Best practices in the analysis of RNA-seq and ChIP-seq data
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The quality of a ChIP-seq data

Ines de Santiago
CRUK Cambridge Research Institute
Ines.desantiago@cruk.cam.ac.uk

UNIVERSITY OF CAMBRIDGE

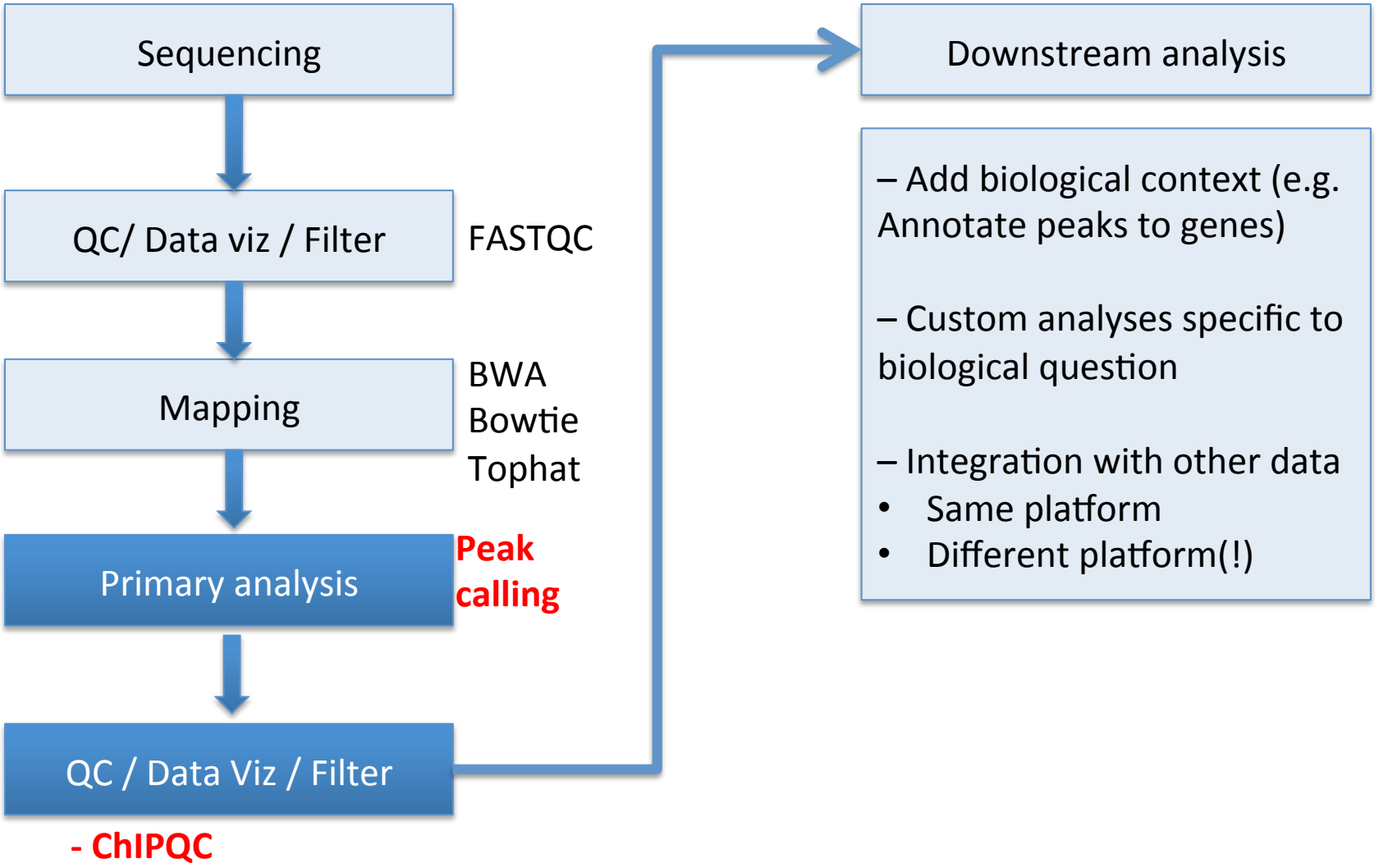
CAMBRIDGE Cancer CENTRE

CANCER RESEARCH UK

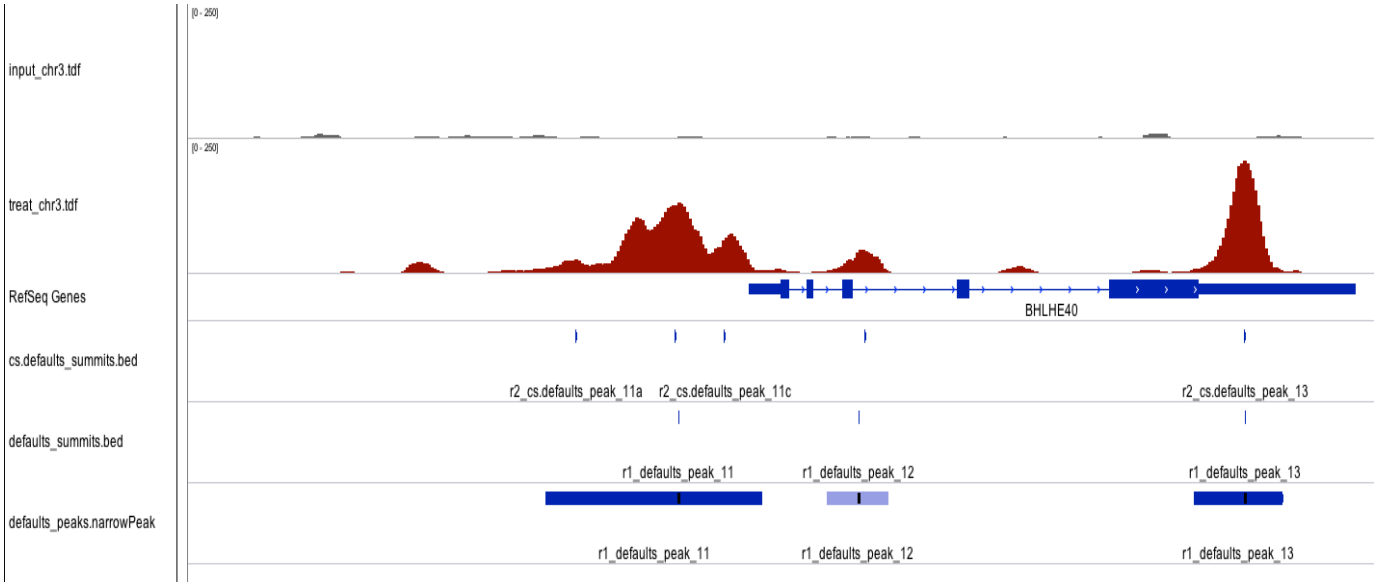
Acknowledgments

- Tom Carroll
  - http://bioconductor.org/help/course-materials/2014/BioC2014/ChIPQC_Presentation.pdf
  - https://github.com/bioinformatics-core-shared-training/ngs-in-bioc/blob/master/Lectures/Lect6b_ChIP-Seq%20Data%20Analysis.pdf
- Shamith Samarajiwa
- Suraj Menon

“Typical” ChIP-Seq Analysis workflow



A good ChIP-seq dataset



Characteristics we can assess quantitatively:

- Reads in peaks
- Peaks higher than background
- Genes close by?
- Enough seq depth?
- Diverse library (duplications)
- Not enriched in the control

What do we want:

- Good quality ChIP-seq enrichment over background

How to quantify ChIP-seq data quality?

- **ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia.**

  (Landt et al – *Genome Research 2012*)

- **ChIPQC –** Tom Carroll and Rory Stark *(Diffbind)*
- **ChIPQC** provides workflow to generate metrics per sample/experiment.
- package **SPP** (for UNIX/LINUX)

What can go wrong?

- **The specificity of the antibody**
  - poor reactivity against the intended target
  - cross-reactivity with other DNA-associated proteins.

- **degree of enrichment** achieved in the affinity precipitation step.

- **Biases during library preparation**:
  - PCR amplification biases
  - Fragmentation biases

ENCODE guidelines for experiments: https://genome.ucsc.edu/ENCODE/experiment_guidelines.html

EVALUATING CHIP-SEQ DATA (QC)

Outline

- **Distribution of Signal**
  - Visualisation of coverage profiles
  - Fraction of reads in peaks (FRIP)
  - Relative enrichment in genomic intervals (REGI)
  - Signal in blacklists (FRIBL)
  - Dispersion of coverage
- **Clustering of Watson/Crick reads.**
- **Other factors affecting site discovery:**
  - Sequencing depth
  - Duplication rate / library complexity
  - Control sample

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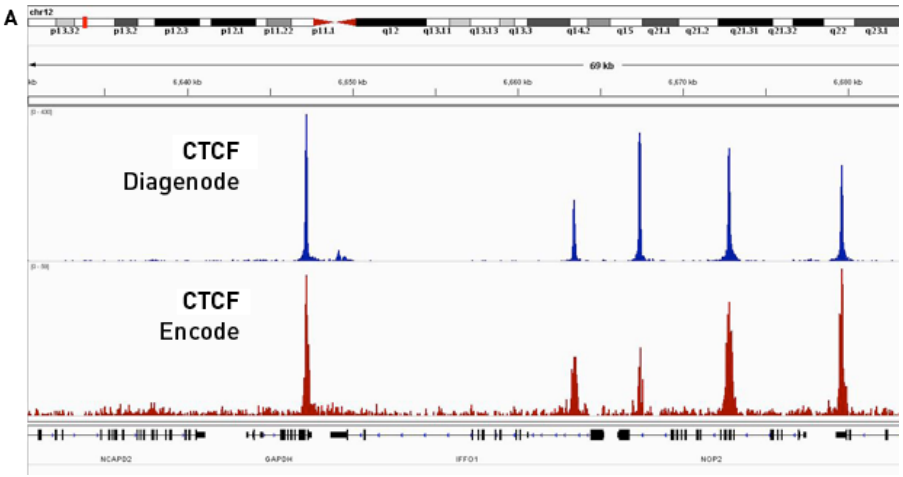
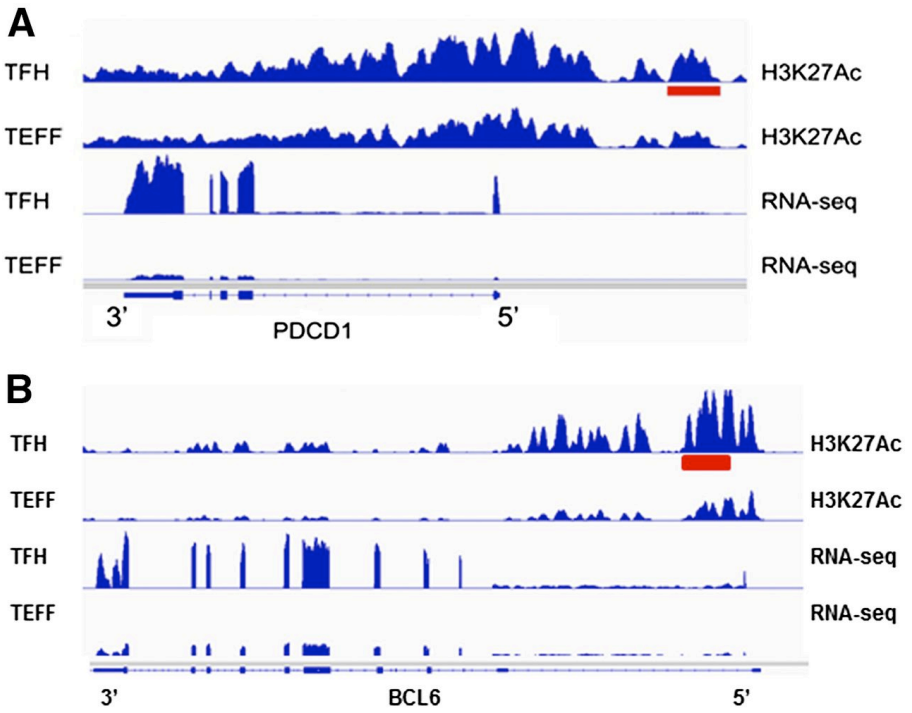
Visualisation of coverage profiles





http://www.bloodjournal.org/content/124/25/3719

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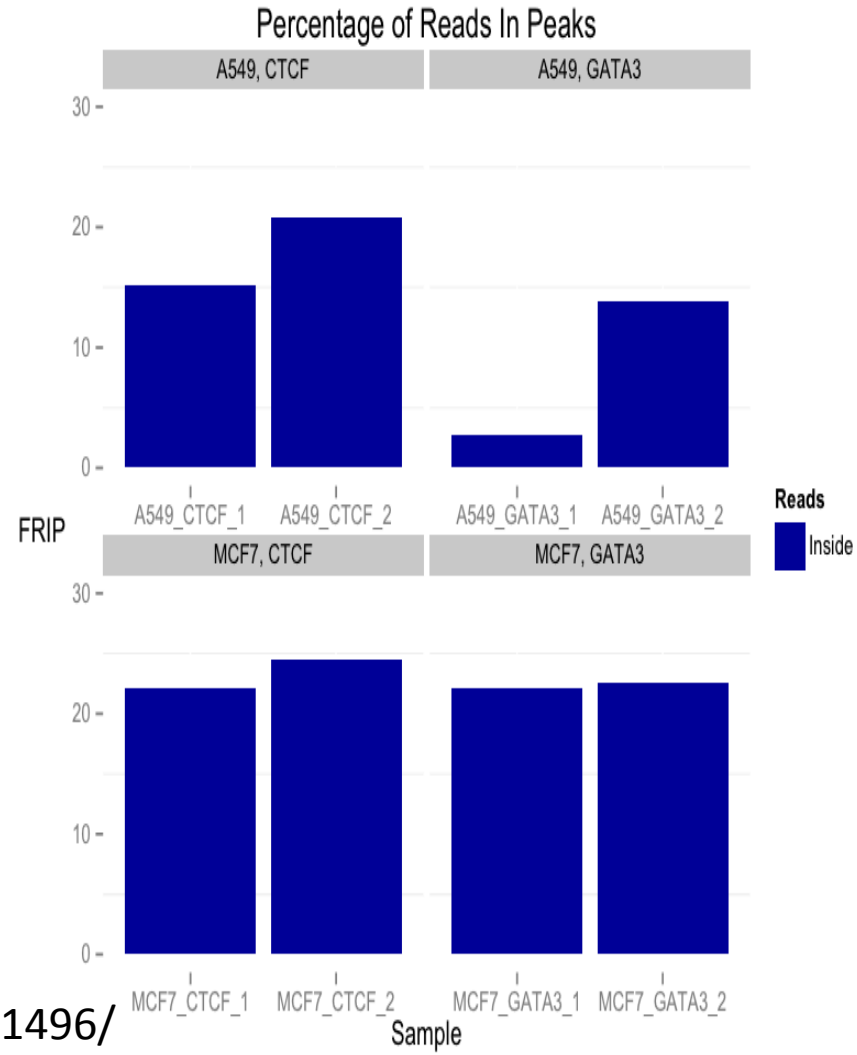
Measuring global ChIP enrichment (FRiP)

- useful and simple first-cut metric for the success of the immunoprecipitation

- Good quality TF > 5%

(guideline, known examples of good data with FRiP < 1% RNAPIII and ZNF274)

Example output from ChIPQ package:



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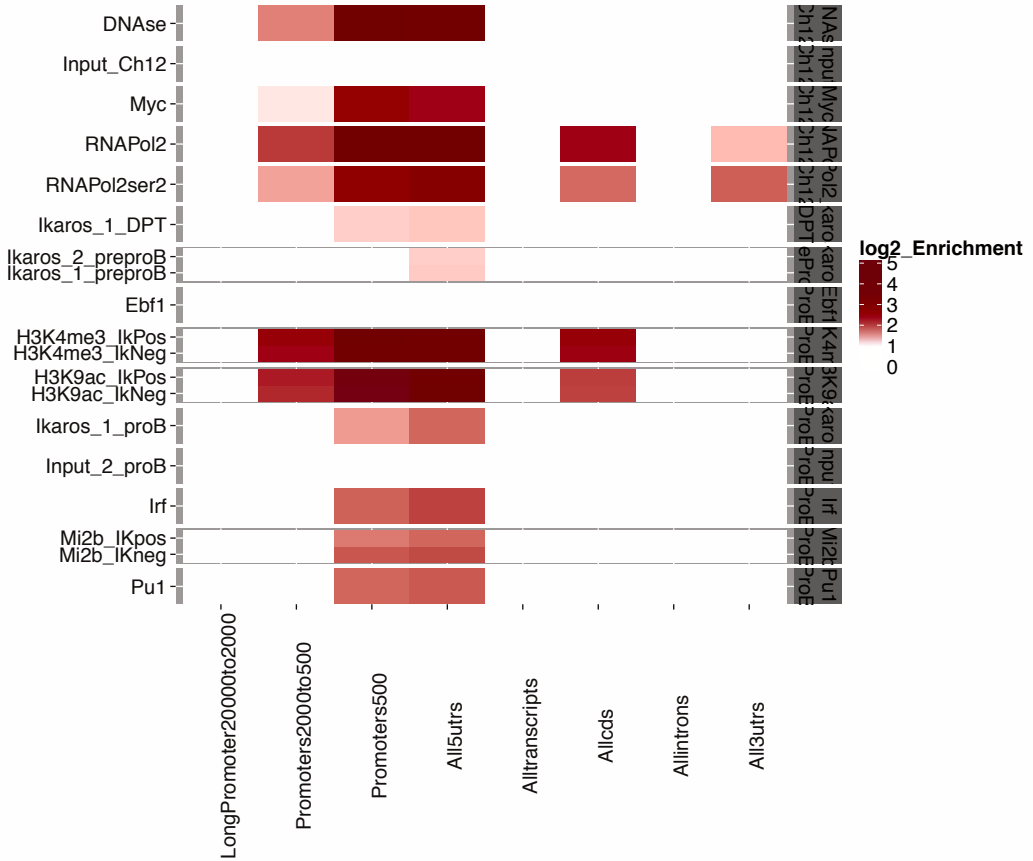
Enrichment in genomic intervals

- Plot relative enrichment of reads in annotated regions.

Example output from ChIPQ package:



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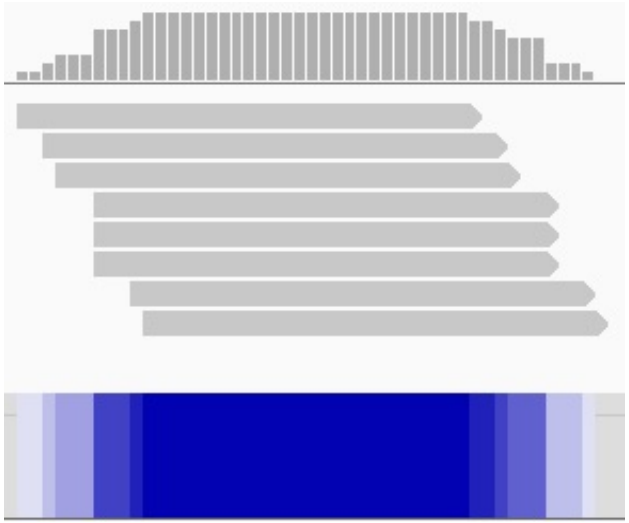
Dispersion of coverage

• depth of coverage: number of fragments at a genomic location.

• Expectation is that for an enriched ChIP sample, depth should show inequality in dispersion across the genome

• Build global profile of signal depth
  - Measure number of base pairs with given depth of signals.
  - Normalise to total number of reads to compare samples

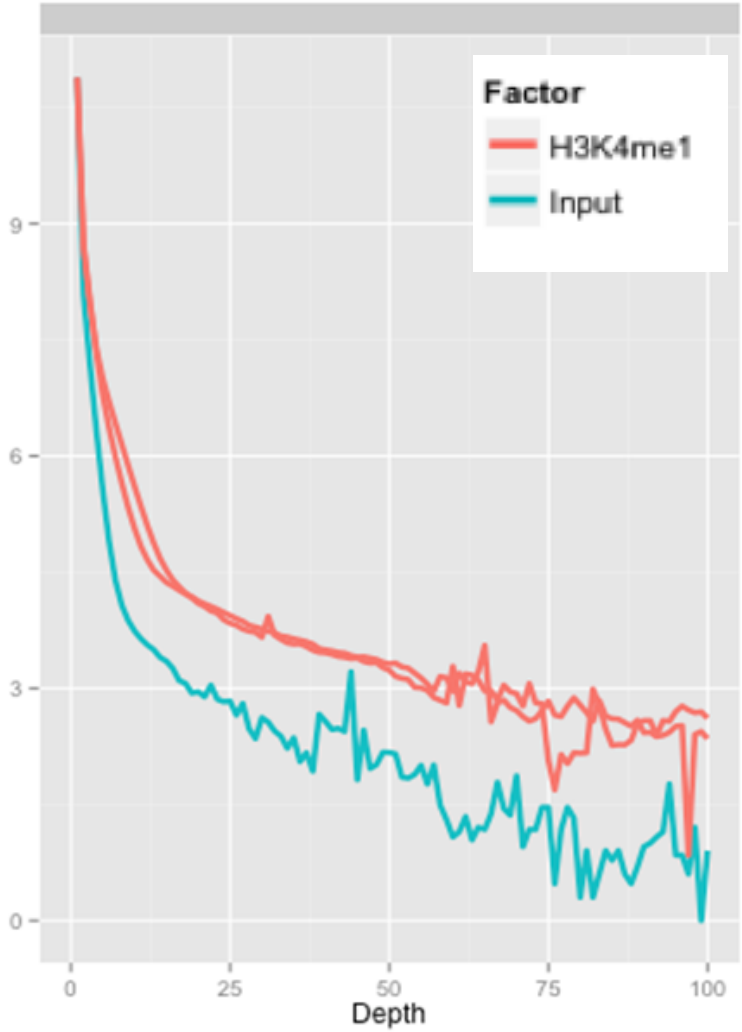


Depth	Base Pairs
1	3
2	4
3	3
5	3
6	4
7	3
8	26

Dispersion of coverage

- Global signal profile “histogram”
- Enriched (ChIP) libraries show higher number of bases at greater depths.
- Profile for inputs (no enrichment) drops off more quickly
- Gap between sample and input indicates enrichment

Example output from ChIPQ package:



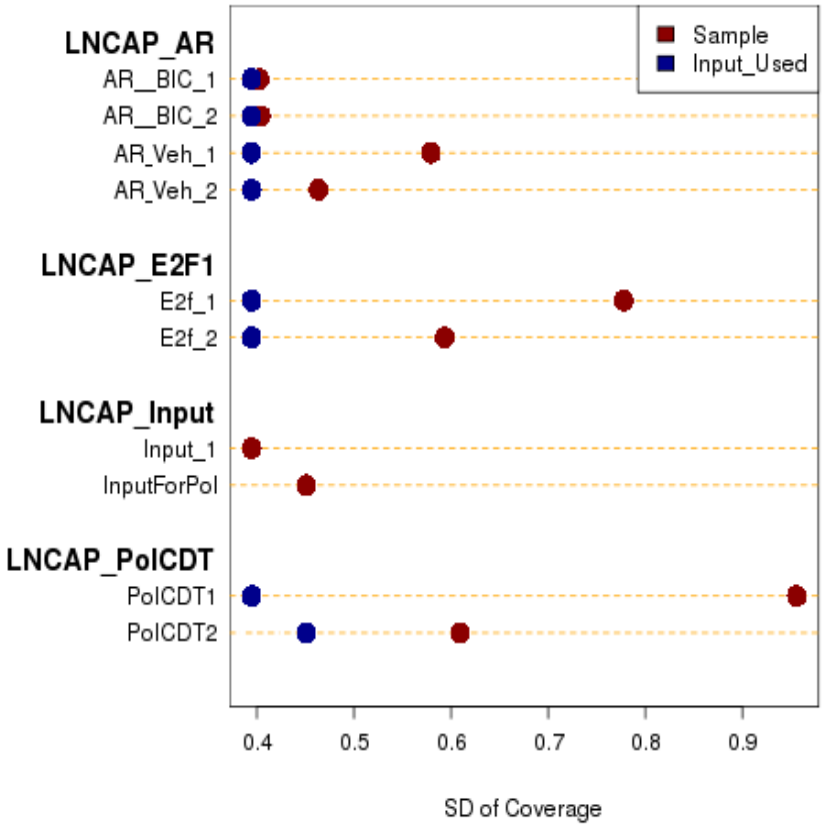
Metric for dispersion of coverage: SSD

- SSD: Standardised Standard Deviation of coverage

- Metric for assessment of dispersion coverage developed in htseqtools package

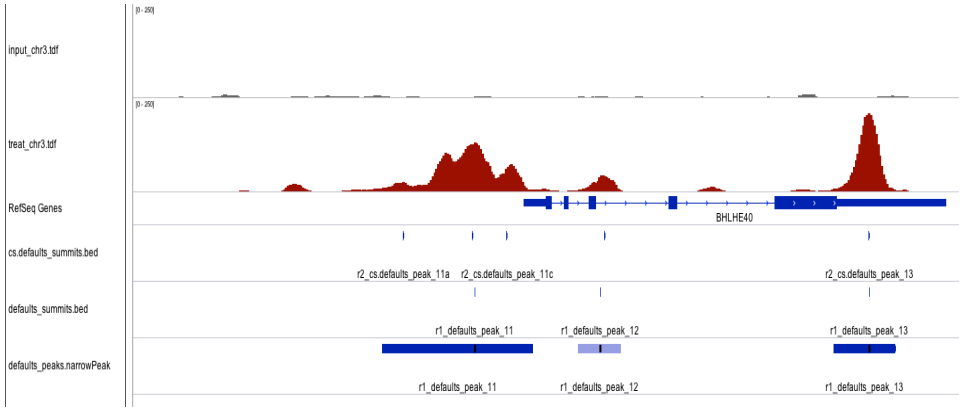
$$SSD = \frac{SD}{\sqrt{n}}$$

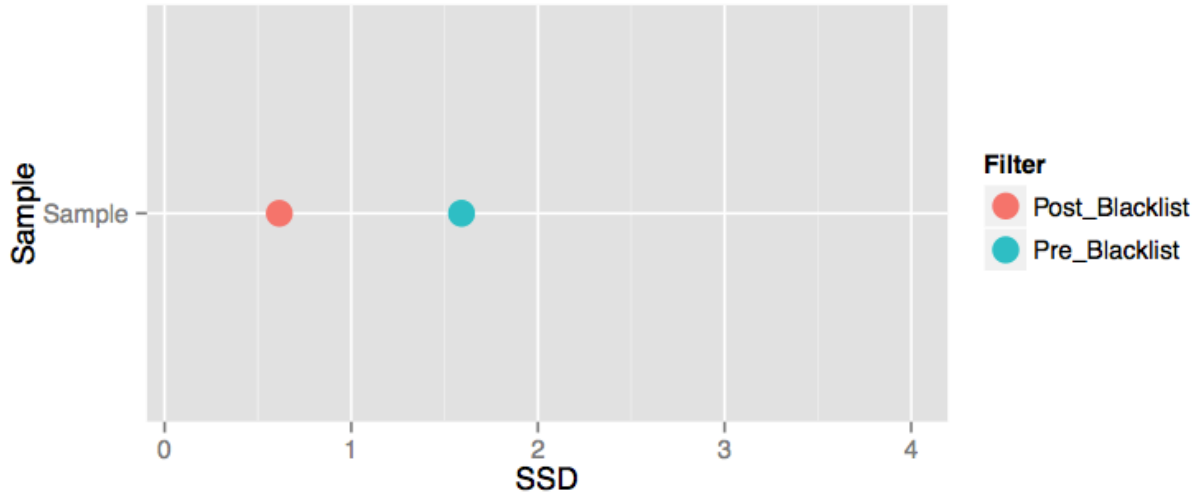
- Provides measure of pile-up across genome
  - High for samples with enriched regions (ChIP)
  - Low for samples with uniform coverage (input)



SSD is highly influenced by blacklists

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





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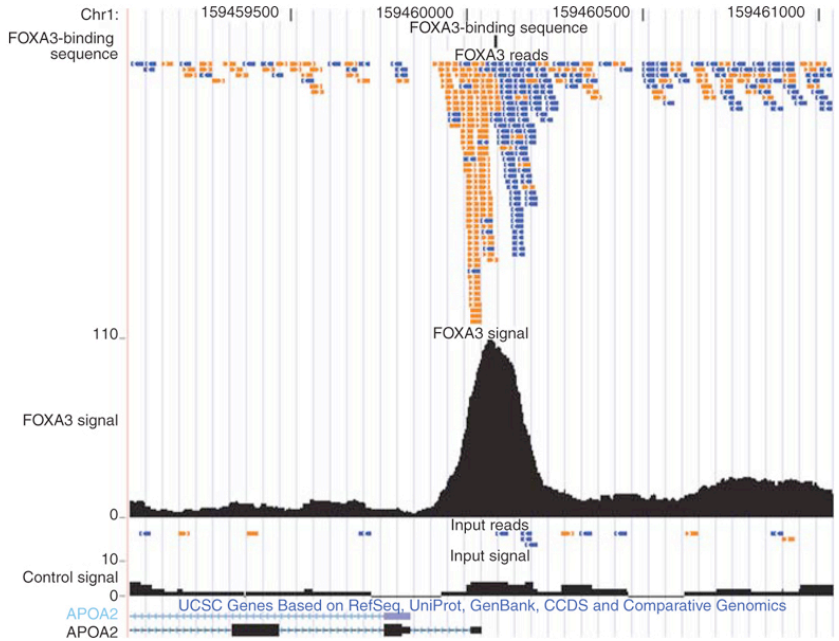
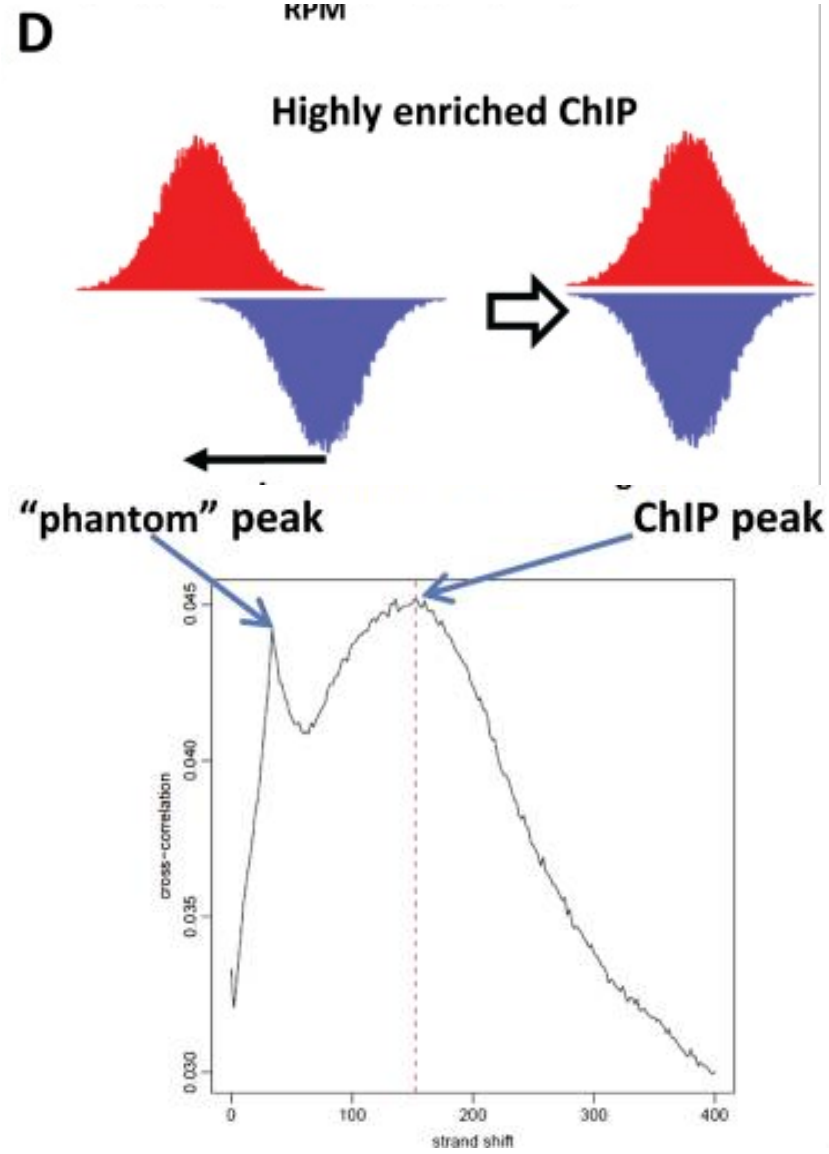
Clustering of Watson/Crick reads



http://www.nature.com/nmeth/journal/v6/n4/images/nmeth.f.247-F2.jpg

http://www.bloodjournal.org/content/124/25/3719

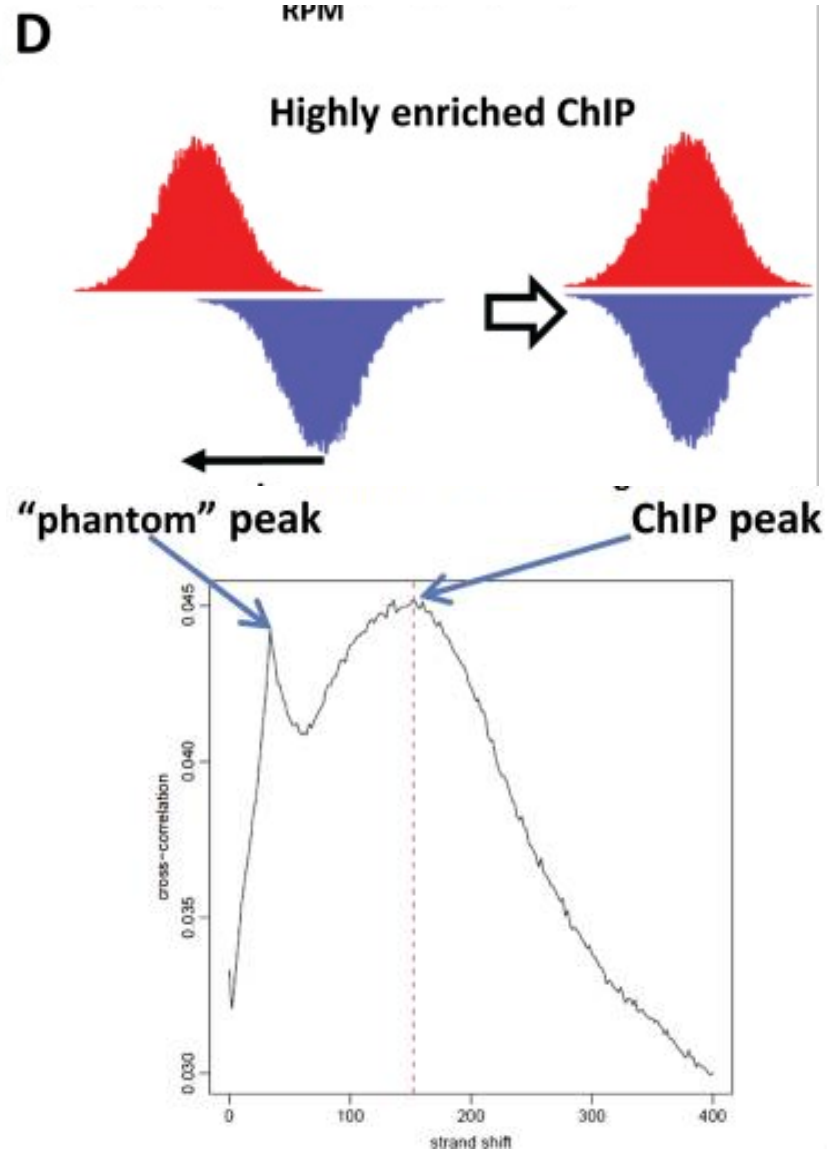
How to make a cross-correlation plot:



Clustering of Watson/Crick reads

- Fragment length can be estimated from data:
  - **Cross-correlations -** Correlation of reads on positive and negative strand after successive read shifts
  - **Cross-coverage -** Coverage of reads on both strand after successive shifts of reads on one strand. Total area covered by reads will be reduced after shifting
- These provide useful QC metrics

How to make a cross-correlation plot:

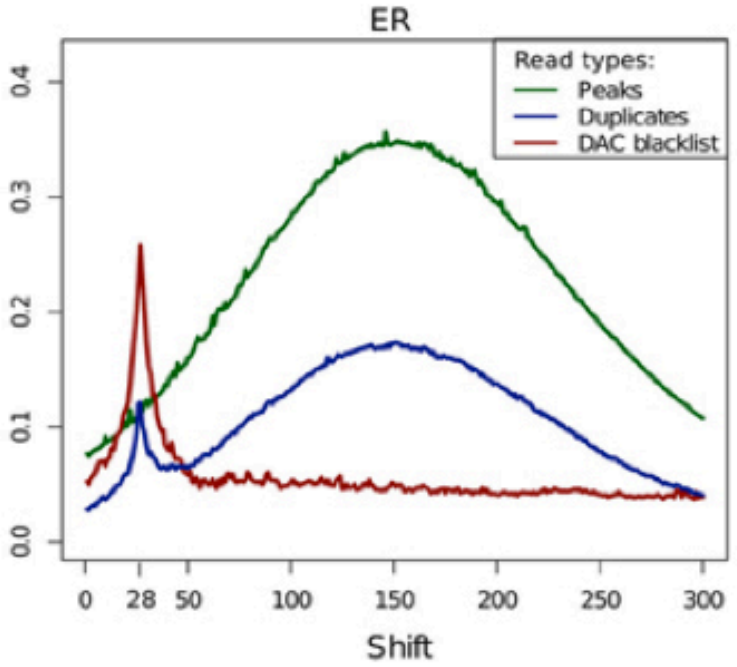


Clustering of Watson/Crick reads

- Cross-coverage score plots are computed by ChIPQC R package

- ChIPQC metrics:
  - *FragCC = $CC_{fragmentlength}$*
  - *RelCC = FragCC/ $Cc_{readlength}$*
  - RelCC > 1 good ChIP-seq

- Blacklisted regions strongly contribute to read length cross-coverage peak



Carroll et al., Front Genet. 2014 Apr 10;5:75.

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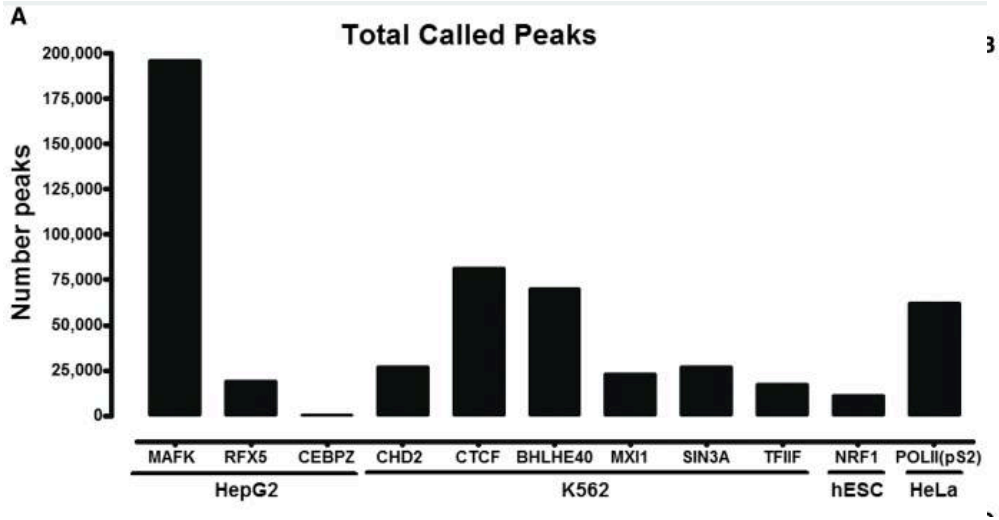
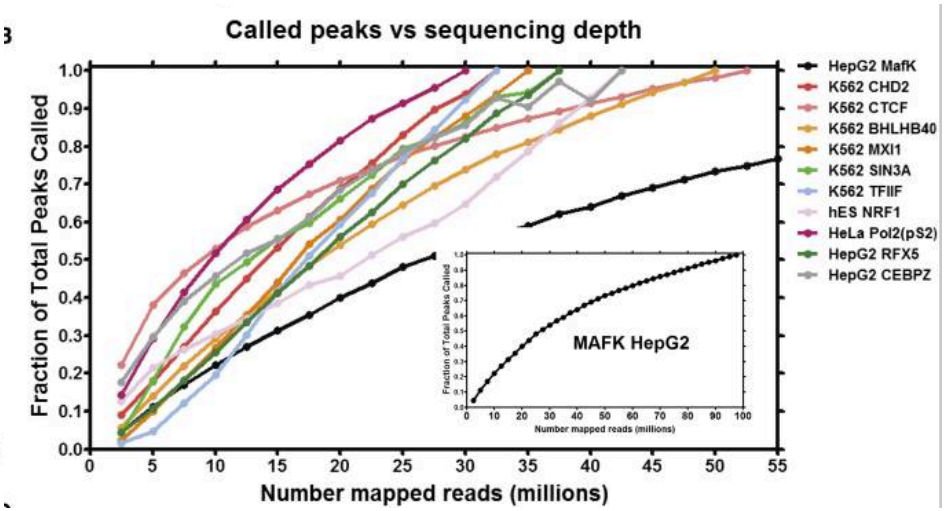
Sequencing Depth

Peak counts depend on sequencing depth.





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Sequencing Depth: guidelines

Sharp peaks (TFs)

- 10M reads
- 2M worms and flies

Broad Peaks (Histones)

- 20M reads mammalian genomes
- 5M worms and flies

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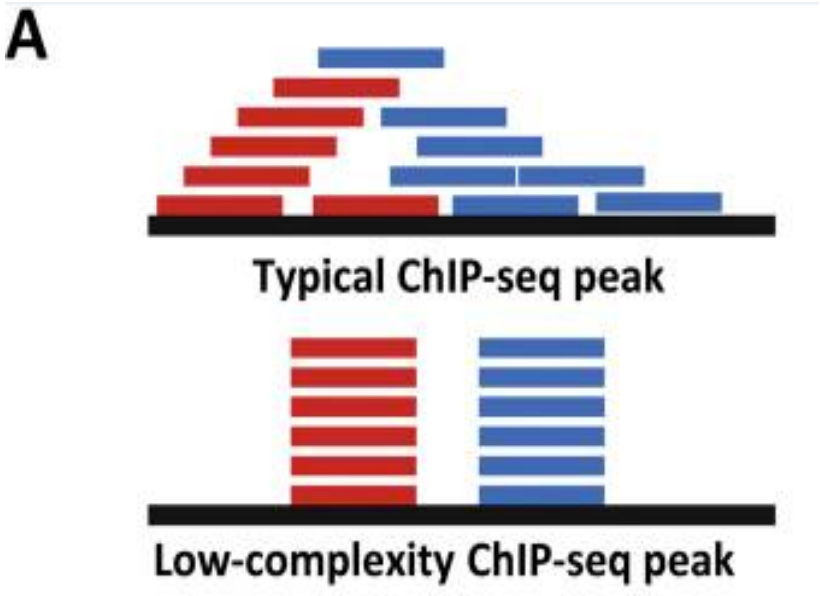
Library complexity (Duplicates)



- Duplication rates are a useful QC metric
  - (Duplicate reads/Total Mapped Reads) *100
  - Expected to be low (<~ 1%) for inputs
- Non-Redundant Fraction (NRF)
  - ENCODE guidelines:

    NRF >= 0.8 for 10M reads

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3431496/

Library complexity (Duplicates)

- Duplicates can be artefacts
- PCR bias: certain genomic regions are preferentially amplified
- Low initial starting material
  - Overamplification -> artificially enriched regions
  - Compounded by PCR bias

- Duplicates can also be 'legitimate'
  - In highly efficient enrichments
  - In deeply sequenced ChIPs

  (Duplication rate increases with sequencing depth)

- Removing these duplicates limits the dynamic range of ChIP signal
  - Max signal for a base is (2*read length)-1

Library complexity (Duplicates)

- So what to do about duplicates?
- Keep in mind enrichment efficiency and read depth
- Thumb-rules
  - Remove duplicates prior to peak calling (some peak callers do this by default)
  - Keep duplicates for differential binding analysis
- A more objective approach:
  - htSeqTools package
  - Estimate duplicate numbers expected for sequencing depth using negative binomial model and attempt to identify significantly anomalous duplicate numbers.

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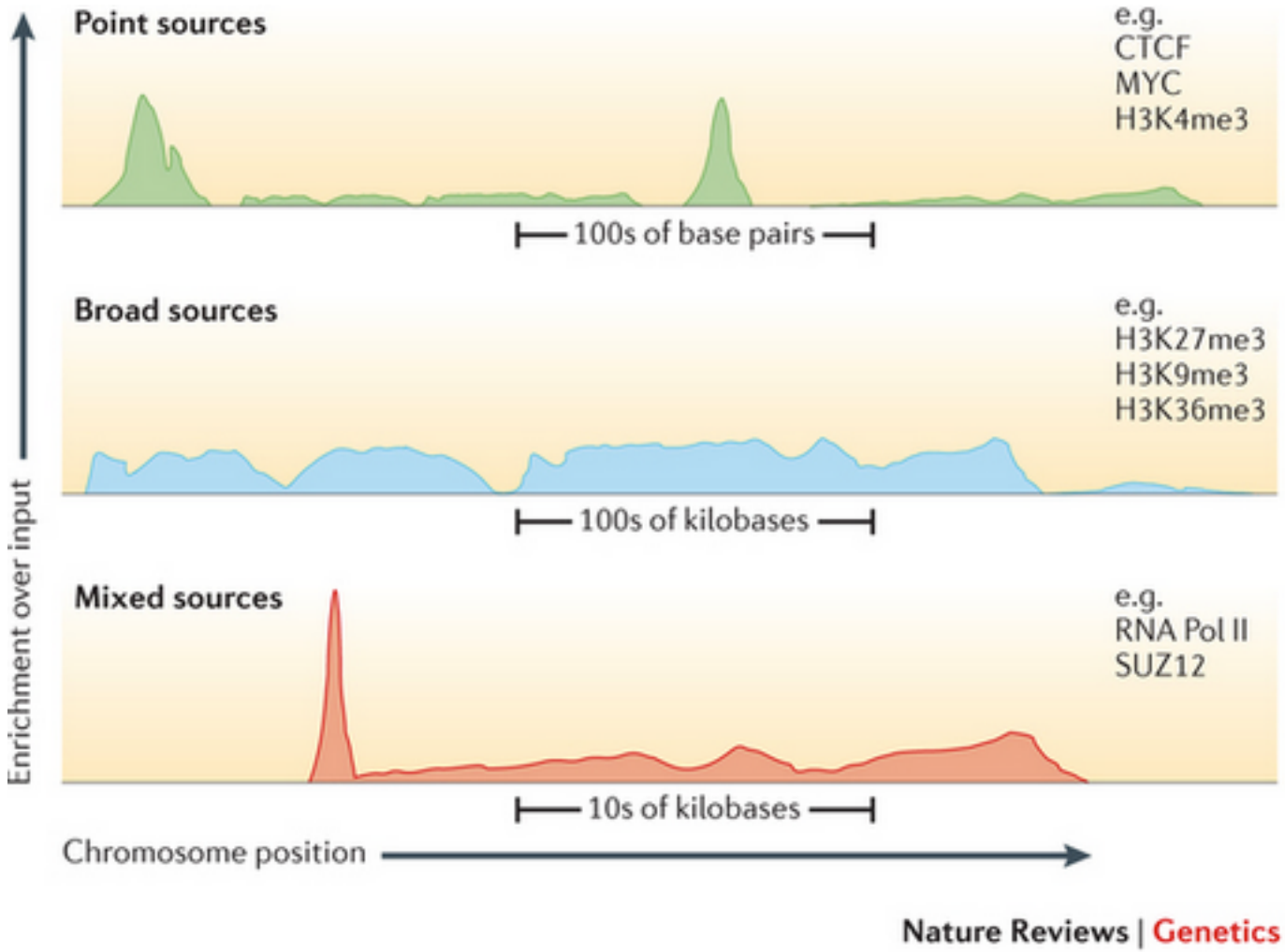
Control sample

- Use of controls **highly** recommended
- **Input DNA**
  - popularly used
  - controls for CNVs, sequencing biases, fragmentation and shearing biases
- **IgG**
  - as with input but also controls for non-specific binding
  - but introduces new biases
- Controls required for
  - different types of samples (e.g. Cell lines, mice, patients)
  - different treatment groups / experimental conditions

PEAK CALLING

Narrow vs Broad peaks



http://www.nature.com/nrg/journal/v15/n2/full/nrg3642.html

Peak Calling:
Which Peak Caller to Use?

- Transcription factor peaks: **MACS** is very popular
- For histone marks with spanning longer regions, **Sicer** is recommended
  - MACS can be used by tweaking parameters
- Several peak callers in R/Bioconductor
  - e.g SPP, TPIC, BayesPeak
  - Not really considered gold-standard (other than SPP)
  - Often impractical: memory hungry and slow

ChIP-Seq Practical

Working with ChIP-Seq Data in R/Bioconductor

chipqc_sweave.pdf