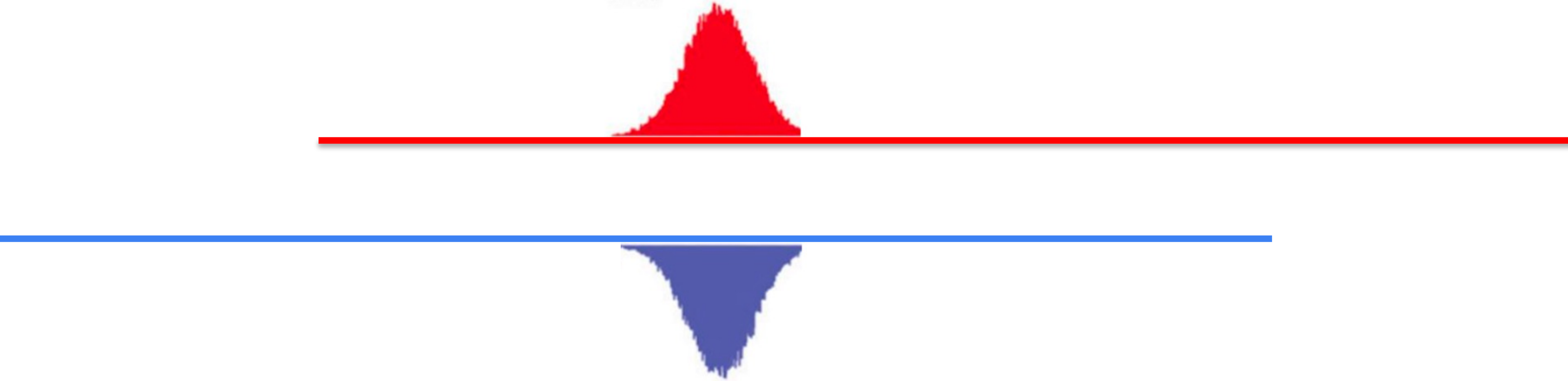
[1,2,3,5,7,8,7,5,3,2,1]

[1,2,3,5,7,8,7,5,3,2,1]




0.10
0.08
0.06





- https://www.youtube.com/watch?v=XWcWn8dt4c8