ChIP-Seq/CUT&Tag/CUT&RUN

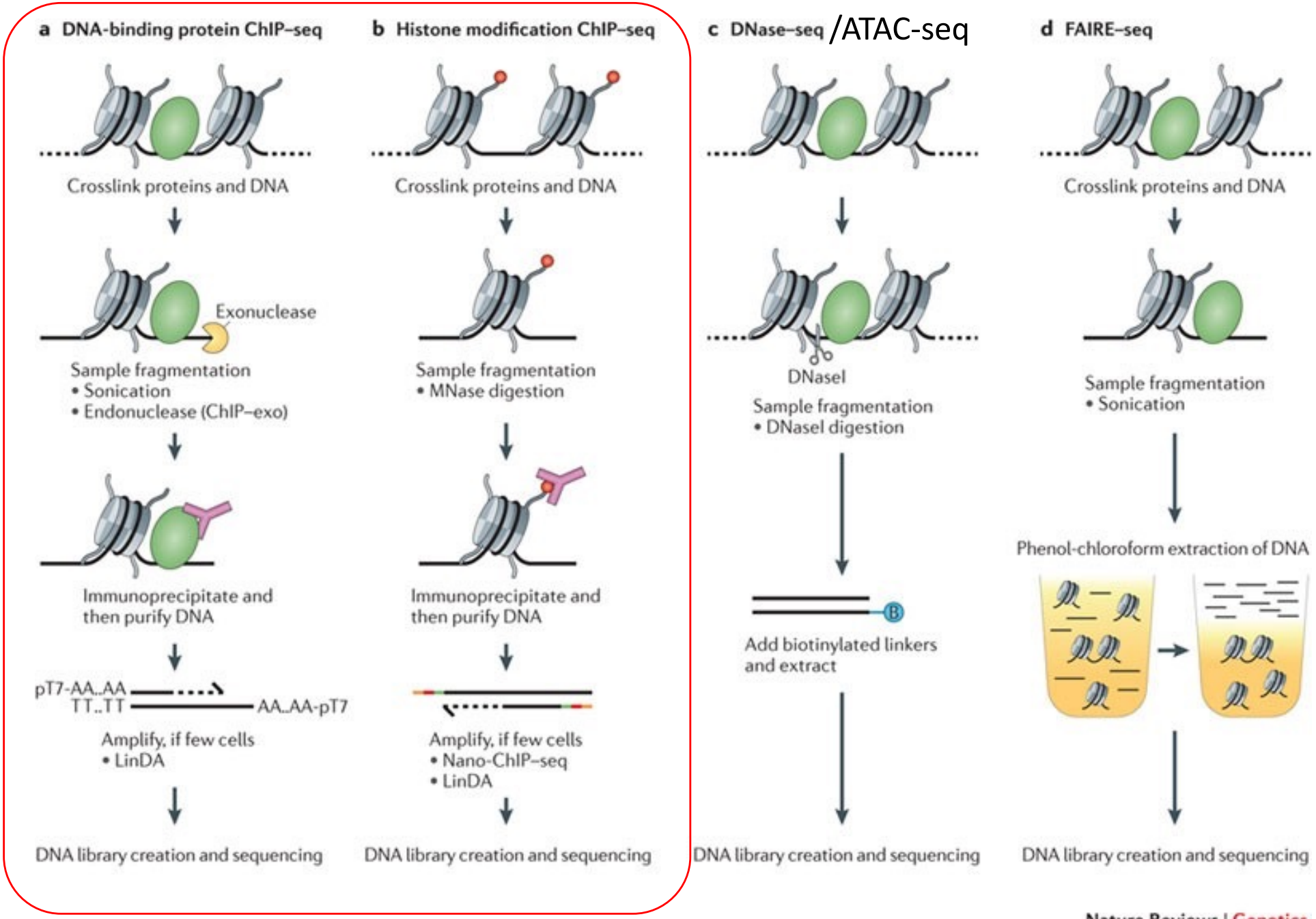
Simon Elsässer, KI/SciLifeLab/Freiburg University

simon.elsasser@scilifelab.se

Excercises – big thanks to Carmen

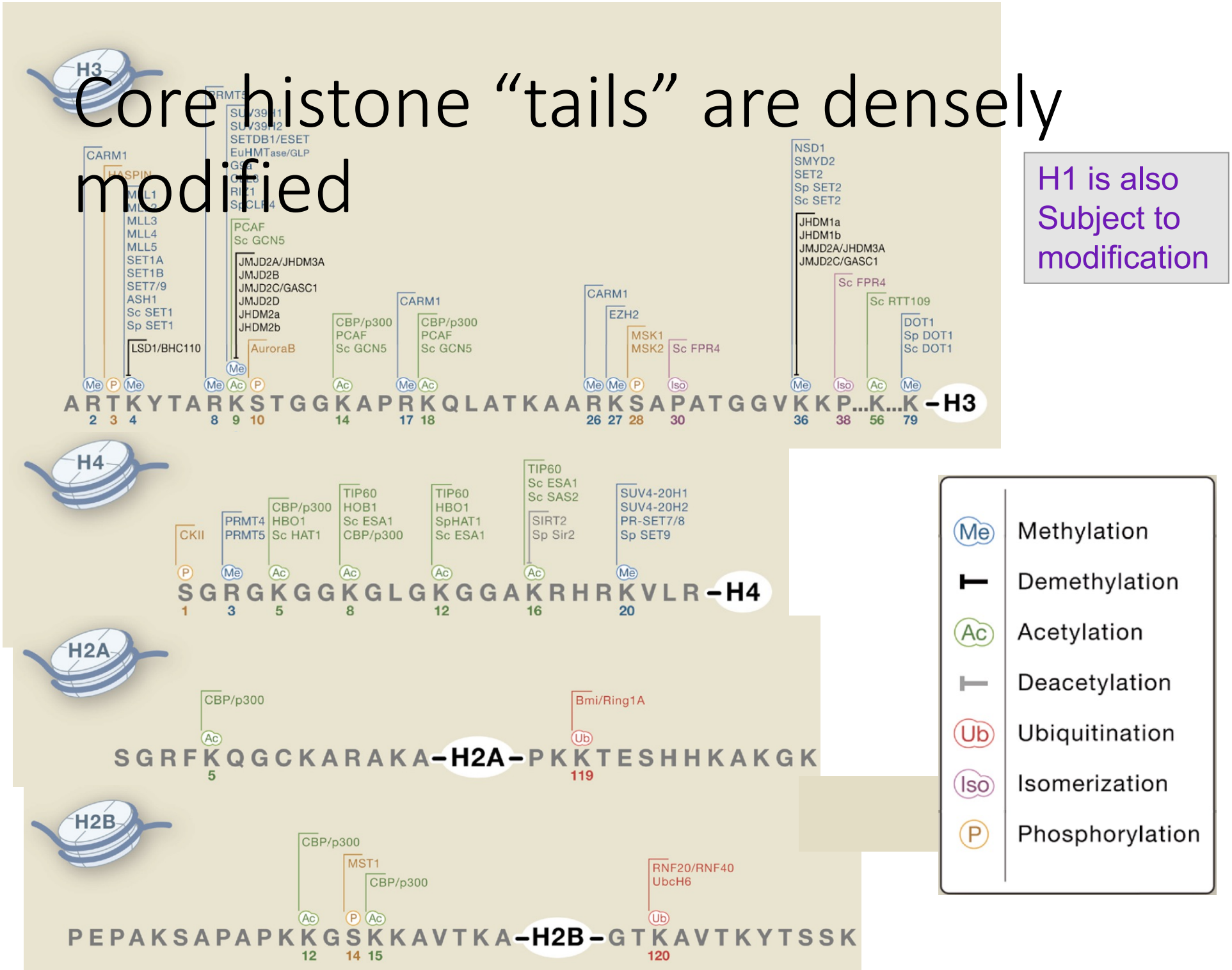
Carmen Navarro

Probing gene regulatory mechanism



Core histone “tails” are densely modified

H1 is also Subject to modification

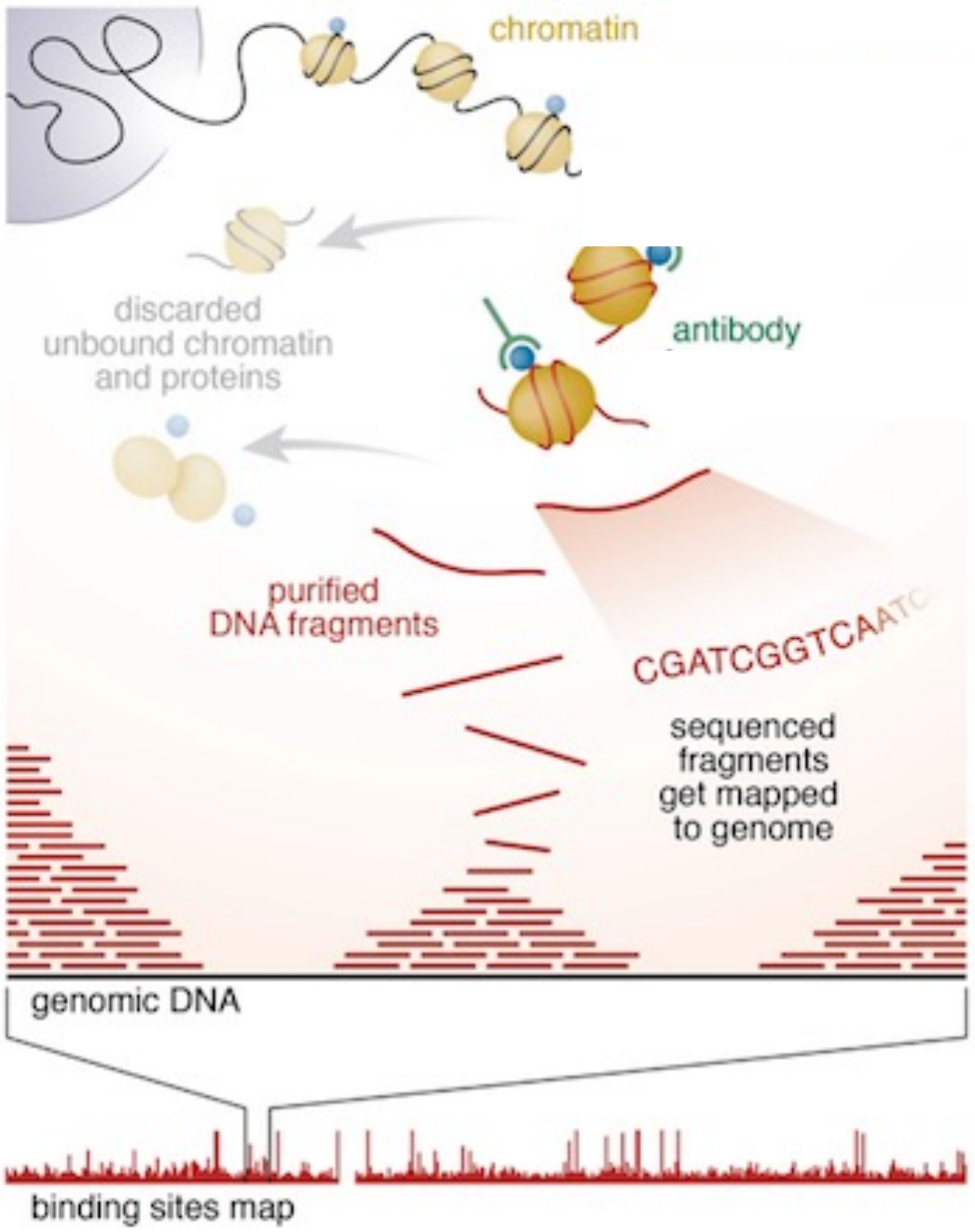


Experimental considerations

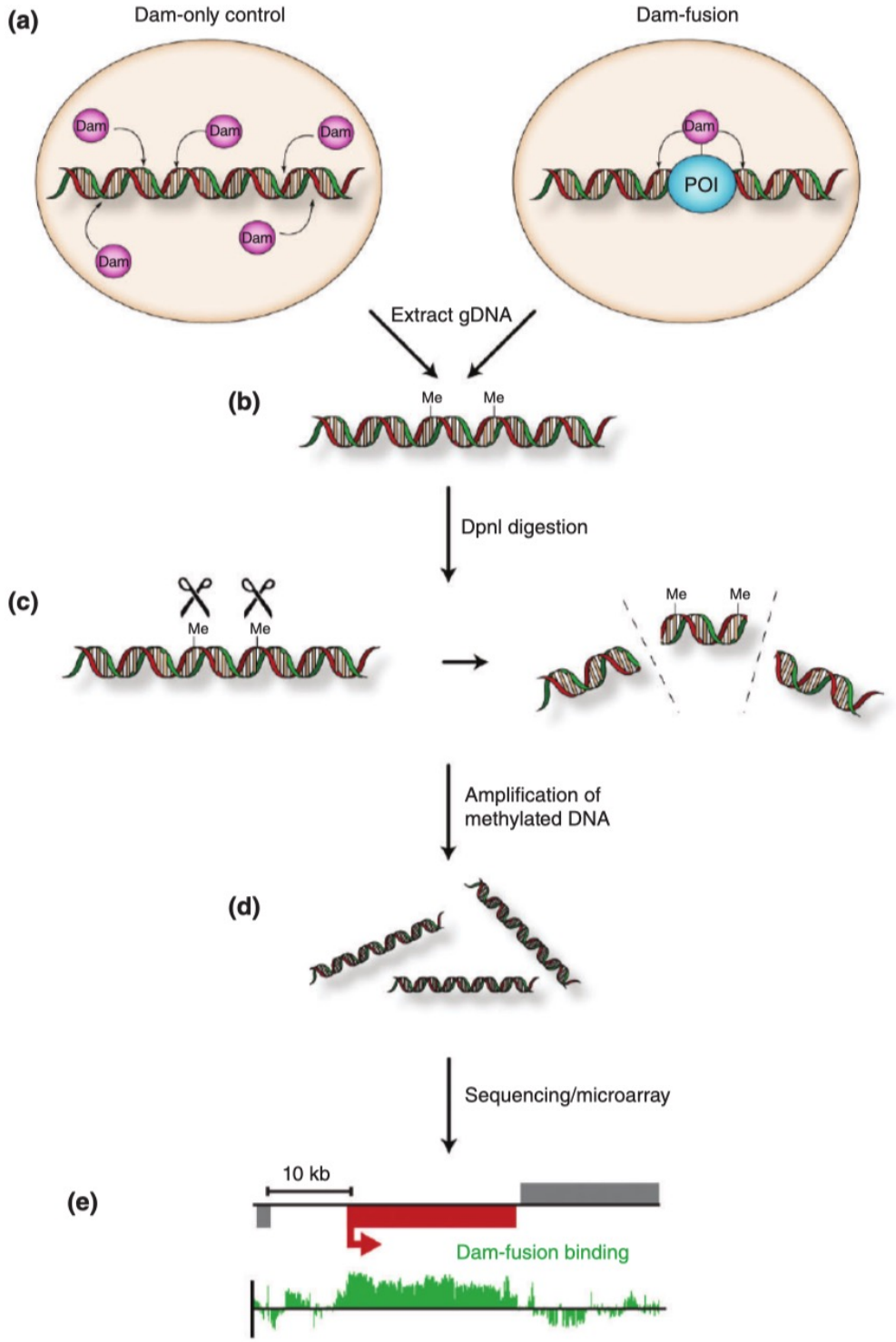
ChIP-Seq and alternatives

- ChIP-seq
- Dam-ID
- CUT&RUN
- CUT&Tag

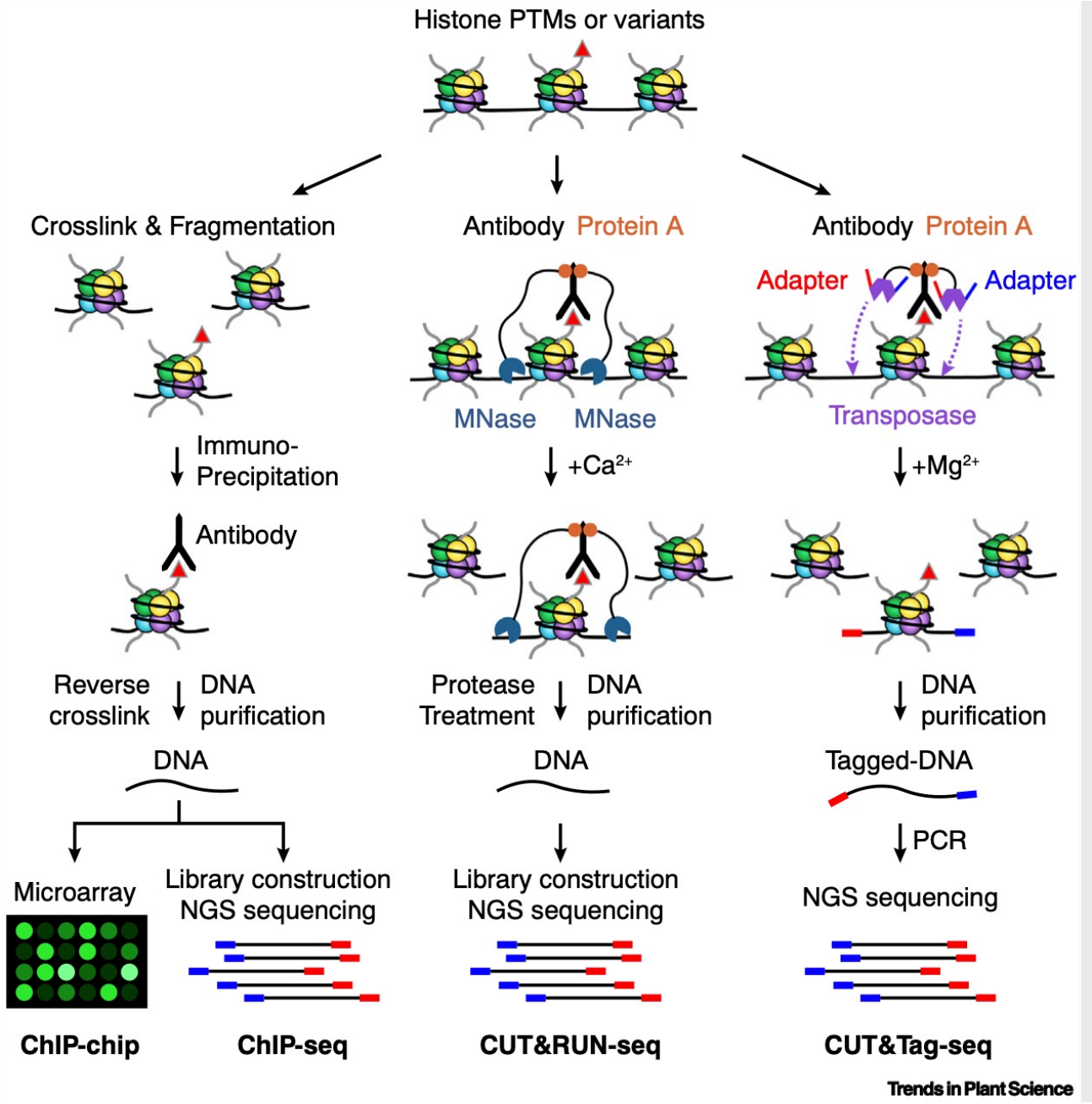
ChIP-seq Principles



Dam-ID


(a)
Dam-only control
Dam-fusion
Dam
POI
Extract gDNA
(b)
Me
DpnI digestion
(c)
Amplification of
methylated DNA
(d)
Sequencing/microarray
(e)
10 kb
Dam-fusion binding

CUT&Run, CUT&Tag



ChIC, ChEC-Seq CUT&Run

Molecular Cell, Vol. 16, 147–157, October 8, 2004, Copyright ©2004 by Cell Press

ChIC and ChEC: Genomic Mapping of Chromatin Proteins

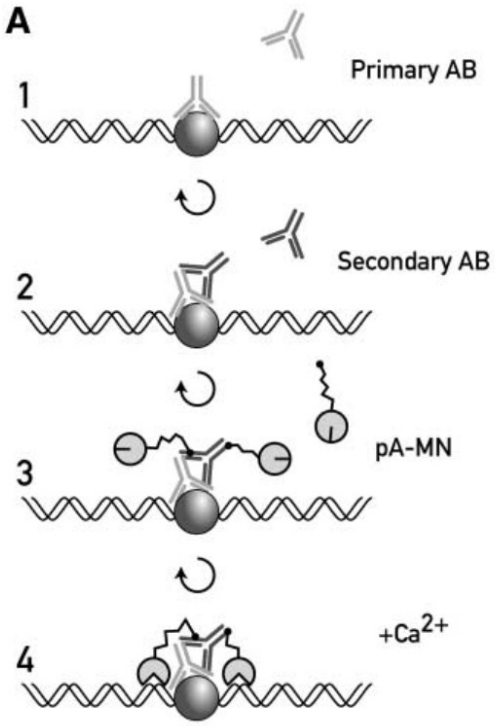
Technique

Manfred Schmid, Thérèse Durussel, and Ulrich K. Laemmli*
Departments of Biochemistry and Molecular Biology
NCCR Frontiers in Genetics
University of Geneva
30, Quai Ernest-Ansermet
CH1211, Geneva 4
Switzerland

ble, and significant amounts are lost into the pellet during centrifugation.

While ChIP is highly successful when applied to soluble proteins, such as transcription regulatory proteins, unpublished experiments with insoluble-type proteins (such as scaffolding components) in this laboratory appeared less promising. ChIP analyses with such ins
ble-type proteins appear afflicted with increased ba

ChIC: Chromatin-immuno cleavage



eLife

ABOUT COMMUNITY SUBMIT MY RESEARCH LOG IN/REGISTER

HOME MAGAZINE INNOVATION

Chromosomes and Gene Expression

An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites

Peter J Skene, Steven Henikoff
Howard Hughes Medical Institute, Basic Sciences Division, Fred Hutchinson Cancer Research Center, United States

Brief Communication | Published: 28 March 2019

Single-cell chromatin immunocleavage sequencing (scChIC-seq) to profile histone modification

Wai Lim Ku, Kosuke Nakamura, Weiwu Gao, Kairong Cui, Gangqing Hu, Qingsong Tang, Bing Ni & Keji Zhao

Nature Methods **16**, 323–325(2019) | Cite this article

Resource

Cell

Profiling of Pluripotency Factors in Single Cells and Early Embryos

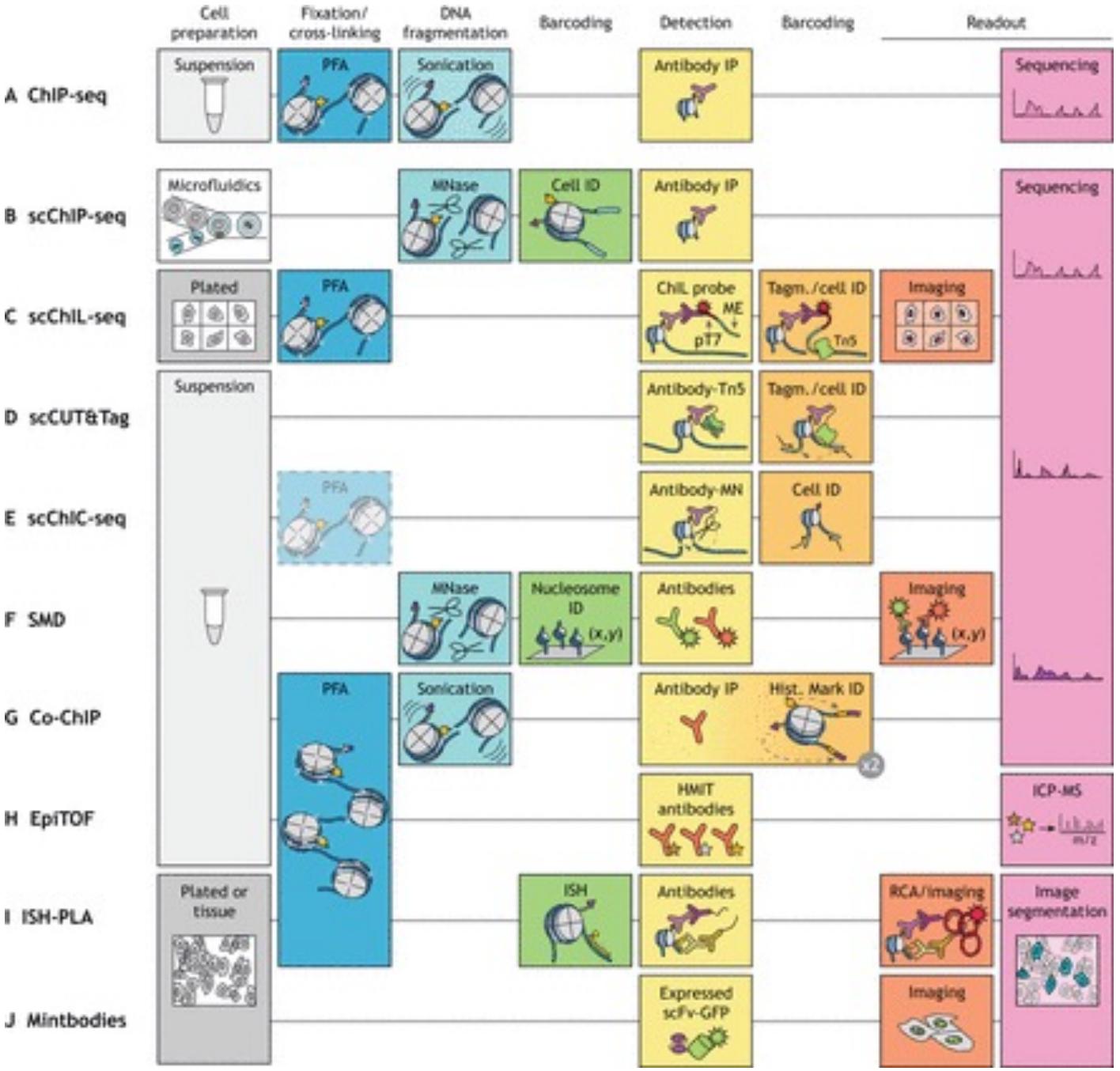
Graphical Abstract

Small Cell

Authors

Sarah J. Hainer, Ana Bošković, Kurtis N. McCannell, Oliver J. Rando,

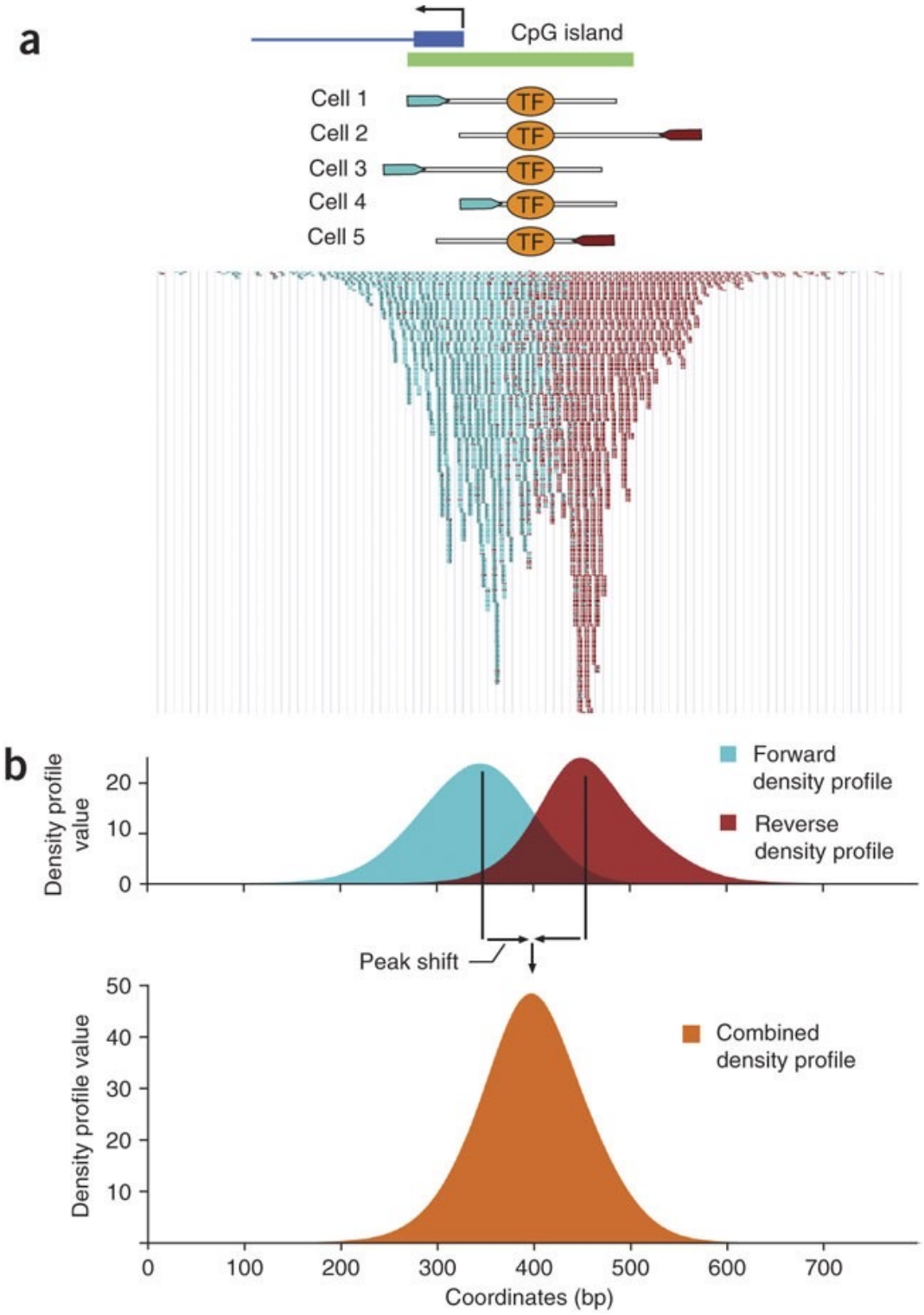
Single-cell revolution



ChIP-seq Principles

Mapping transcription factor binding:

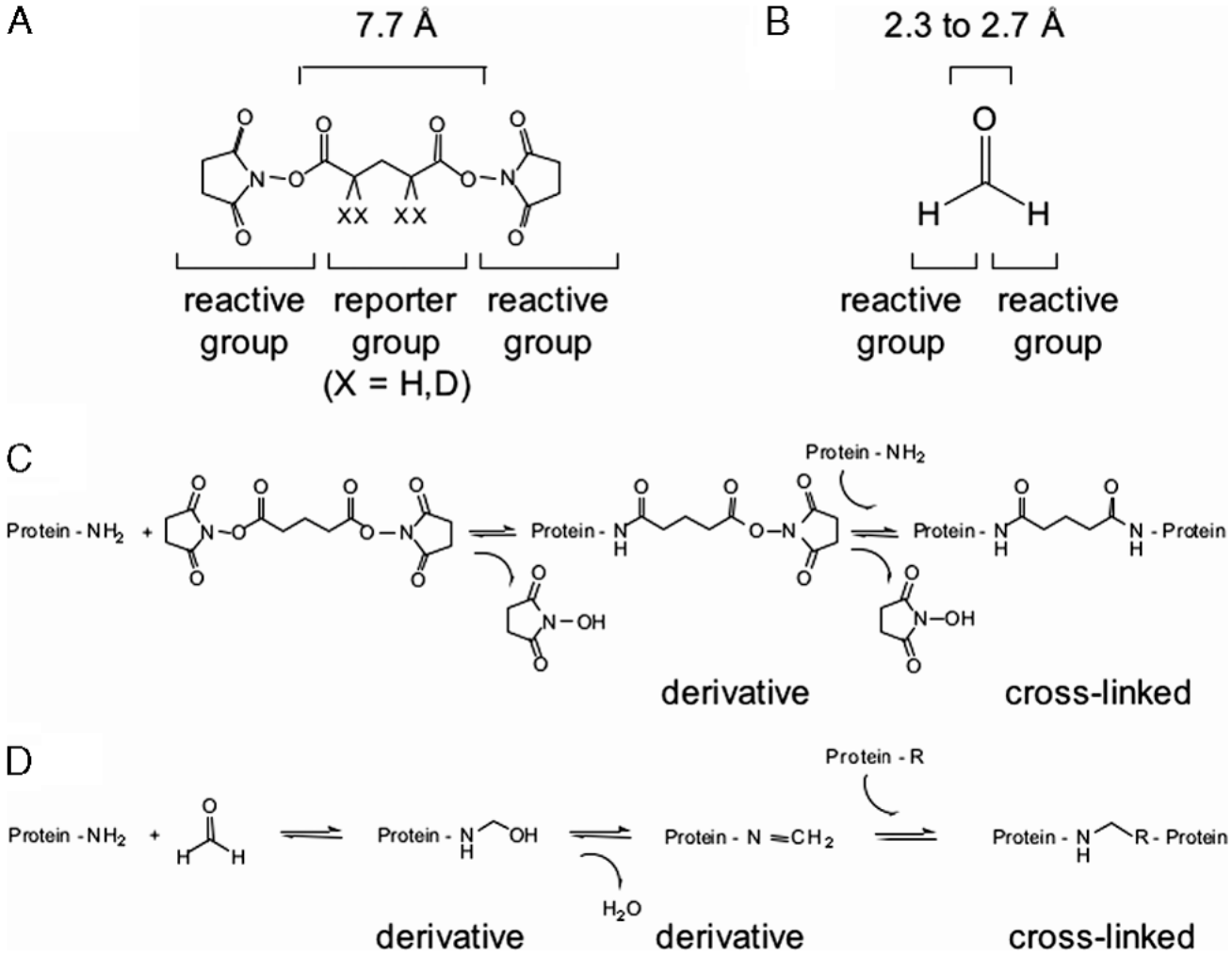
- TF-DNA interactions typically don't survive lysis and immunoprecipitation conditions --> we must crosslink the TF to the DNA beforehand



ChIP-seq Principles

Mapping transcription factor binding:

- TF-DNA interactions typically don't survive lysis and immunoprecipitation conditions --> we must crosslink the TF to the DNA beforehand
  - Formaldehyde
  - ESG
  - DSG



Crosslinking versus native ChIP

Crosslinking

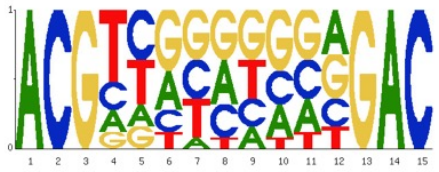
- Formaldehyde fixation
- Shearing (sonications) or MNase
- Increasing signal for weak/transient histone/DNA- interacting proteins
- fragment ends not informative with sonication

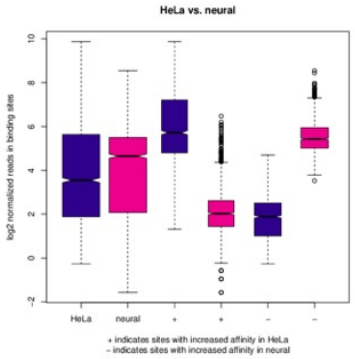
Native

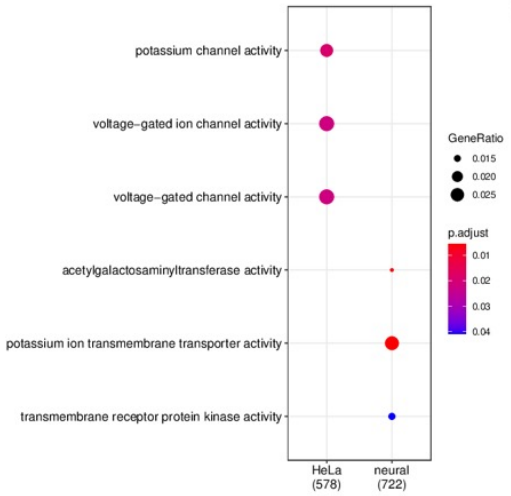
- Lower salt/detergent
- Mnase fragmentation
- Better signal-to-noise for strong chromatin interactors, histones
- Fragment ends demarcate footprint, e.g. nucleosome position

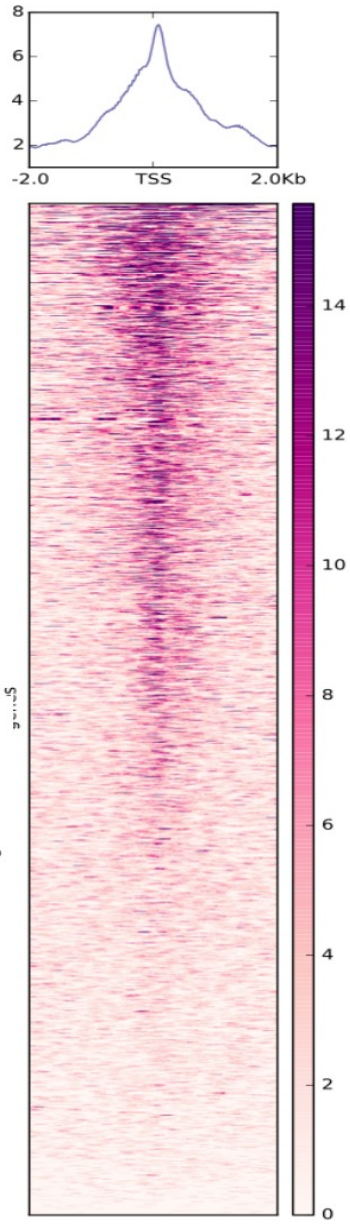
ChIP-seq peak calling downstream analyses

- Validation (wet lab)
- Downstream analysis
  - Motif discovery
  - Annotation
  - Integration of binding and expression data
  - Integration of various binding datasets
  - Differential binding





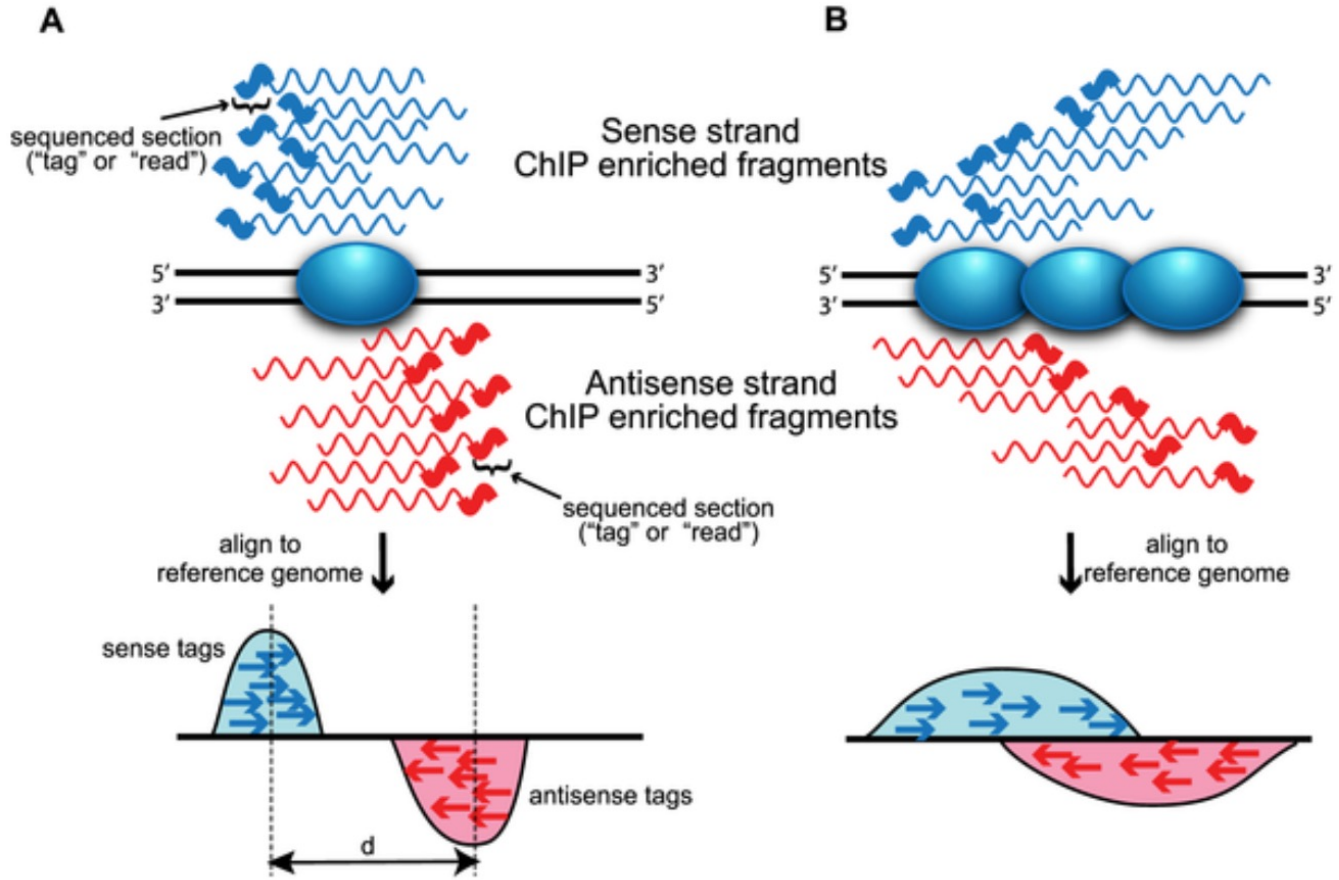




Peak detection

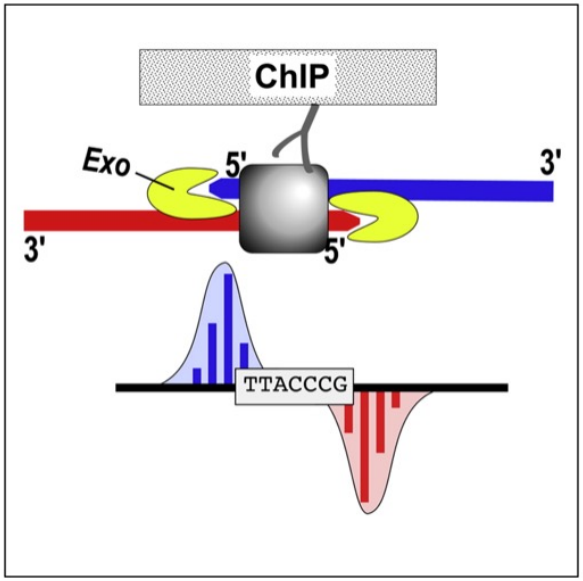
Sequence-specific binding (TFs)

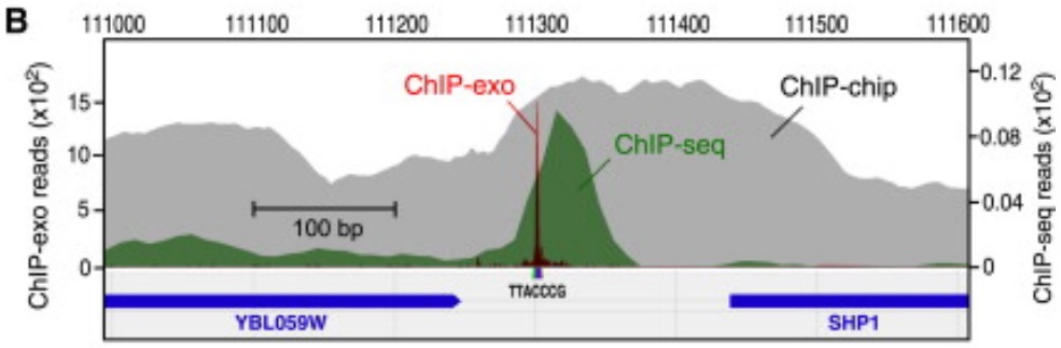
Distributed binding (histones, RNApol2)

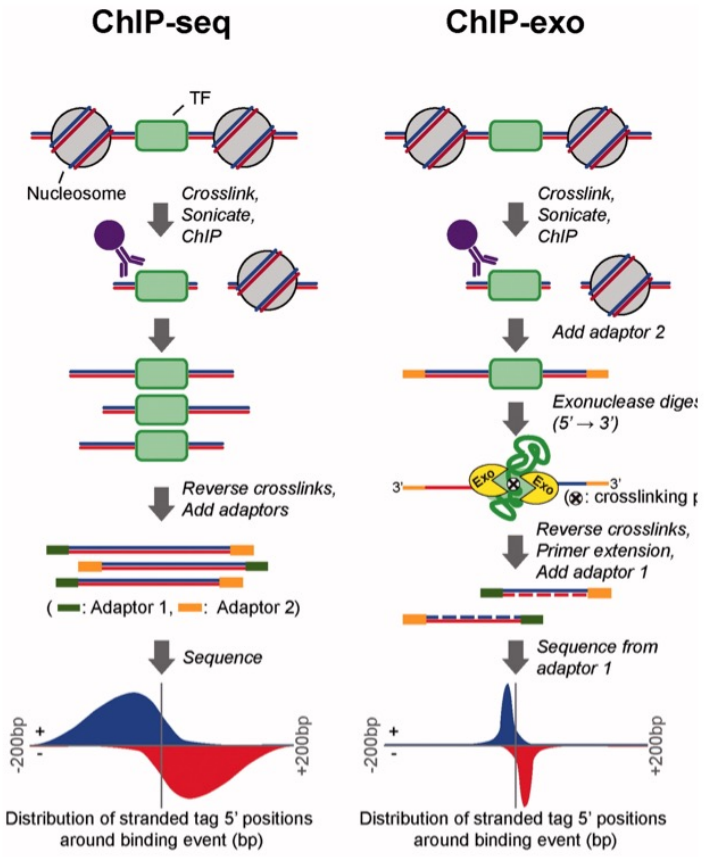


Wilbanks 2010

ChIP-exo: improvement in binding site identification



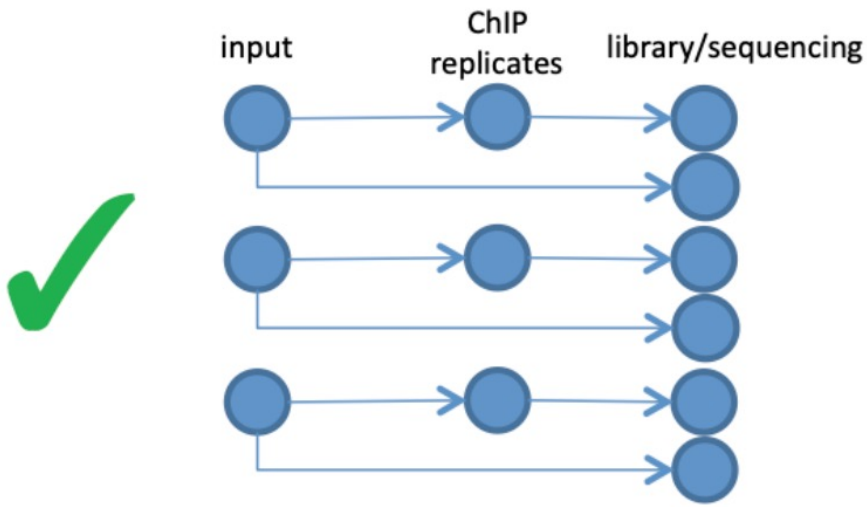


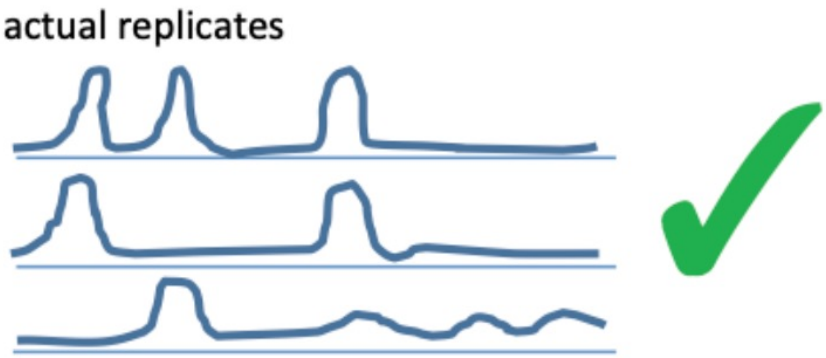


Pugh 2015
Rhee and Pugh, Cell 2011

Experiment design

- Sound experimental design: replication, randomisation, control and blocking (R.A. Fisher, 1935)
- In the absence of a proper design, it is essentially impossible to partition biological variation from technical variation
- Please visit section *Experimental Design and Data Management* on the course website for more information

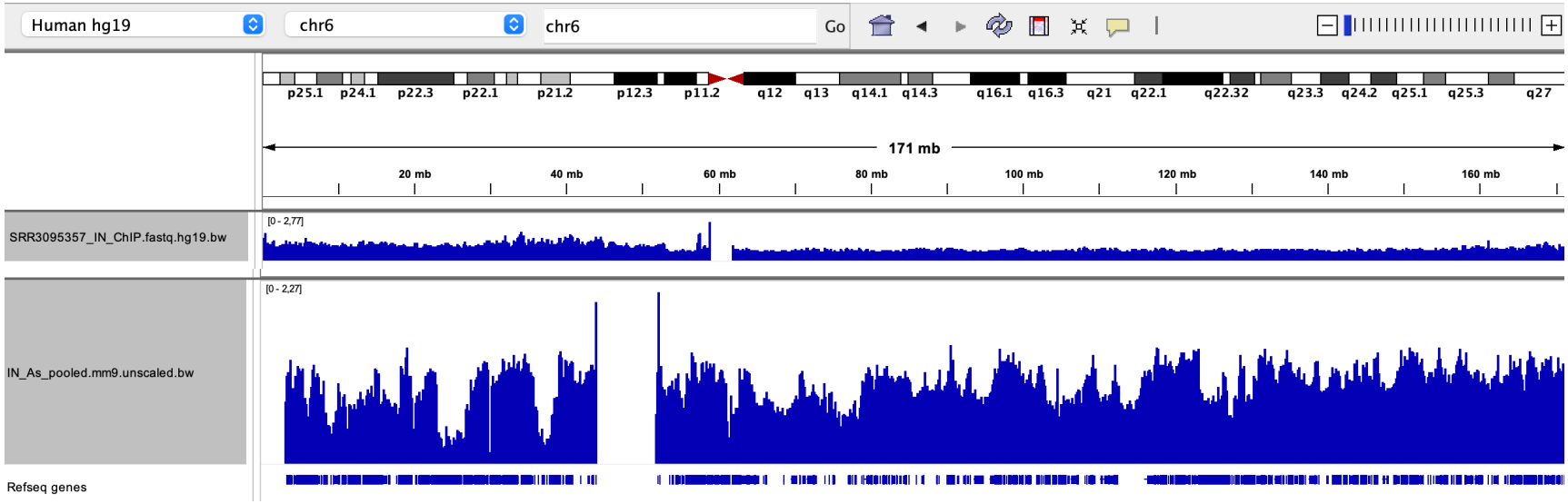


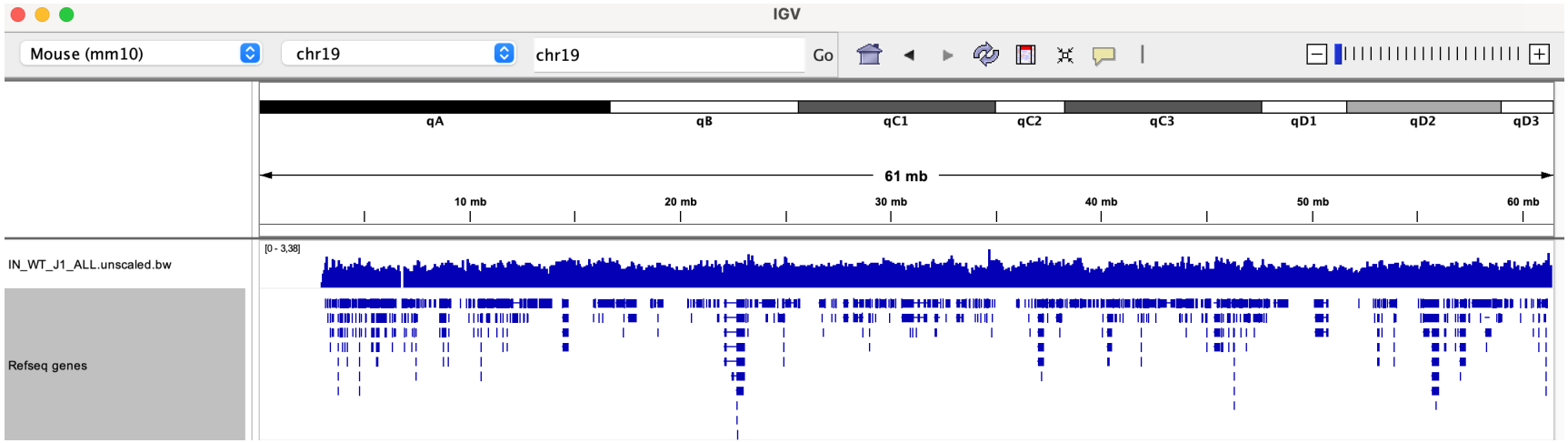


Importance of Input

Input genomic DNA is skewed by technical and biological factors

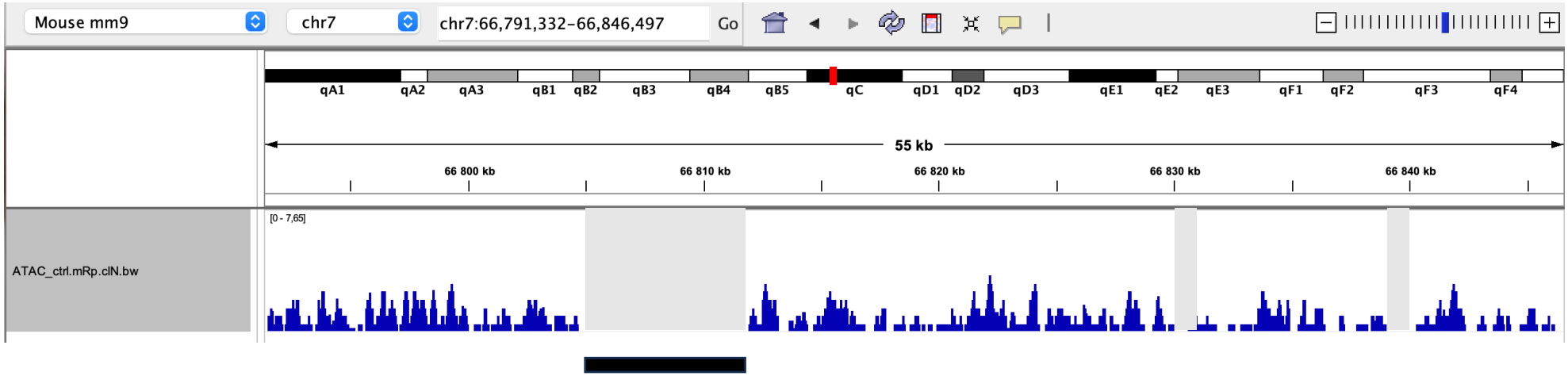
Different Input Examples





The problem of missing data vs zerp signal

- some tools are using ”NA” in bigwig files wherever there is no read mapping (nf-core ATAC)
- some tools make blacklisted regions “0” value
- Bigwig analysis tools usually ignore NAs while “0” counts towards the calculation, e.g. bin average
- The correct way (I think) is to NA blacklisted regions but use “0” for non-covered regions



Blacklisted region

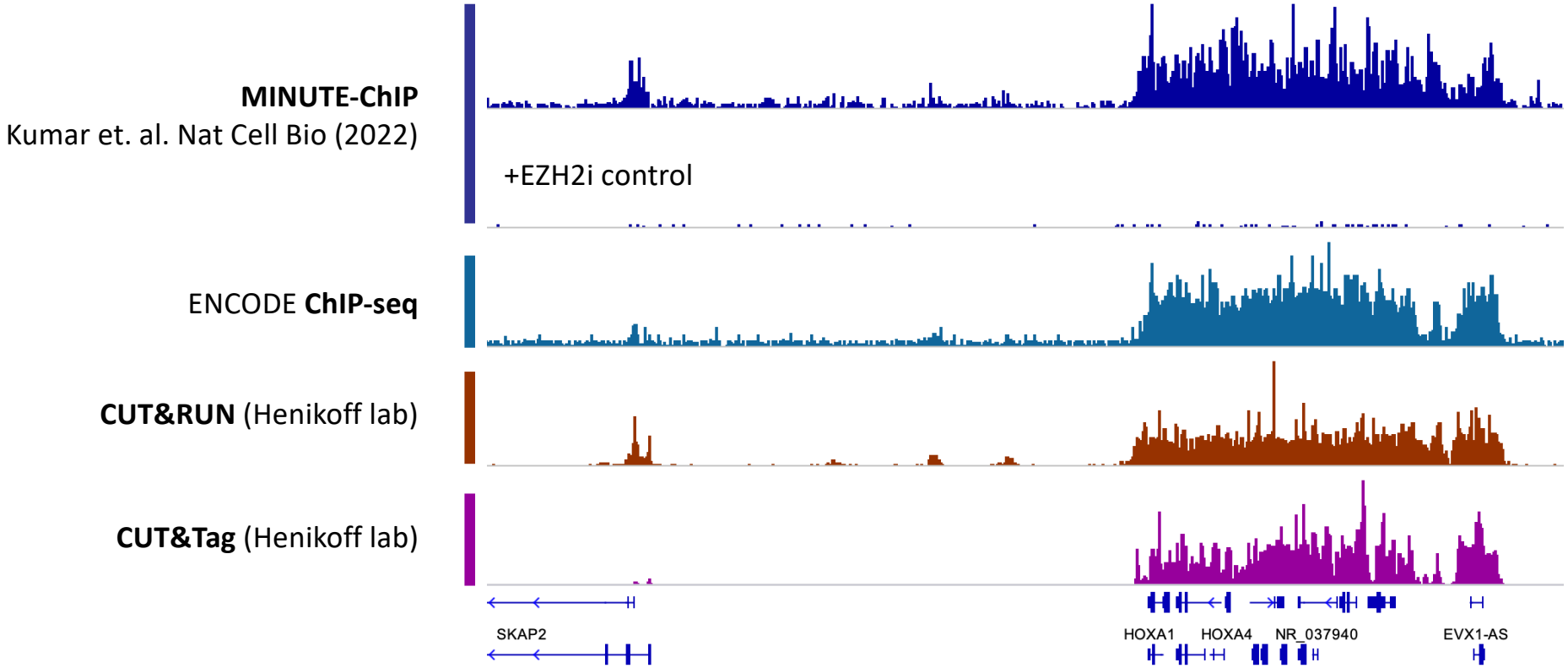
No read aligning here

```
Chr1 1000345 4
Chr1 1000350 0
Chr1 1000355 0
Chr1 1000360 0
Chr1 1000365 0
Chr1 1000370 9
```

```
Chr1 1000345 4
Chr1 1000350 NA
Chr1 1000355 NA
Chr1 1000360 NA
Chr1 1000365 NA
Chr1 1000370 9
```

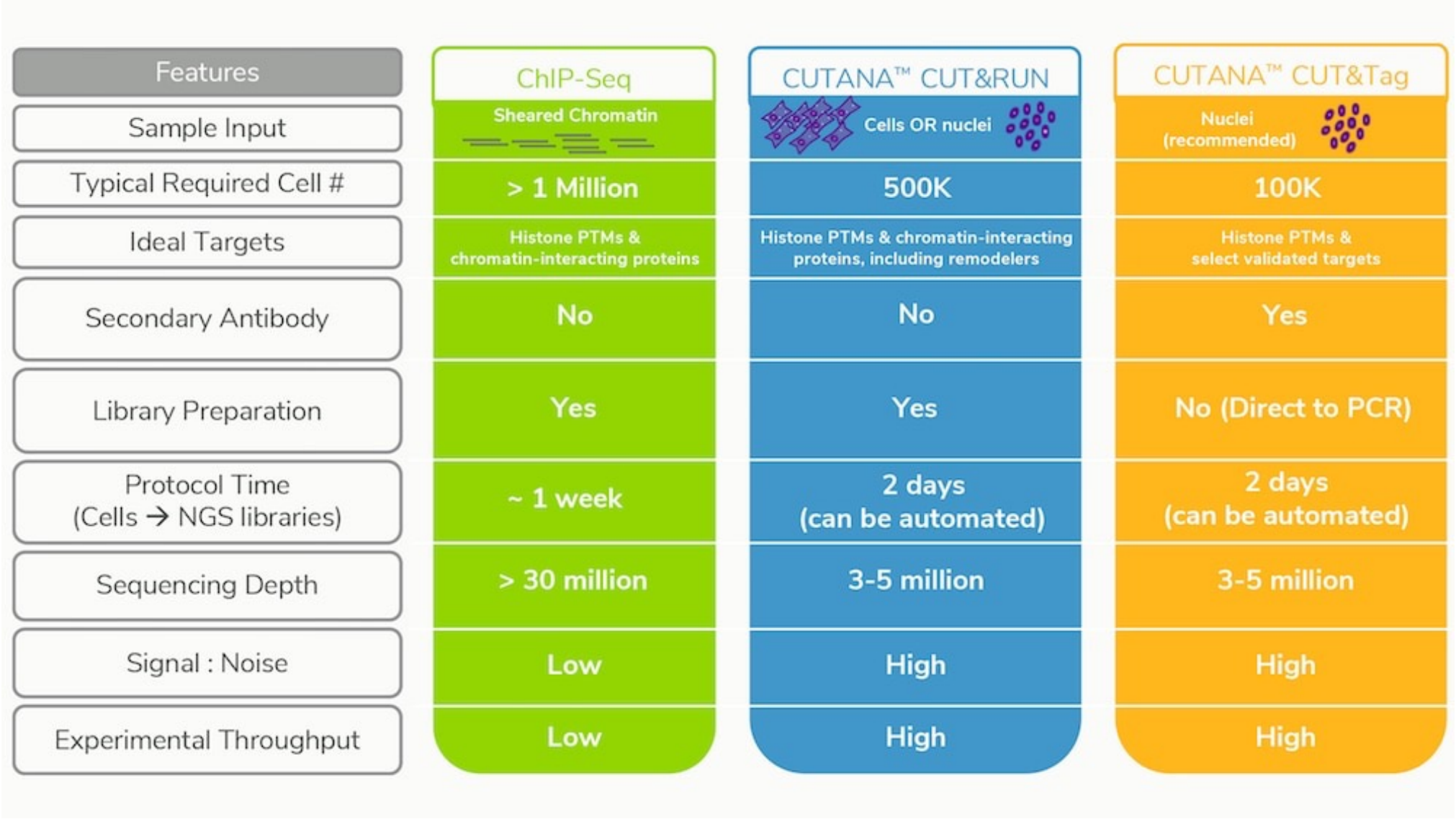
Excercise – CUT&RUN vs CUT&Tag vs ChIP

Comparison: H3K27me3 signal in human embryonic stem cells across different methods



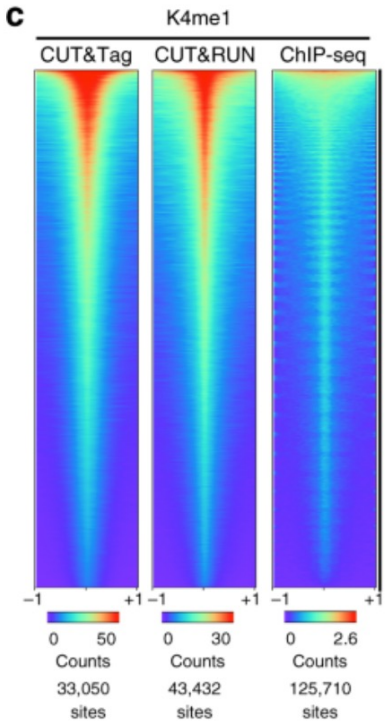
https://nbis-workshop-epigenomics.readthedocs.io/en/latest/content/tutorials/quantitativeChip/cut-and-tag-data.html

ChIP-seq vs CUT&Run, CUT&Tag



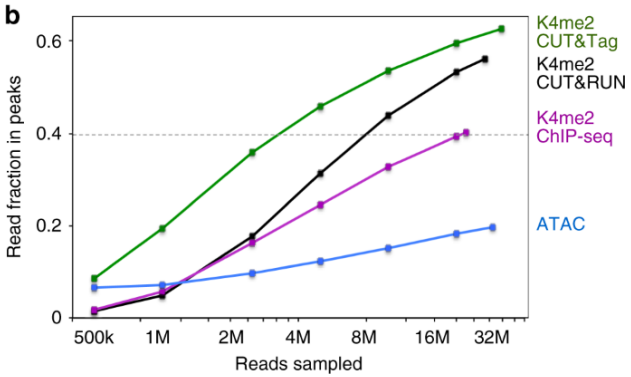
Weighing pros and cons

- Cell number needed
- Time aspect
- Signal-to-noise
- Background (think technical versus biological!)
- Reproducibility
- QCability (e.g. no input for CUT&Tag, CUT&RUN)



CUT&Tag for efficient epigenomic profiling of small samples and single cells

Hatice S. Kaya-Okur, Steven J. Wu, Christine A. Codomo, Erica S. Pledger, Terri D. Bryson, Jorja G. Henikoff, Kami Ahmad & Steven Henikoff



New Results

Tn5 transposase-based epigenomic profiling methods are prone to open chromatin bias

Meng Wang, Yi Zhang

doi: https://doi.org/10.1101/2021.07.09.451758

CUT&Tag recovers up to half of ENCODE ChIP-seq peaks

Di Hu, Leyla Abbasova, Brian M Schilder, Alexi Nott, Nathan G Skene, Sarah J Marzi

doi: https://doi.org/10.1101/2022.03.30.486382

JOURNAL ARTICLE

Untargeted CUT&Tag reads are enriched at accessible chromatin and restrict identification of potential G4-forming sequences in G4-targeted CUT&Tag experiments

Matthew D Thompson, Alicia K Byrd

Nucleic Acids Research, Volume 53, Issue 14, 12 August 2025, gkaf678,
https://doi.org/10.1093/nar/gkaf678

Analysis

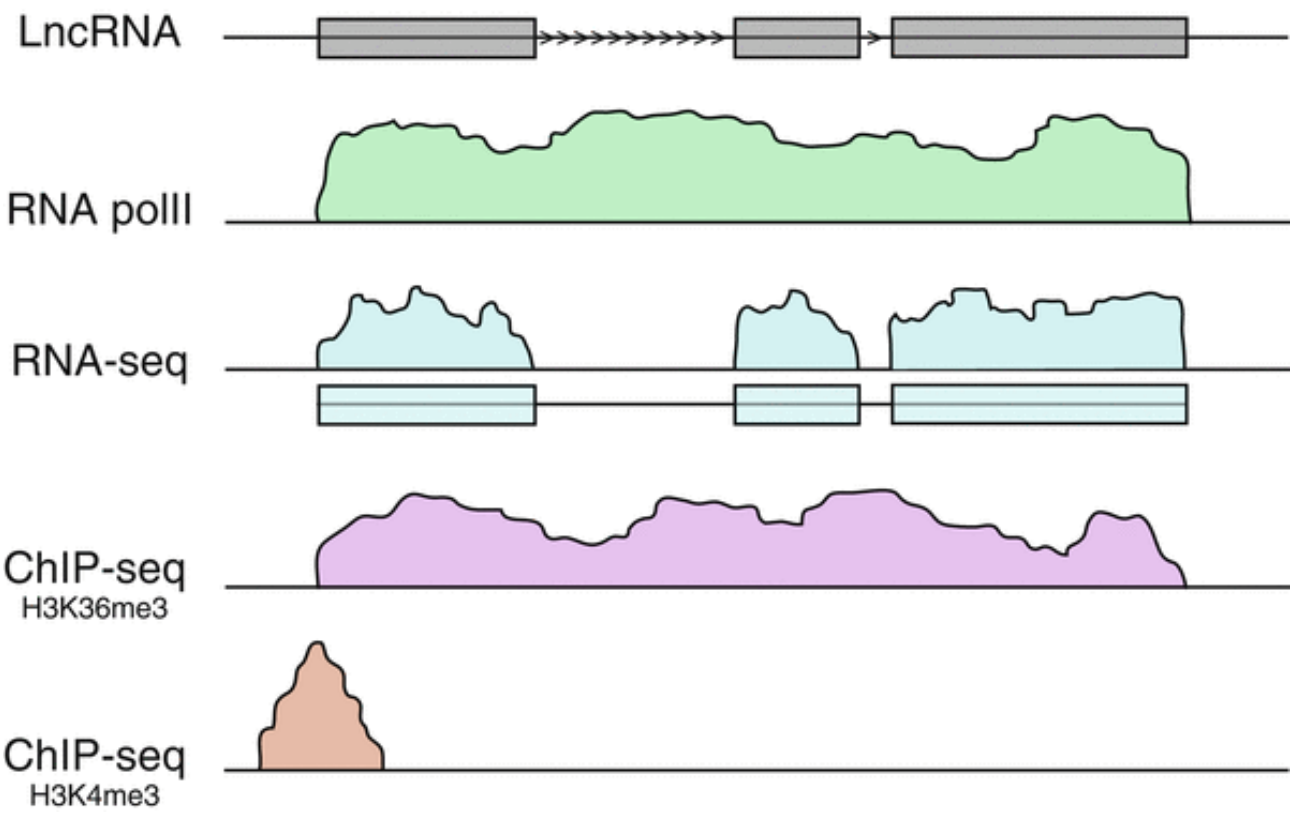
Applications of ChIP-Seq and related methods

- Map features to genome (--> knowing where a features is *may* imply function)
- Specific versus genome-wide feature
- Discover genome-wide correlations (--> generate experimental hypothesis --> test to establish causation)
  - Think about the meaning: “repressive chromatin”, “activating mark”, “silencing factor”
  - A histone PTM “recruits” a factor
  - A factor ‘protects’ a gene from spurious transcription
- Compare conditions, e.g. how does a knockout, inhibitor, external change in condition affect epigenome?

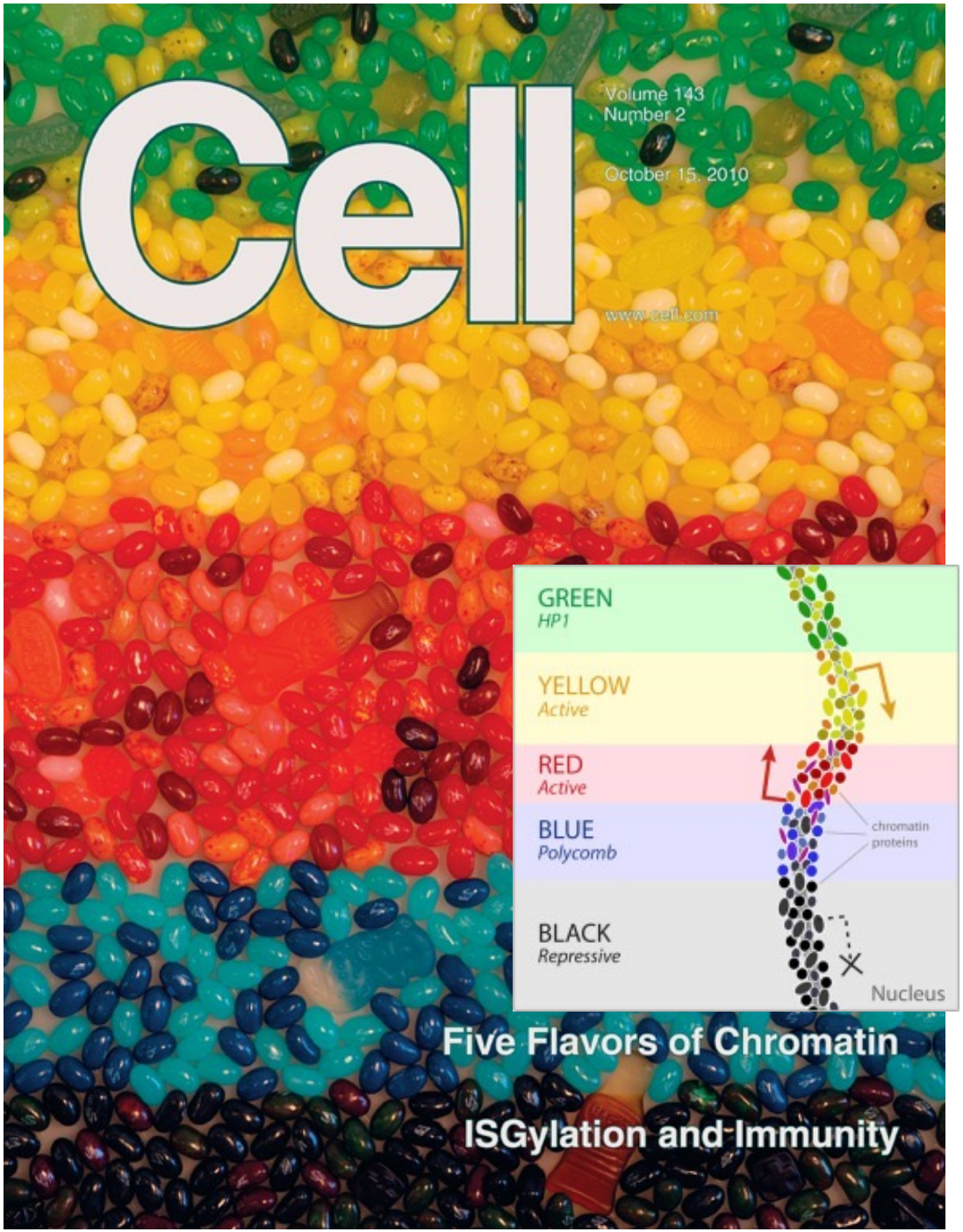
Using histone modifications to predict functional regions in the genome

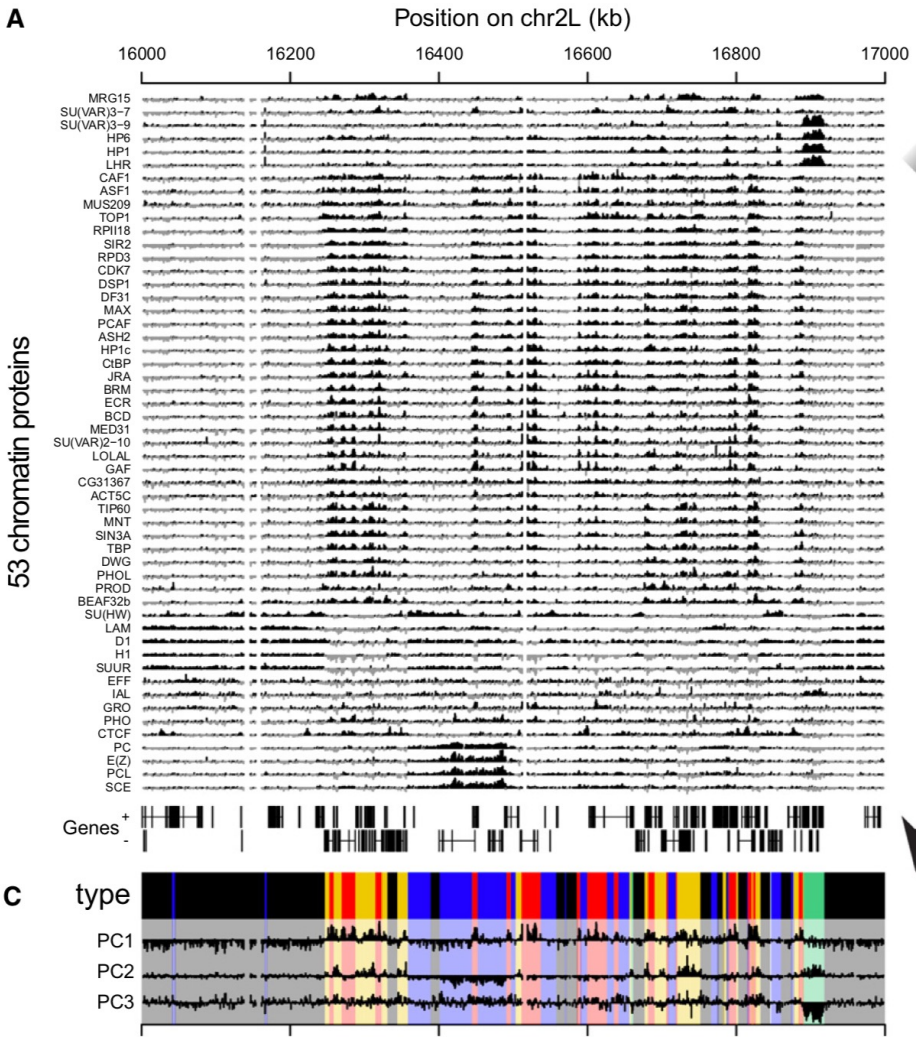
Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals

Mitchell Guttman[1,2], Ido Amit[1], Manuel Garber[1], Courtney French[1], Michael F. Lin[1], David Feldser[3], Maite Huarte[1,6],

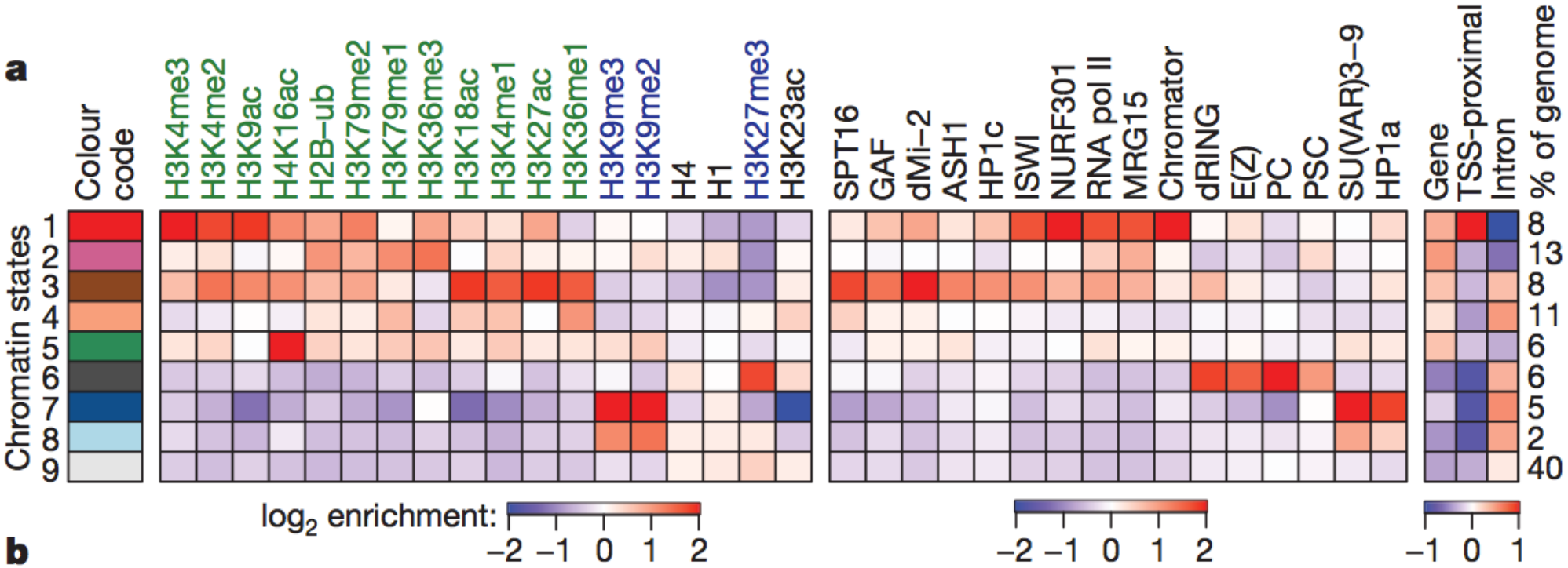


Chromatin ’states’ define functional regions





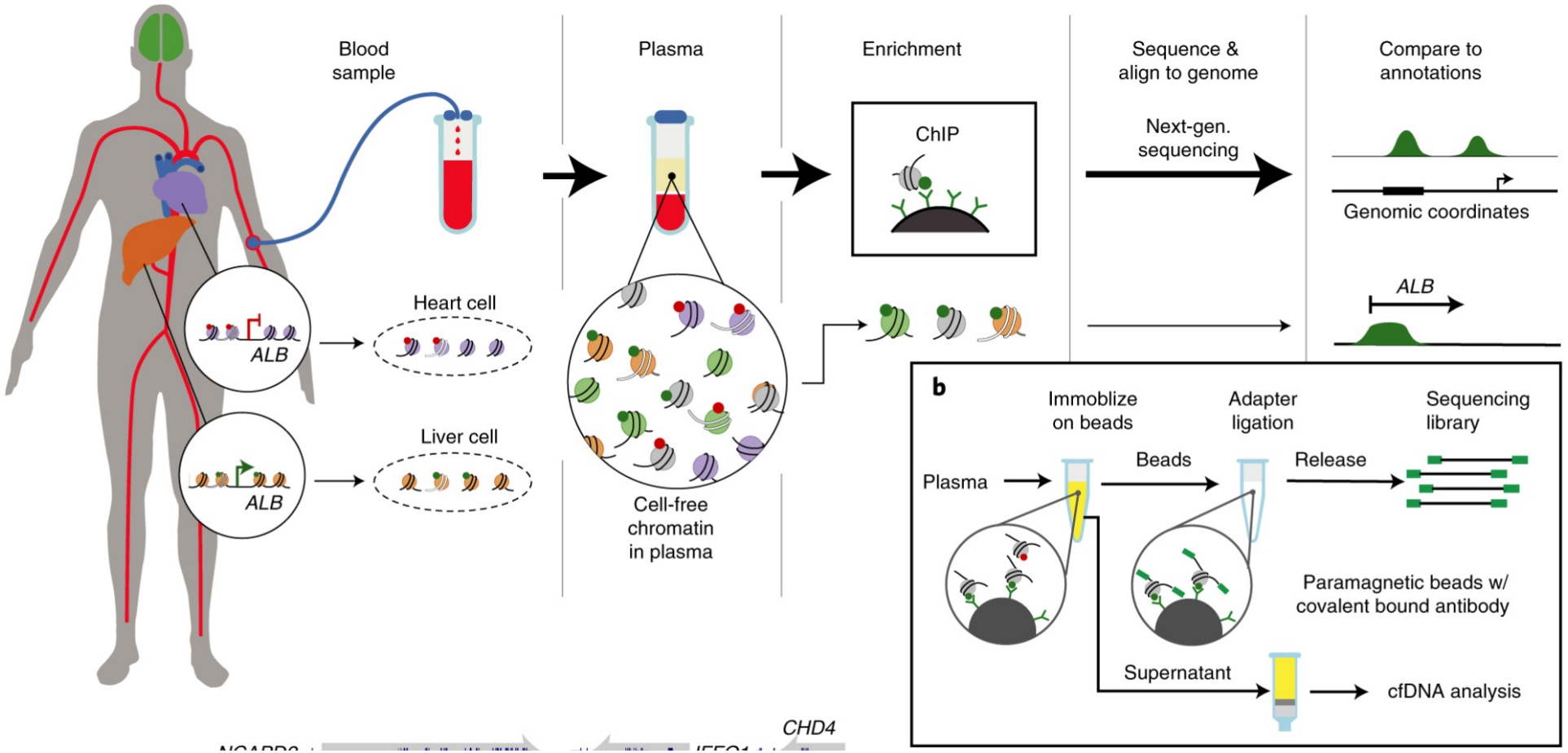
9 chromatin states defined by a combinatorial pattern of enrichment and depletion for specific chromatin marks



Article | Published: 11 January 2021

ChIP-seq of plasma cell-free nucleosomes identifies gene expression programs of the cells of origin

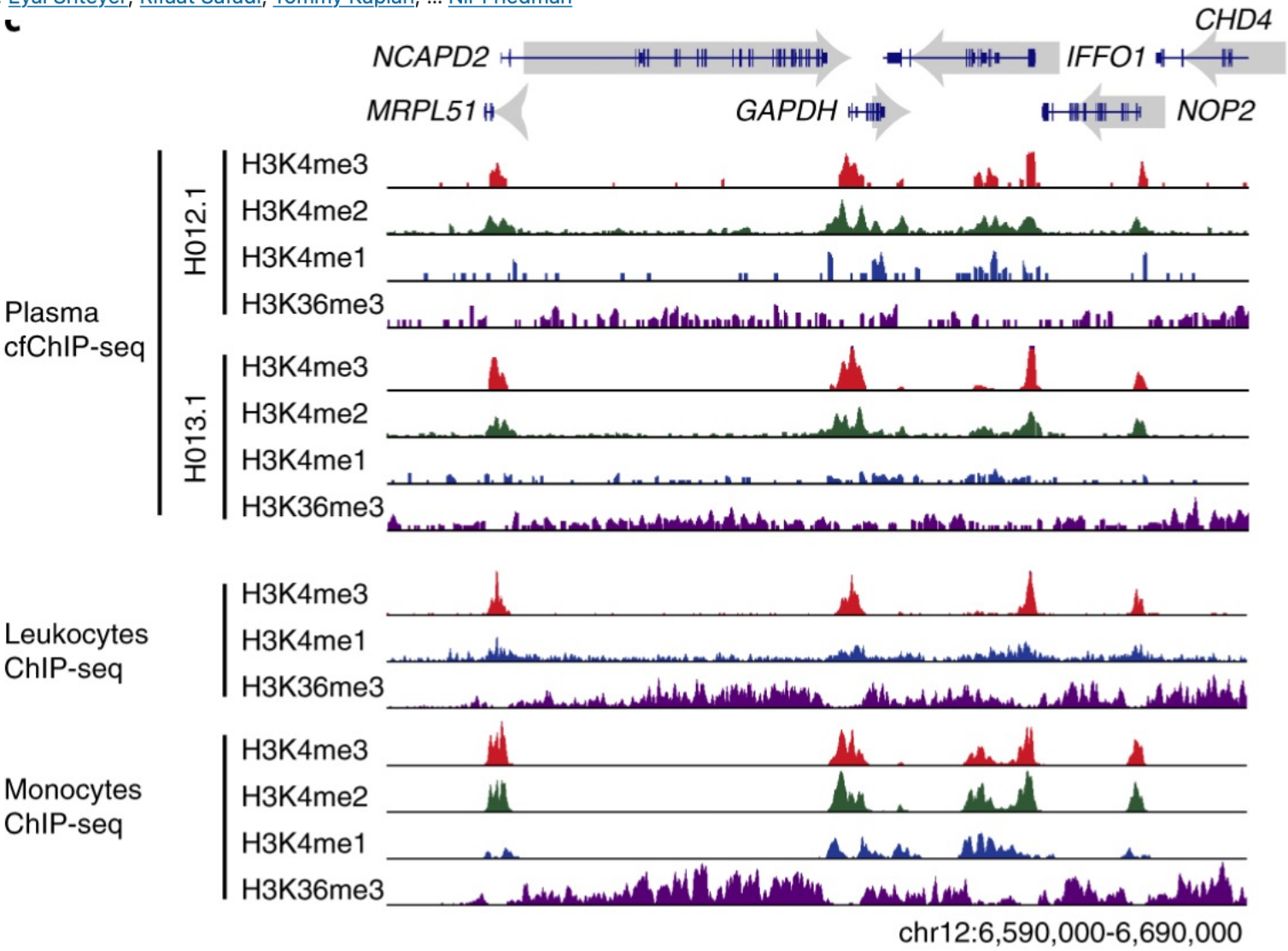
Ronen Sadeh, Israa Sharkia, Gavriel Fialkoff, Ayelet Rahat, Jenia Gutin, Alon Chappleboim, Mor Nitzan, Ilana Fox-Fisher, Daniel Neiman, Guy Meler, Zahala Kamari, Dayana Yaish, Tamar Peretz, Ayala Hubert, Jonathan E. Cohen, Azzam Salah, Mark Temper, Albert Grinshpun, Myriam Maoz, Samir Abu-Gazala, Ami Ben Ya'acov, Eyal Shteyer, Rifaat Safadi, Tommy Kaplan, ... Nir Friedman



Article | Published: 11 January 2021

ChIP-seq of plasma cell-free nucleosomes identifies gene expression programs of the cells of origin

Ronen Sadeh, Israa Sharkia, Gavriel Fialkoff, Ayelet Rahat, Jenia Gutin, Alon Chappleboim, Mor Nitzan, Ilana Fox-Fisher, Daniel Neiman, Guy Meler, Zahala Kamari, Dayana Yaish, Tamar Peretz, Ayala Hubert, Jonathan E. Cohen, Azzam Salah, Mark Temper, Albert Grinshpun, Myriam Maoz, Samir Abu-Gazala, Ami Ben Ya'acov, Eyal Shteyer, Rifaat Safadi, Tommy Kaplan, ... Nir Friedman



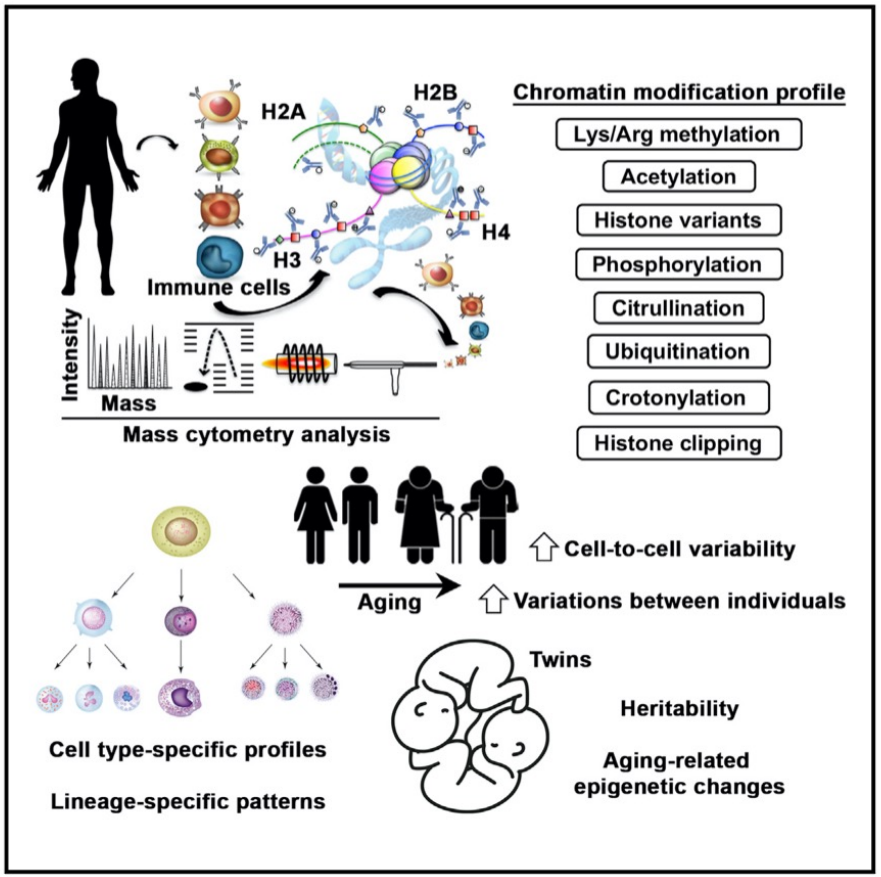
Histone modifications in diagnostics

Article

Cell

Single-Cell Chromatin Modification Profiling Reveals Increased Epigenetic Variations with Aging

Graphical Abstract



Authors

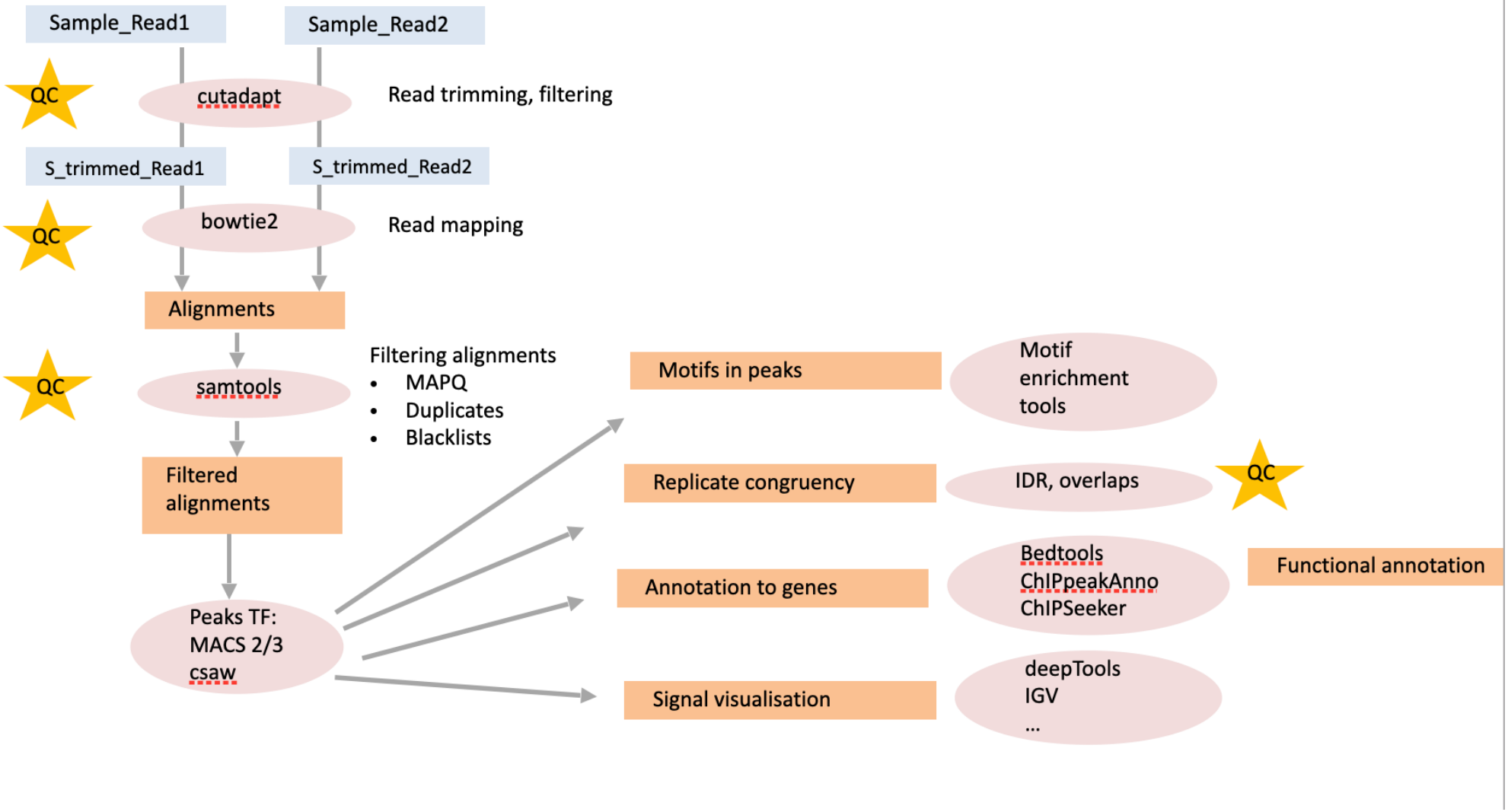
Peggie Cheung, Francesco Vallania, Hayley C. Warsinske, ..., Paul J. Utz, Purvesh Khatri, Alex J. Kuo

Correspondence

pjutz@stanford.edu (P.J.U.), pkhatri@stanford.edu (P.K.), alex0229@stanford.edu (A.J.K.)

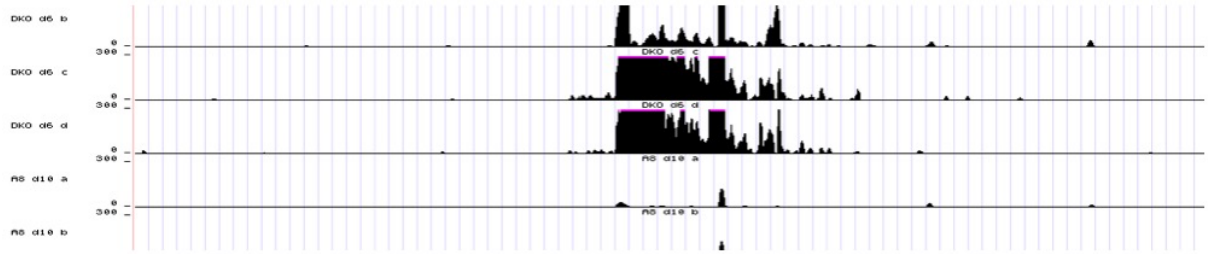
In Brief

A global look at human chromatin shows that variability in histone modification patterns accrues with age and depends on non-heritable factors.

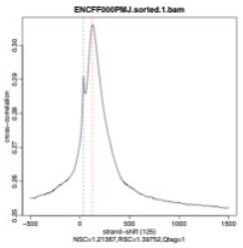

Sample_Read1
Sample_Read2
QC
cutadapt
Read trimming, filtering
S_trimmed_Read1
S_trimmed_Read2
QC
bowtie2
Read mapping
Alignments
QC
samtools
Filtering alignments
MAPQ
Duplicates
Blacklists
Filtered alignments
Peaks TF: MACS 2/3 csaw
Motifs in peaks
Motif enrichment tools
Replicate congruency
IDR, overlaps
QC
Annotation to genes
Bedtools
ChIPpeakAnno
ChIPSeeker
Functional annotation
Signal visualisation
deepTools
IGV
...

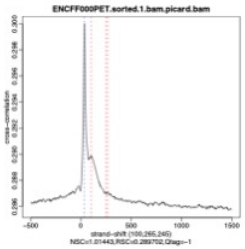
ChIP-seq QC: did the ChIP work?

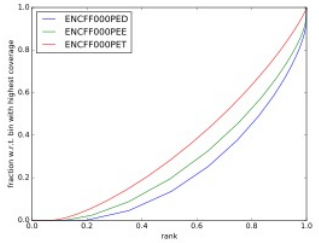
- 1. Inspect the signal (mapped reads, coverage profiles) in genome browser



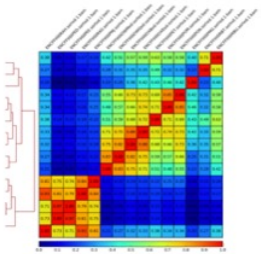
- 2. Compute peak-independent quality metrics (cross correlation, cumulative enrichment)



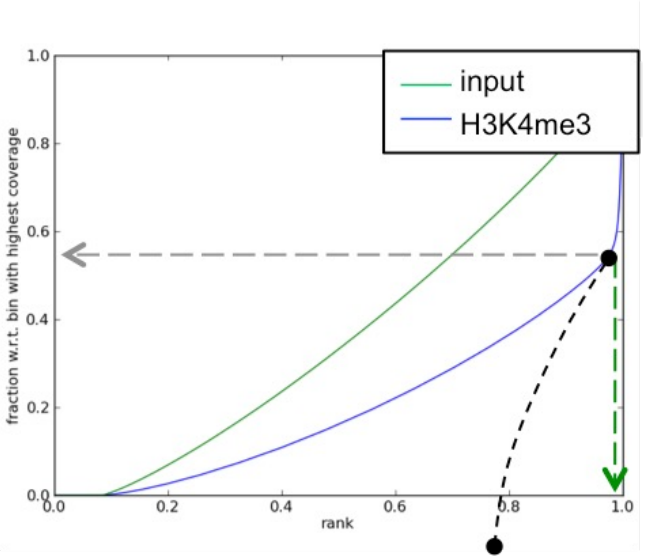




- 3. Assess replicate consistency (correlations between replicates of the same condition)

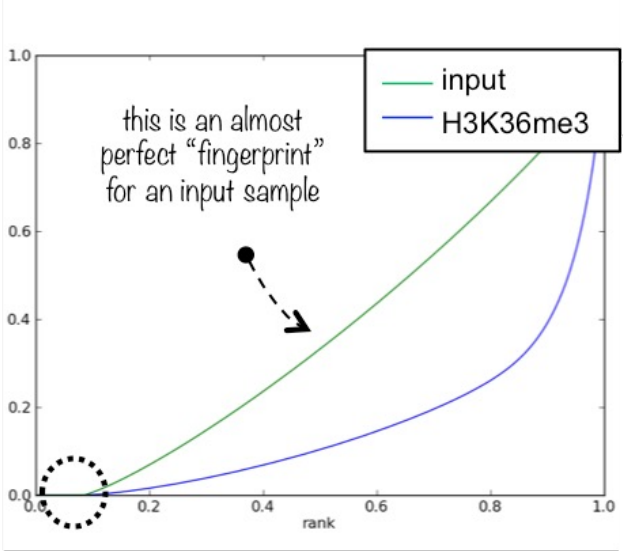


Fingerprint plot (deepTools)

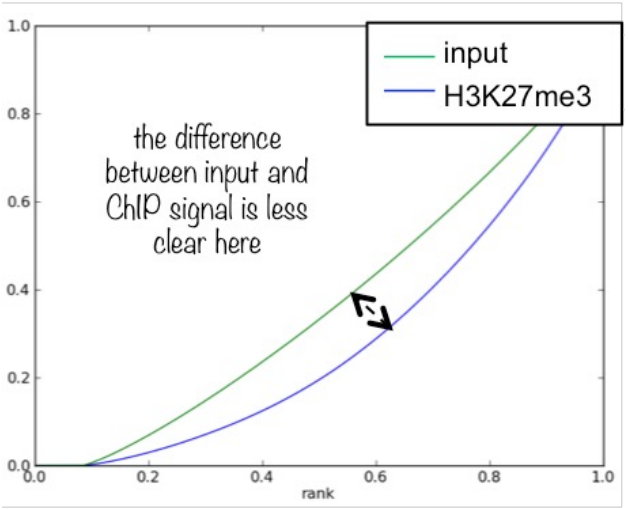


when counting the reads contained in **97%** of all genomic bins, only ca. **55%** of the maximum number of reads are reached, i.e. 3% of the genome contain a very large fraction of reads!

→ this indicates very localized, very strong enrichments!
(as every biologist hopes for in a ChIP for H3K4me3)



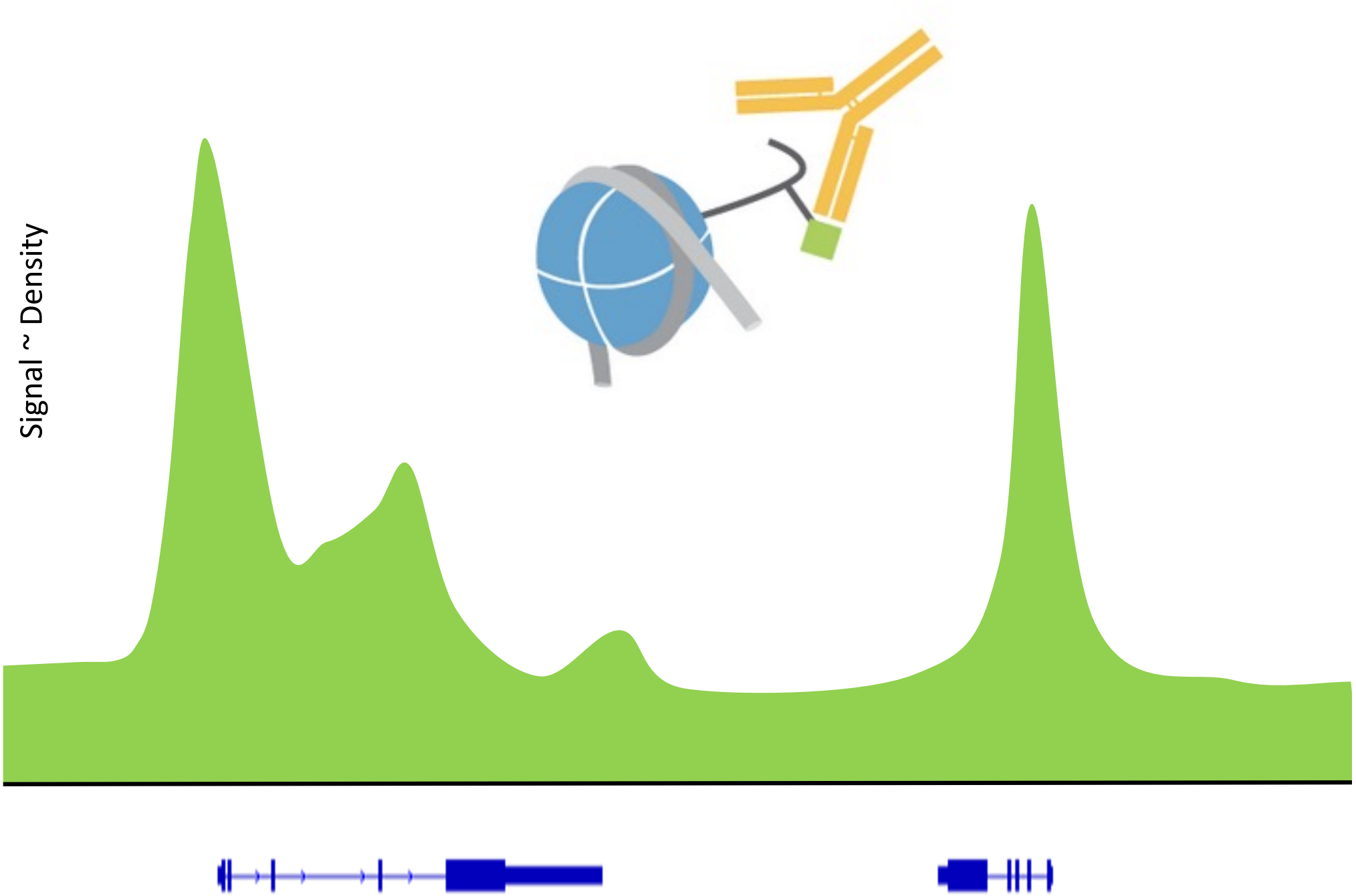
pay attention to where the curves start to rise – this already gives you an assessment of how much of the genome you have not sequenced at all (i.e. bins containing zero reads – for this example, ca. 10% of the entire genome do not have any read)



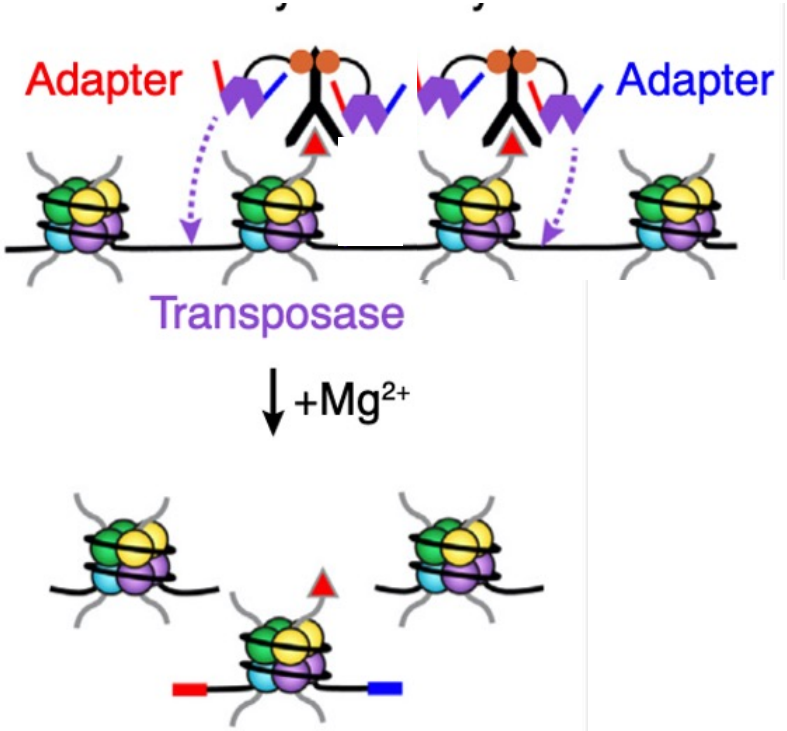
H3K27me3 is a mark that yields broad domains instead of narrow peaks

→ it is more difficult to distinguish input and ChIP, it does not mean, however, that this particular ChIP experiment failed

ChIP-Seq signal (histone PTM density or Binding Protein occupancy)



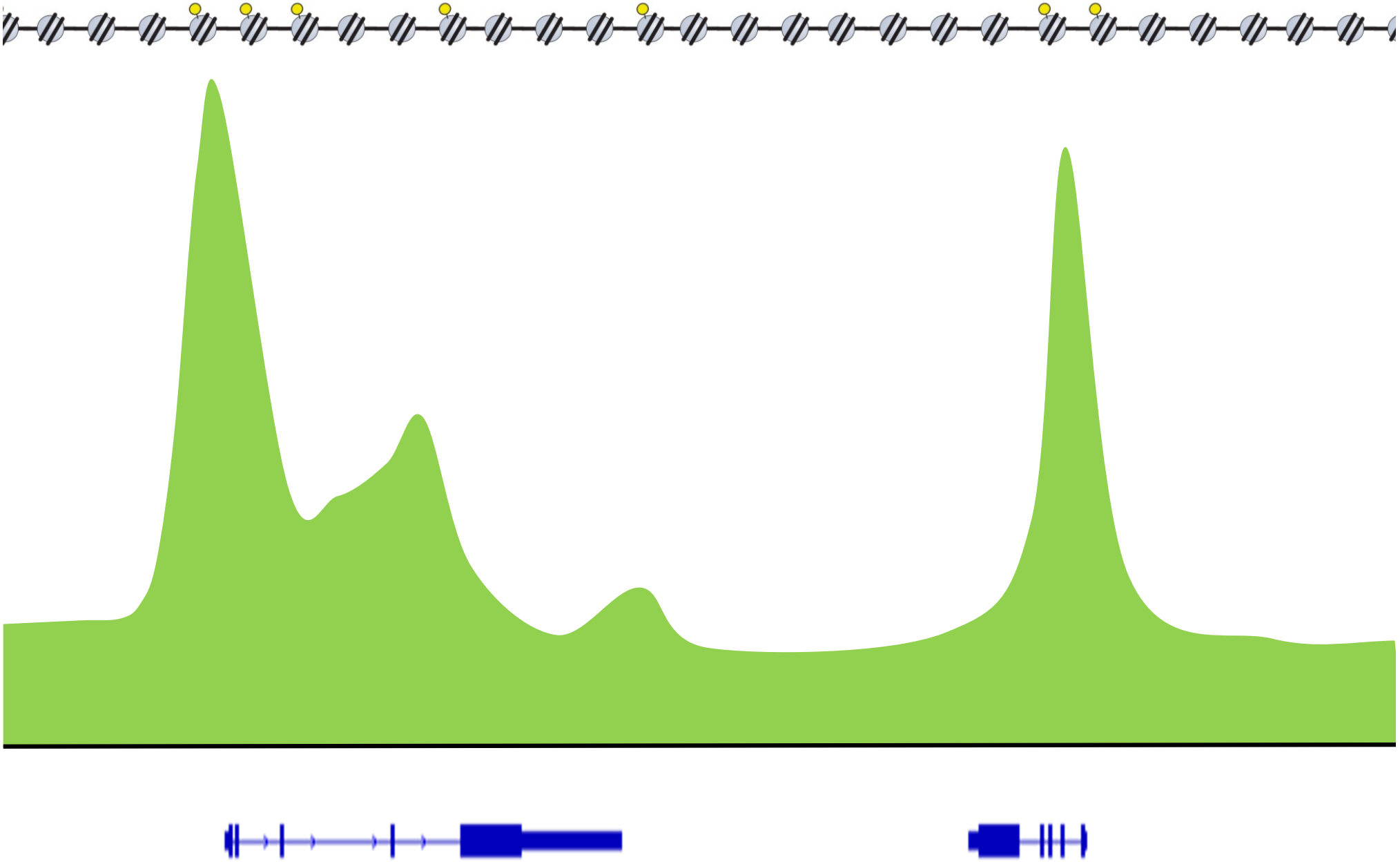
CUT&Tag signal – is it proportional or square-proportional to the epitope density?

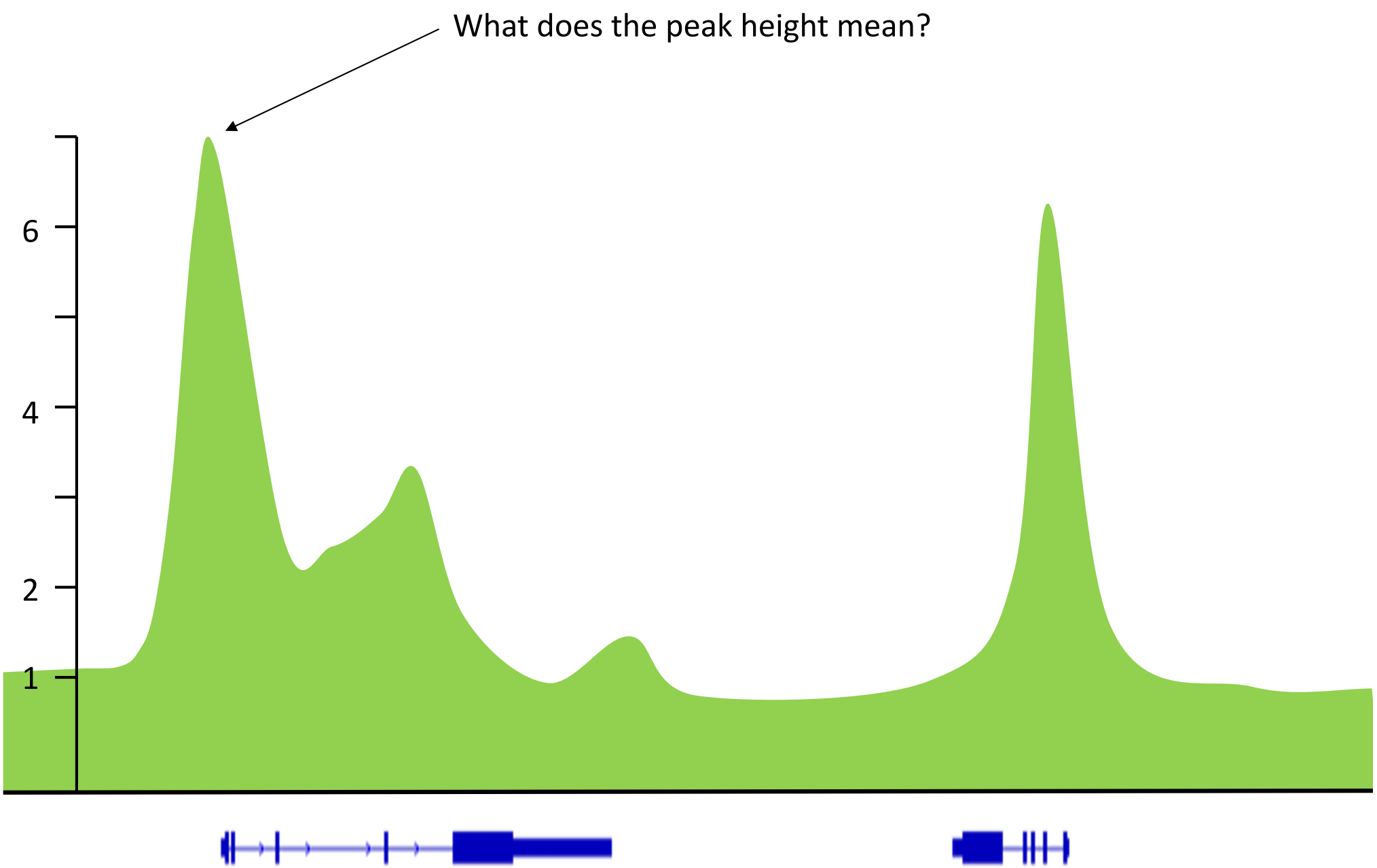


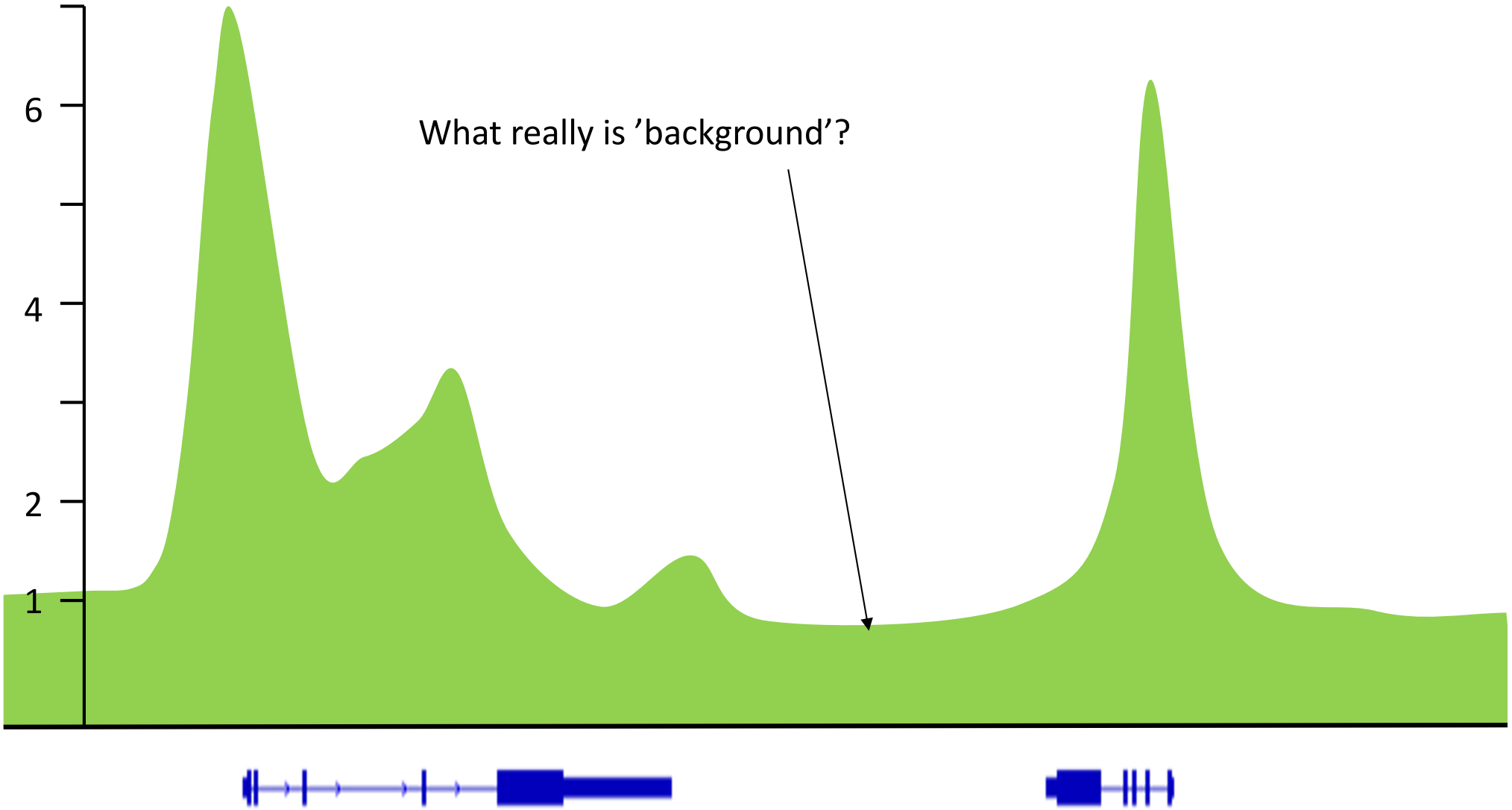
2 integration events needed!

Signal ~ Density^2

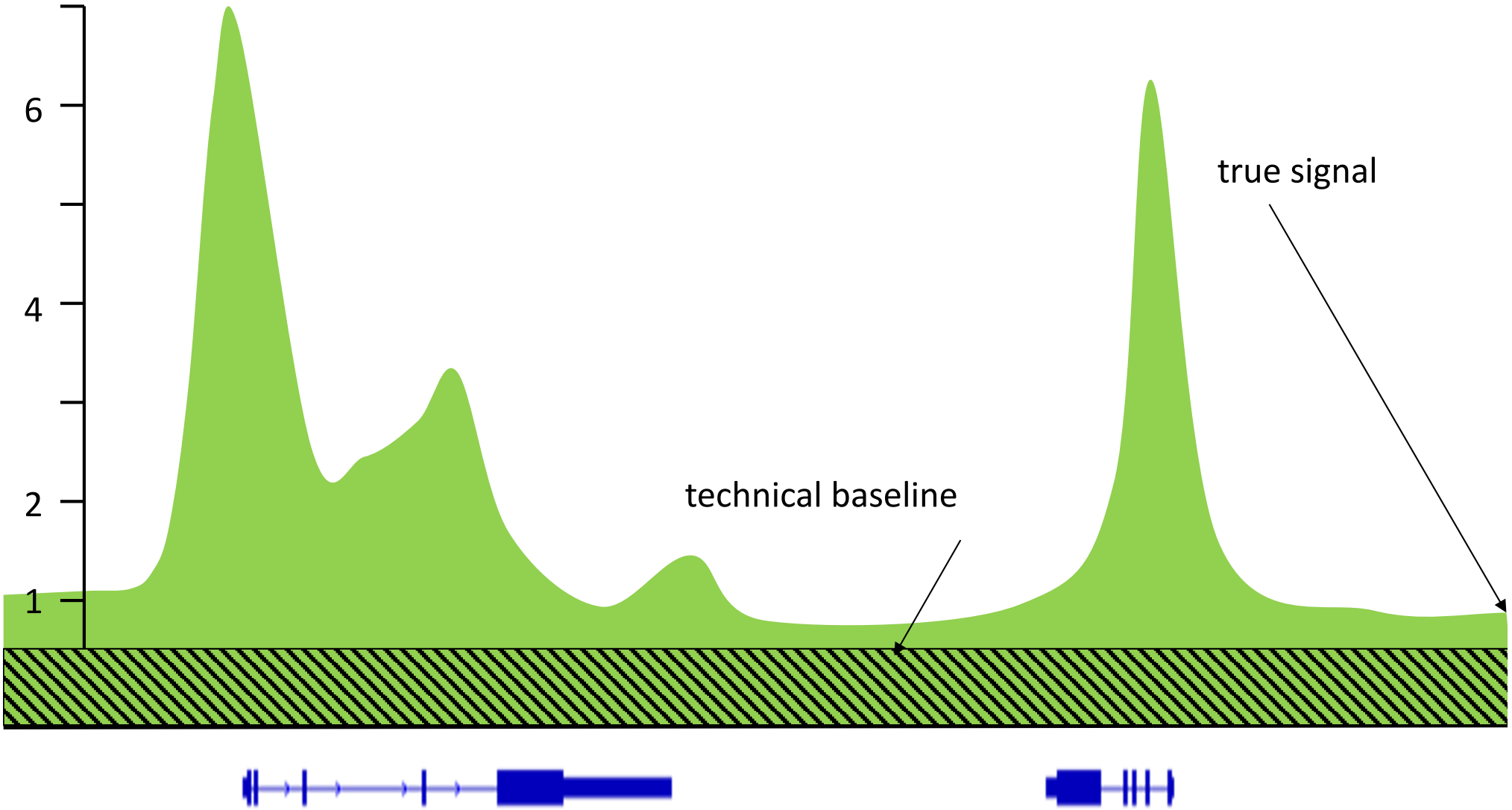
Interpreting ChIP-Seq signal

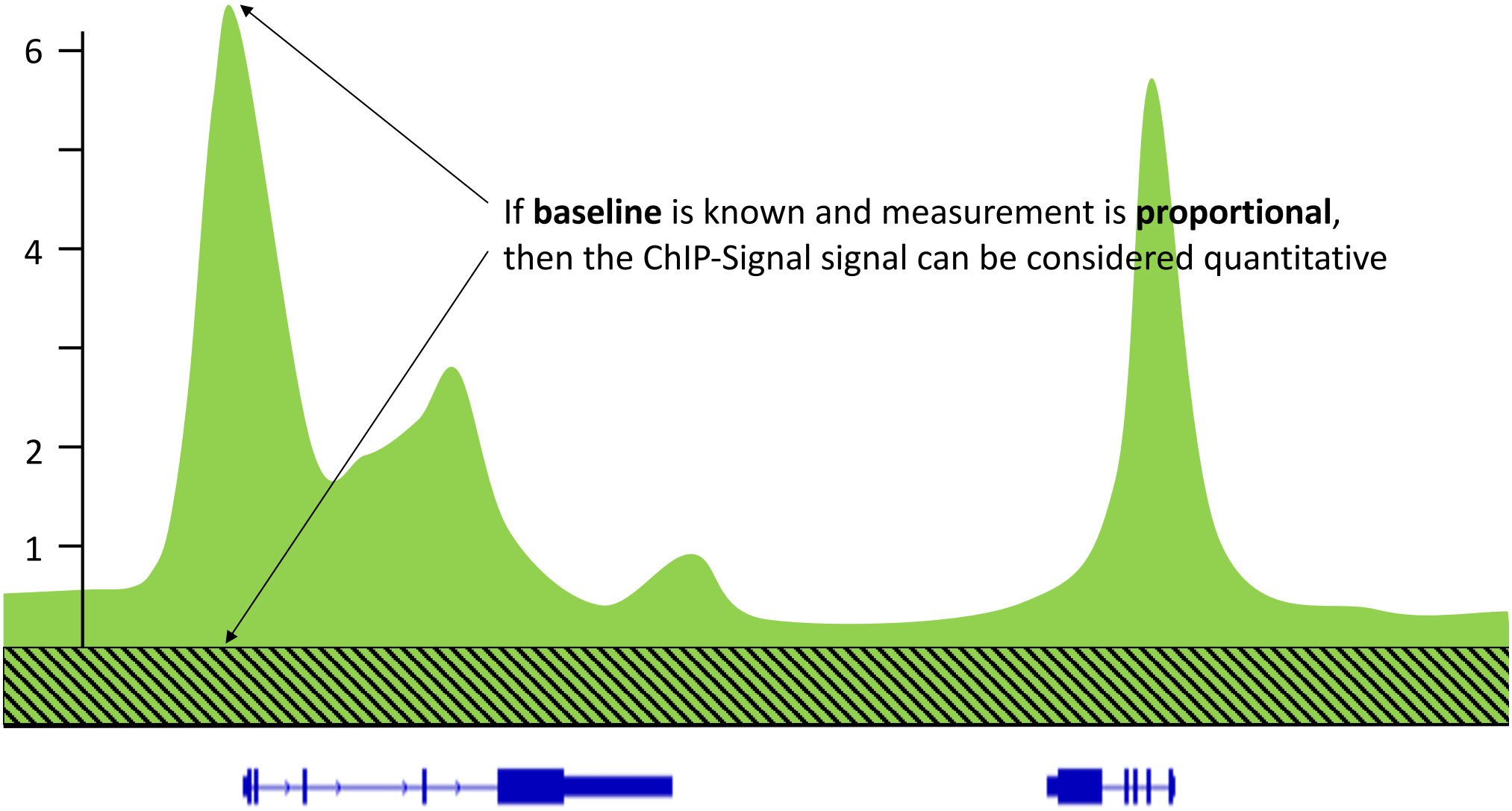



What does the peak height mean?
6
4
2
1


6
4
2
1
What really is ’background’?

What really is ’background’?


6
4
2
1
true signal
technical baseline


6
4
2
1
If **baseline** is known and measurement is **proportional**,
then the ChIP-Signal signal can be considered quantitative

Good ChIP requires good Antibody

Potential problems with antibodies:

- specificity modified histone (crossreactivity for other histone sites/modifications)
- specificity for target protein
- cross-reactivity to other epitopes
- Affinity/Avidity of the interaction and stability against harsh wash conditions
- sensitivity to formaldehyde modification of the target protein

2010

Cell

ATR-X Syndrome Protein Targets Tandem Repeats and Influences Allele-Specific Expression in a Size-Dependent Manner

Martin J. Law,[1,8] Karen M. Lower,[1,8] Hsiao P.J. Voon,[1] Jim R. Hughes,[1] David Garrick,[1] Vip Viprakasit,[3] Matthew Mitson,[1] Marco De Gobbi,[1] Marco Marra,[7] Andrew Morris,[4] Aaron Abbott,[4] Steven P. Wilder,[5] Stephen Taylor,[2] Guilherme M. Santos,[6] Joe Cross,[1] Helena Ayyub,[1] Steven Jones,[7] Jiannis Ragoussis,[4] Daniela Rhodes,[6] Ian Dunham,[5] Douglas R. Higgs,[1] and Richard J. Gibbons[1,*]

2013

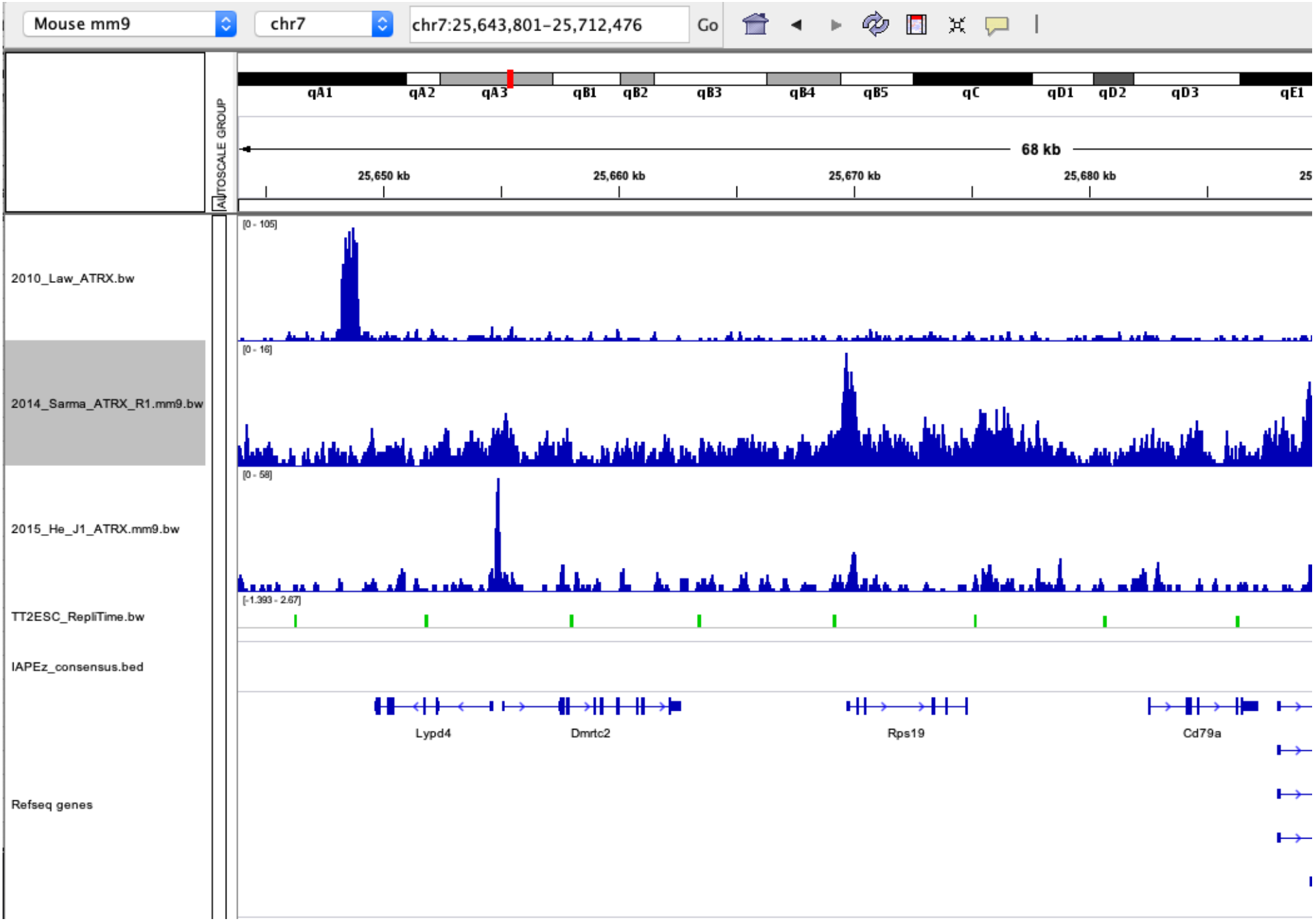
Cell

Article

ATRX Directs Binding of PRC2 to Xist RNA and Polycomb Targets

Kavitha Sarma,[1,2,3] Catherine Cifuentes-Rojas,[1,2,3] Ayla Ergun,[2,3] Amanda del Rosario,[5] Yesu Jeon,[1,2,3] Forest White,[5] Ruslan Sadreyev,[2,3,4] and Jeannie T. Lee[1,2,3,4,*]


Mouse mm9
chr7
chr7:14,647,688-44,008,663
Go
qA1 qA2 qA3 qB1 qB2 qB3 qB4 qB5 qC qD1 qD2 qD3 qE1 qE2 qE3 qF1 qF2
29 mb
20 mb
30 mb
2010_Law_ATRX.bw
[0 - 9.23]
2014_Sarma_ATRX_R1.mm9.bw
[0 - 4.41]
2015_He_J1_ATRX.mm9.bw
[0 - 3.61]
IAPEz_consensus.bed
PEz-int IAPEz-int IAPEz-int IAPEz-int IAPEz-int IAPEz-int IAPEz-int IAPEz-int IAPEz-int IAPEz-int
TT2ESC_RepliTime.bw
[-1.393 - 2.67]
Refseq genes
2a3 Obox2 Bbc3 Psg29 Igfl3 Mypop Bcl3 Vmn1r155 Gm5891 Gm5725 Zfp235 Grik5 Axl Nlrp9a Blvrb Fbl Rinl Zfp84 Etv2 Scgb2b7 Scgb2b15 Wtip Pepd Tshz3 Uri1
Sult2a8 Crx Stm4 Ceacam13 Eml2 Vmn1r95 Gm4214 Gm8677 V1rd19 Zfp574 Nlrp9a Blvrb Gmfg Yif1b Zfp82 Lsr Scgb2b17 Cebpa 1600014C10
Vmn1r90 Crxos Stm4 Ceacam12 Vasp Gm8453 Gm4175 Vmn1r137 Zfp180 Pou2f2 Nlrp9a Dyrk1b Zfp27 Dmkn Cebpa
Meis3 Dmpk Vmn1r130 Vmn1r130 Nlrp5 Zfp428 Lipe BC024978 Gm19897 Zfp27 Dmkn Cebpa
Meis3 Mir3100 Vmn1r107 Vmn1r107 Nlrp5 Arhgef1 BC024978 Ffar2 Cebpa
Clasrp Zfp180

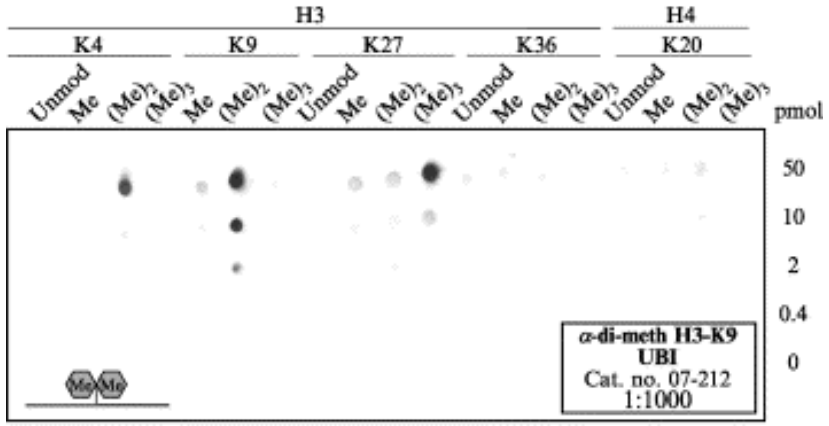

Mouse mm9
chr7
chr7:25,643,801-25,712,476
Go
AUTOSCALE GROUP
qA1
qA2
qA3
qB1
qB2
qB3
qB4
qB5
qC
qD1
qD2
qD3
qE1
68 kb
25,650 kb
25,660 kb
25,670 kb
25,680 kb
25
[0 - 105]
2010_Law_ATRX.bw
[0 - 16]
2014_Sarma_ATRX_R1.mm9.bw
[0 - 58]
2015_He_J1_ATRX.mm9.bw
[-1.393 - 2.67]
TT2ESC_RepliTime.bw
IAPEz_consensus.bed
Lypd4
Dmrtc2
Rps19
Cd79a
Refseq genes

Good ChIP requires good Antibody

Potential problems with antibodies:

background affinity for unmodified peptide epitope proximal to the site of modification

•specificity for seq. context



Good ChIP requires good Antibody

The worse the antibody, the more ChIP will look like input

And normalizing for uneven input is tricky! Options

- ratio ChIP versus background
- background subtraction

Cistrome Data Browser

Tips

- Check what factors regulate your gene of interest, what factors bind in your interval or have a significant binding overlap with your peak set. Have a try at CistromeDB Toolkit.
- If you have a **T**ranscription **F**actor ChIP-seq (and TF perturbed expression) data, Cistrome-GO help you predict the function of this TF.
- Please help us curate the samples which has incorrect meta-data annotation by clicking the button on the inspector page. Thank you!

Containing word(s): Search Options

Species	Biological Sources	Factors
All	All	All
Homo sapiens	1-cell pronuclei	AATF
Mus musculus	1015c	ABCC9
	10326	ACSS2
	1064Sk	ACTB
	106A	ADNP

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	

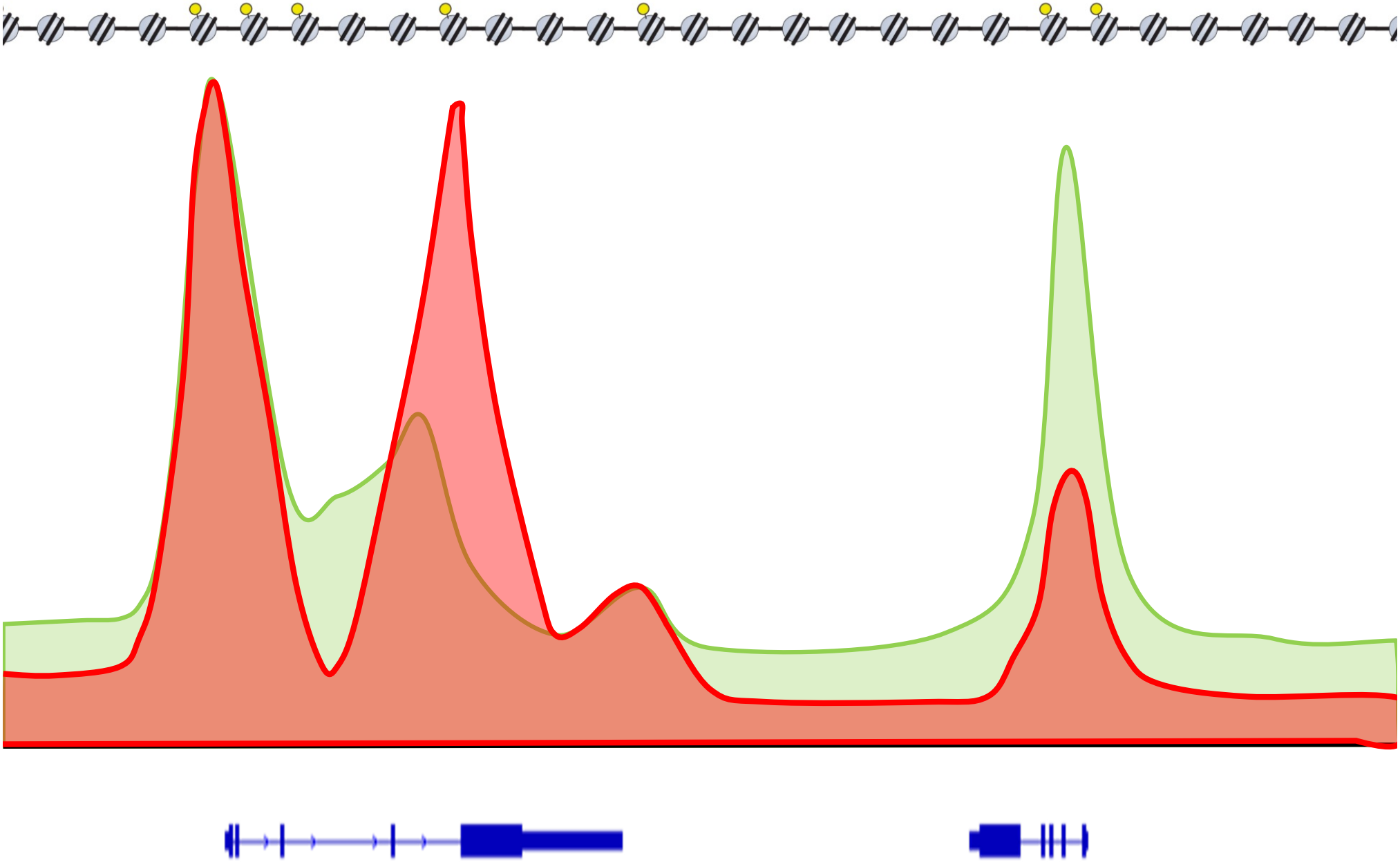
http://cistrome.org/db/#/

Applications of ChIP-Seq and related methods

- Map features to genome (--> knowing where a features is *may* imply function)
- Specific versus genome-wide feature
- Discover genome-wide correlations (--> generate experimental hypothesis --> test to establish causation)
  - Think about the meaning: “repressive chromatin”, “activating mark”, “silencing factor”
  - A histone PTM “recruits” a factor
  - A factor ‘protects’ a gene from spurious transcription
- Compare conditions, e.g. how does a knockout, inhibitor, external change in condition affect epigenome?

These applications imply that you are interested in quantitatively comparing occupancies or levels of PTMs!

Interpreting ChIP-Seq signal



Relative quantitation Epigenomic profiles can be compared

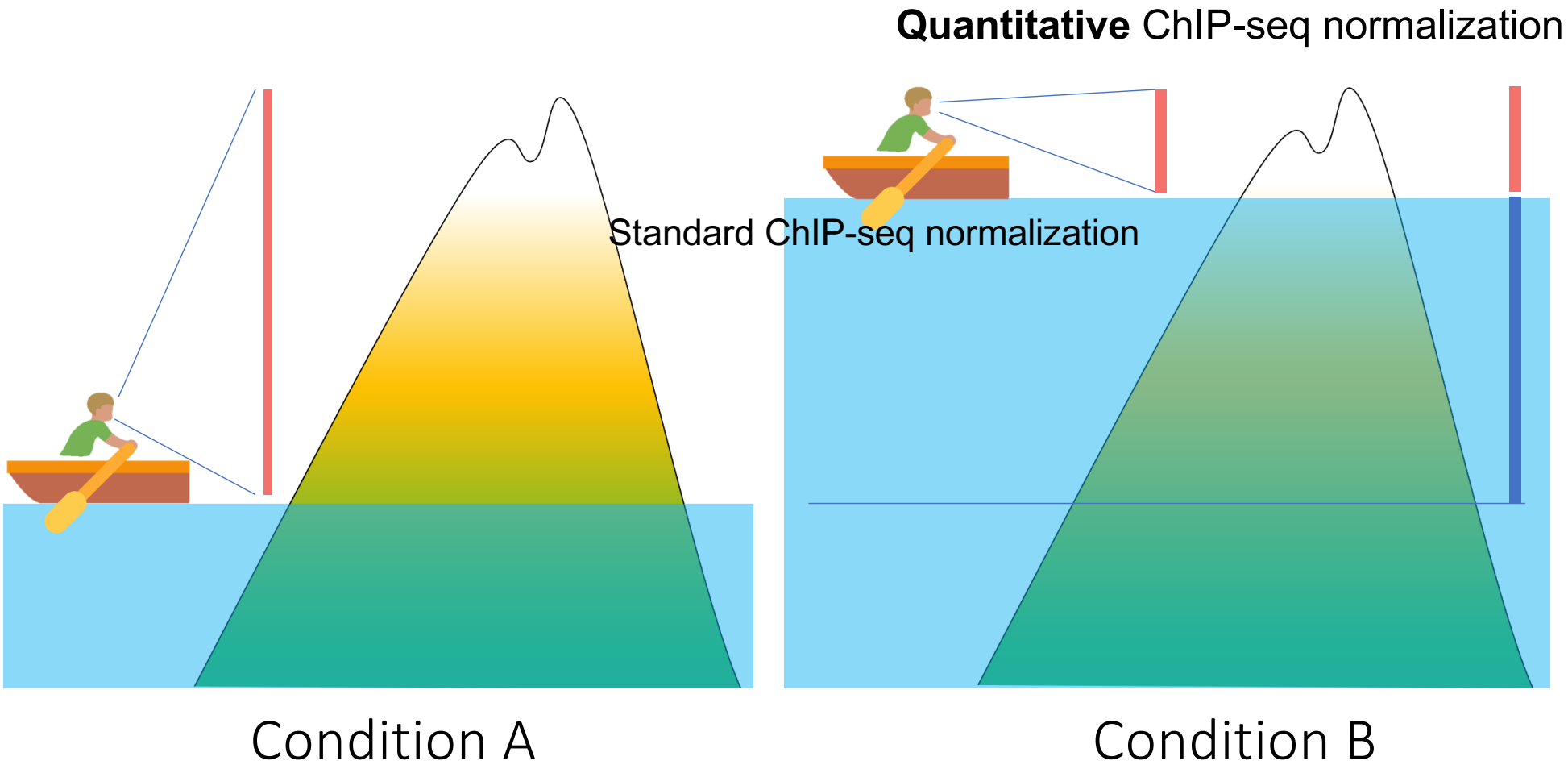
Before comparing, we need to ensure that samples are normalized. Traditional normalization brings all samples to the same effective sequencing depth, and it is assumed that then the samples can be compared quantitatively.

Normalization methods used

- RPKM/FPKM (Reads/Fragments Per Kilobase Million)
- RPGC (Reads Per Genome Coverage, “1x normalization)

Assumes that relative signal changes but global levels and background do not change!

Capturing quantitative epigenome landscapes



Quantitative ChIP-Seq

- Measured signal (=read density) scales linear (proportional) with 'true' signal
- Signal is comparable quantitatively between samples
- Technical or batch does not influence the quantitative answer

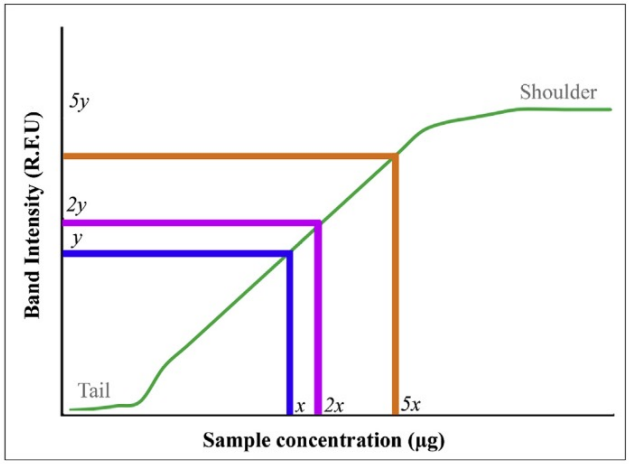


Fig. 2. Linear relationship between sample concentration and band intensity. The signal derived from the protein bands on a Western blot varies with the amount of sample extract loaded onto the protein gel. The illustrated graph depicts a linear and proportional relationship between amount of sample loaded (x, $2x$, $5x$) and the relative fluorescence units (R.F.U) captured from the target bands (y, $2y$, $5y$). Tail and shoulder end of the data curve capture noise and saturated signal, respectively. µg, micrograms; R.F.U, Relative fluorescence units.

Absolute quantification further requires that the output can be understood in a real-world unit (e.g. binding occupancy or PTM density in fraction/percent)

Quantitative ChIP with *Drosophila* Spike-in

Biological chromodynamics: a general method for measuring protein occupancy across the genome by calibrating ChIP-seq

Bin Hu, Naomi Petela, Alexander Kurze, Kok-Lung Chan, Christophe Chapard, Kim Nasmyth

Nucleic Acids Research, Volume 43, Issue 20, 16 November 2015, Page e132,
https://doi.org/10.1093/nar/gkv670
Published: 30 June 2015 **Article history**

ChIPSeqSpike: A R/Bioconductor package for ChIP-Seq data scaling according to spike-in control

Descostes N, Tsirigos A, Reinberg D

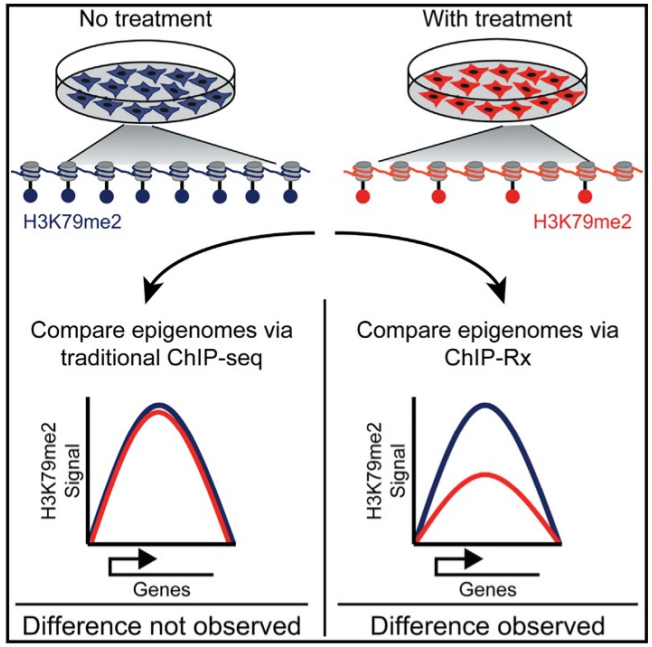
Preprint from **bioRxiv**, 22 Feb 2018

Resource

Cell Reports

Quantitative ChIP-Seq Normalization Reveals Global Modulation of the Epigenome

Graphical Abstract



Highlights

ChIP-seq is a prevailing methodology to investigate and compare epigenomic states

Lack of an empirical normalization strategy has limited the usefulness of ChIP-seq

ChIP-Rx allows genome-wide quantitative comparisons of histone modification status

Authors

David A. Orlando, Mei Wei Chen, ..., James E. Bradner, Matthew G. Guenther

Correspondence

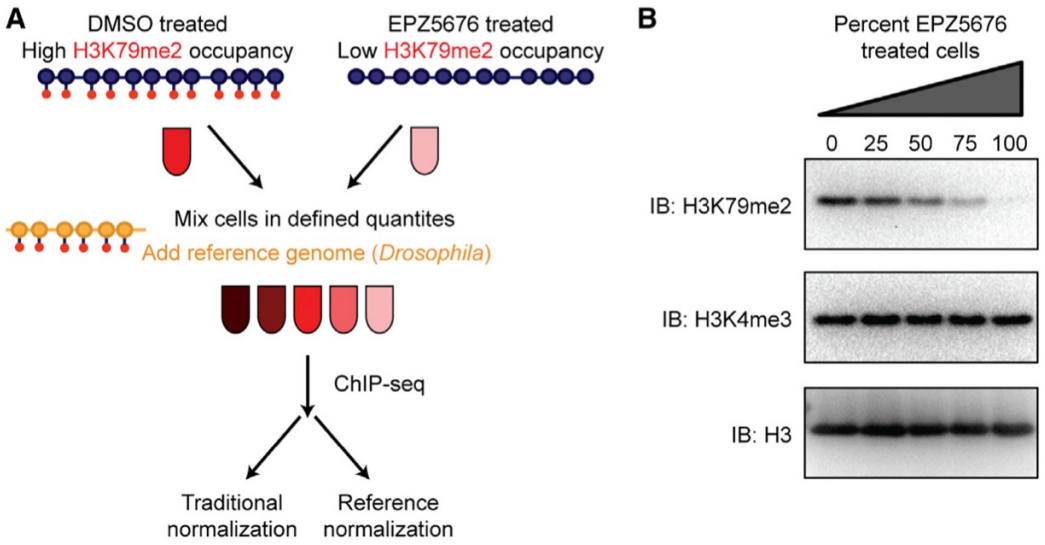
dorlando@syros.com (D.A.O.),
mguenther@syros.com (M.G.G.)

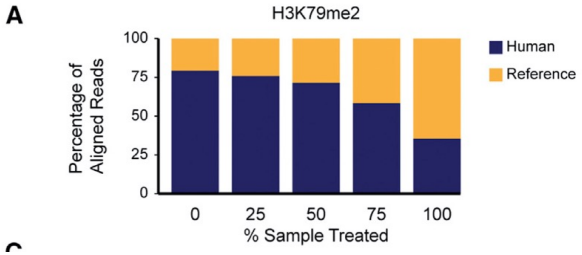
In Brief

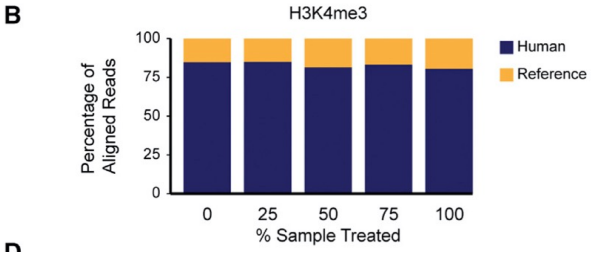
The lack of an empirical methodology to enable normalization among chromatin immunoprecipitation coupled with massively parallel DNA sequencing (ChIP-seq) experiments has limited the precision and comparative utility of this technique. Orlando et al. describe a method, called ChIP with reference exogenous genome (ChIP-Rx), that allows one to perform genome-wide quantitative comparisons of histone modification status across cell populations using defined quantities of a reference epigenome. They use the method to detect disease-relevant epigenomic changes following drug treatment.

Accession Numbers

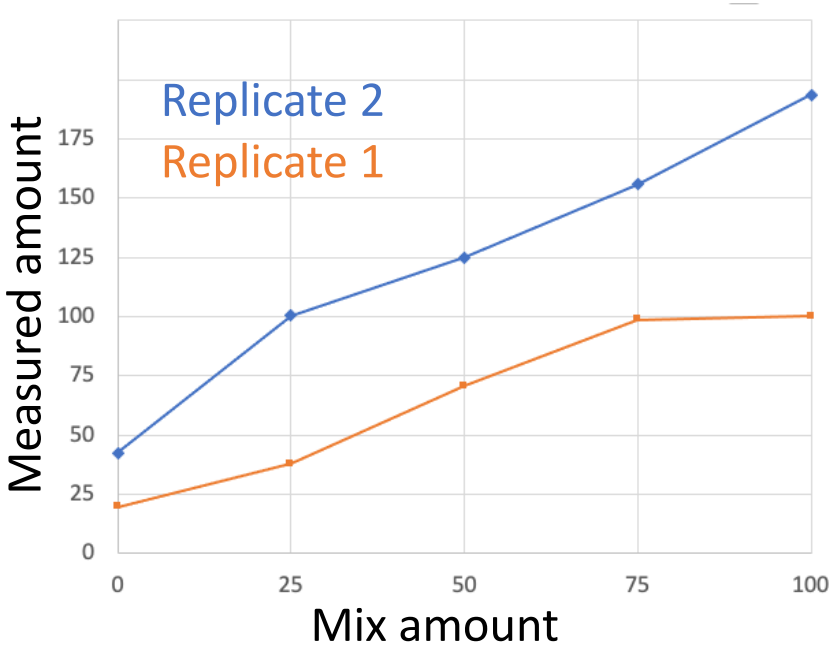
GSE60104







Multiple replicates are essential for correct spike-in normalization!



Practical solutions to quantitative scaling

- BAM-based, then carry along scale factor determined by BAM read counts for scaling on-the-fly

- BigWig-based, scaled bigwig file can be used in any downstream analysis

Limitations of Spike-in ChIP

- Spike-in amount has to be accurate in relation to chromatin amount (think about error in cell counting, protein assay or pipetting)
- Alternatively, fraction Drosophila reads spiked-in needs to be determined experimentally by sequencing input. Some confusion in the field exists if or not input is taken into account
- Some confusion exists if and when a background normalization using input can be done while also using spike-in normalization (how to normalize input? divide or subtract?)
- Antibody must crossreact with the spike-in species.

Excercise – reanalysis of Orlando data using Bioconductor package

ChIP-seq with exogenous chromatin spike

This tutorial is included from previous workshop. Thanks!

ChIPSeqSpike: A R/Bioconductor package for ChIP-Seq data scaling according to spike-in control

Descostes N, Tsirigos A, Reinberg D

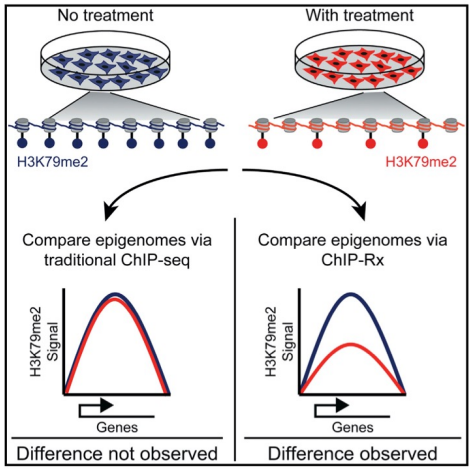
Preprint from **bioRxiv**, 22 Feb 2018

Resource

Cell Reports

Quantitative ChIP-Seq Normalization Reveals Global Modulation of the Epigenome

Graphical Abstract



Highlights

ChIP-seq is a prevailing methodology to investigate and compare epigenomic states

Lack of an empirical normalization strategy has limited the usefulness of ChIP-seq

ChIP-Rx allows genome-wide quantitative comparisons of histone modification status

Authors

David A. Orlando, Mei Wei Chen, ..., James E. Bradner, Matthew G. Guenther

Correspondence

dorlando@syros.com (D.A.O.), mguenther@syros.com (M.G.G.)

In Brief

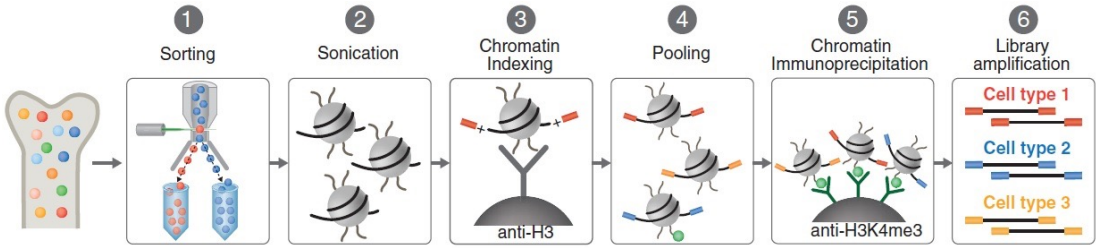
The lack of an empirical methodology to enable normalization among chromatin immunoprecipitation coupled with massively parallel DNA sequencing (ChIP-seq) experiments has limited the precision and comparative utility of this technique. Orlando et al. describe a method, called ChIP with reference exogenous genome (ChIP-Rx), that allows one to perform genome-wide quantitative comparisons of histone modification status across cell populations using defined quantities of a reference epigenome. They use the method to detect disease-relevant epigenomic changes following drug treatment.

Accession Numbers

GSE60104

Barcode-first methods

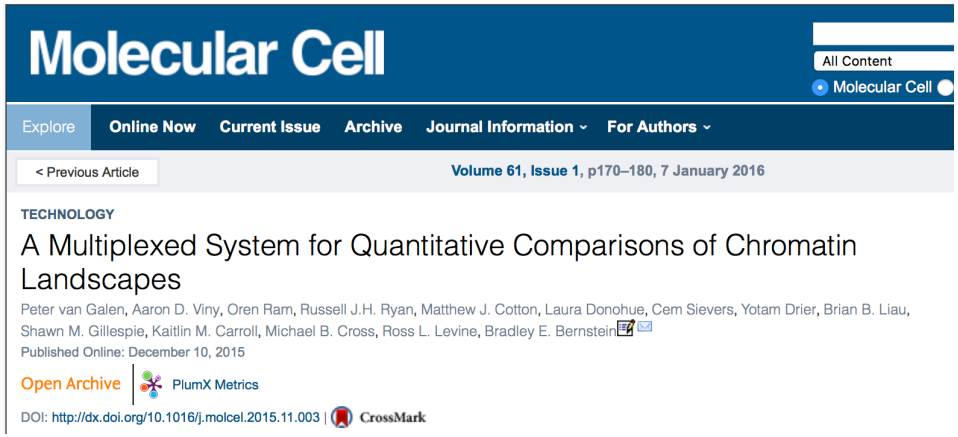
I-ChIP: on-beads barcoding



One-pot methods ChIP

A high−throughput ChIP−Seq for large−scale chromatin studies

Christophe D Chabbert, Sophie H Adjalley, Bernd Klaus, Emilie S Fritsch, Ishaan Gupta, Vicent Pelechano, Lars M Steinmetz



Resource

Cell Reports

Quantitative Multiplexed ChIP Reveals Global Alterations that Shape Promoter Bivalency in Ground State Embryonic Stem Cells

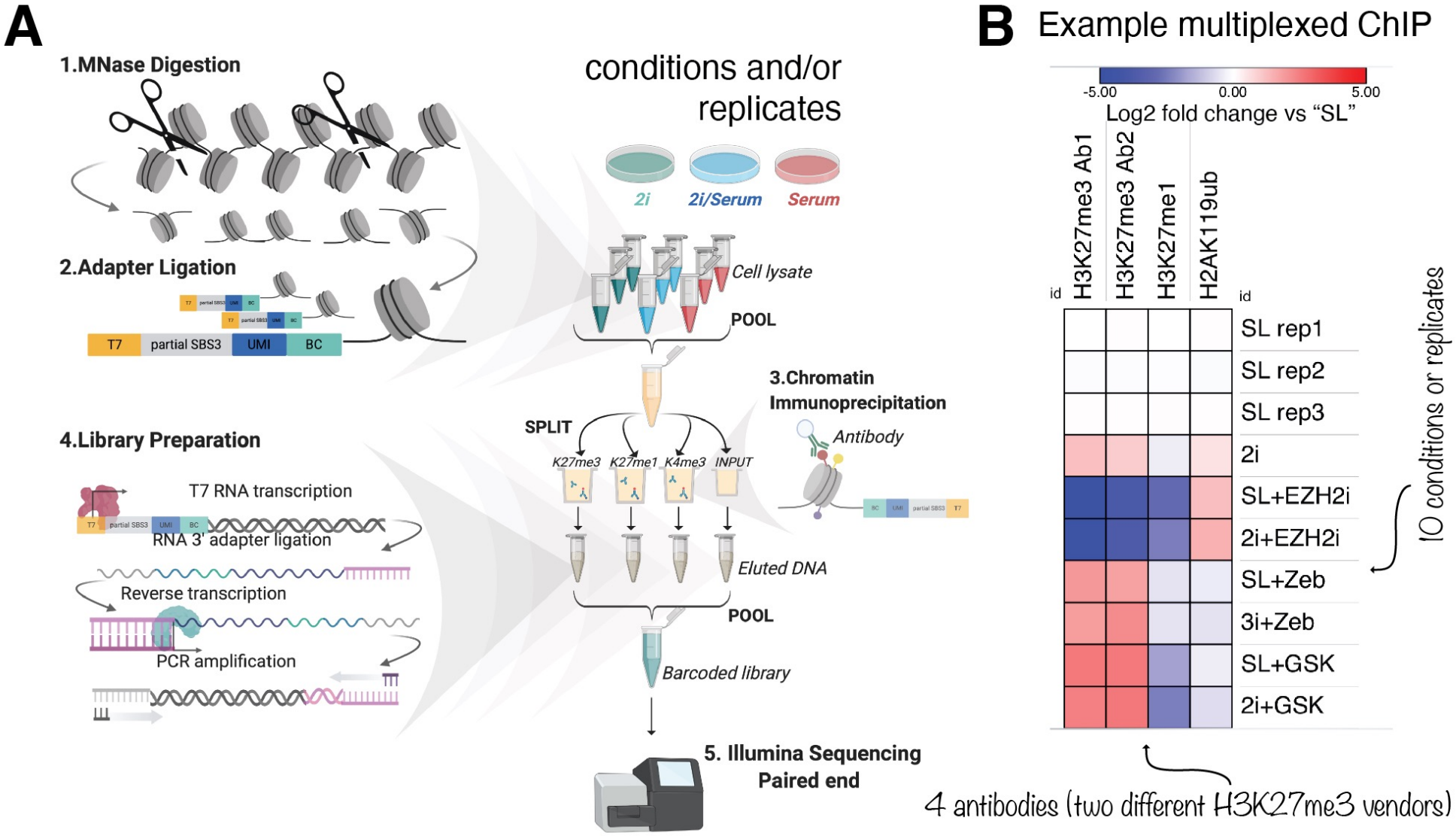
Graphical Abstract

Quantitative ChIP with large linear dynamic range

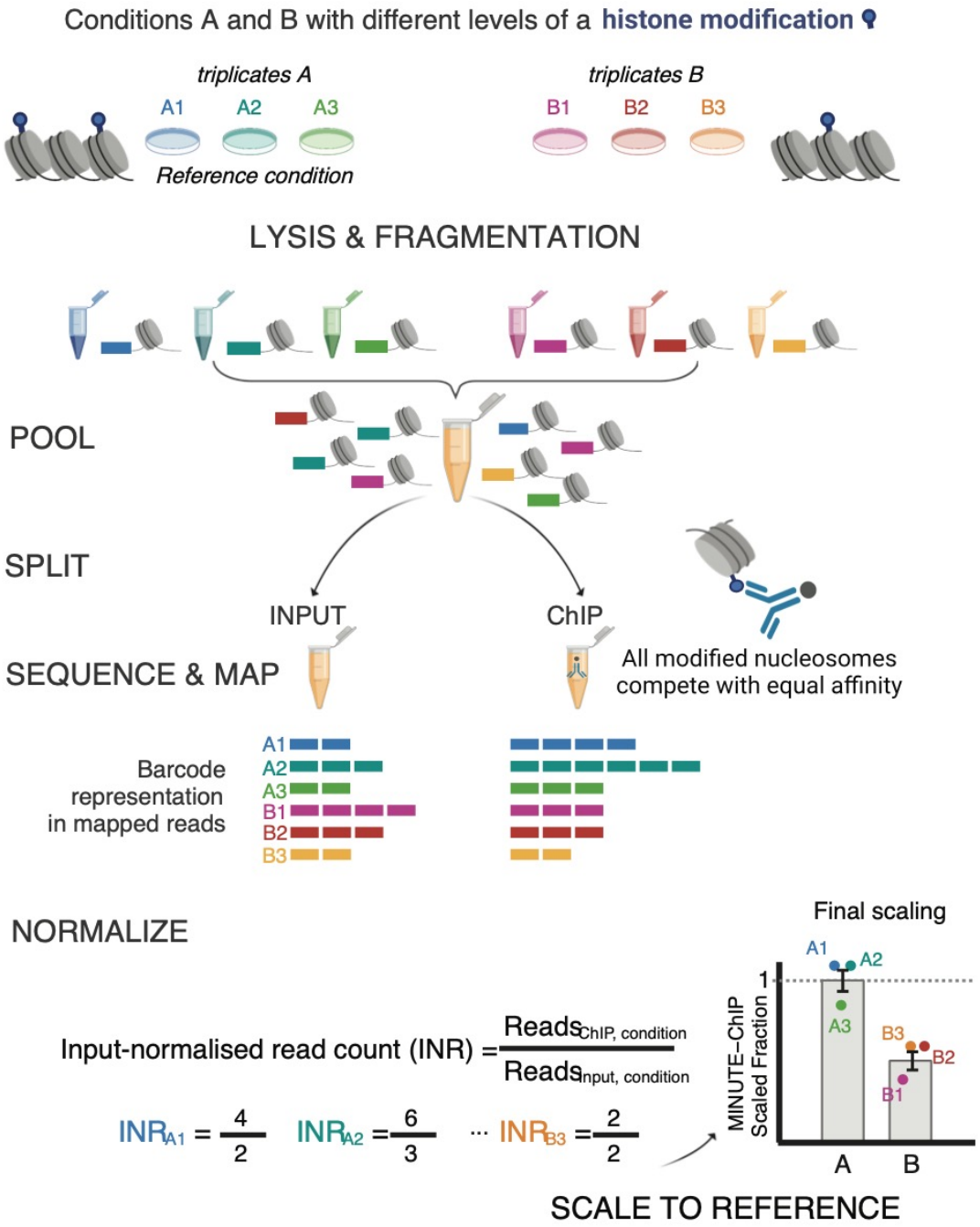
Authors

Banushree Kumar, Simon J. Elsässer

Multiplexed ChIP (MINUTE-ChIP)



MINUTE-ChIP Quantification

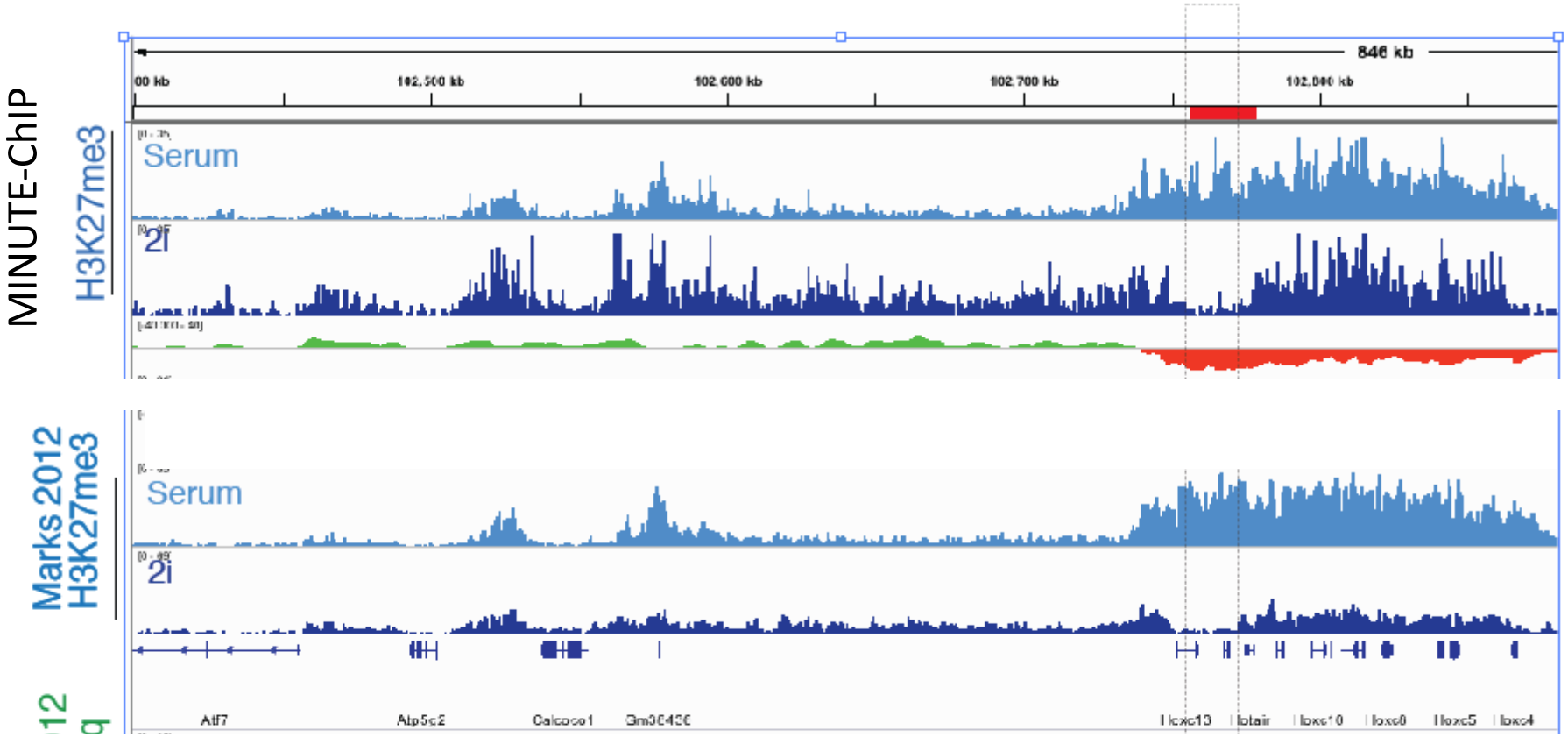


Nature Protocols upcoming:

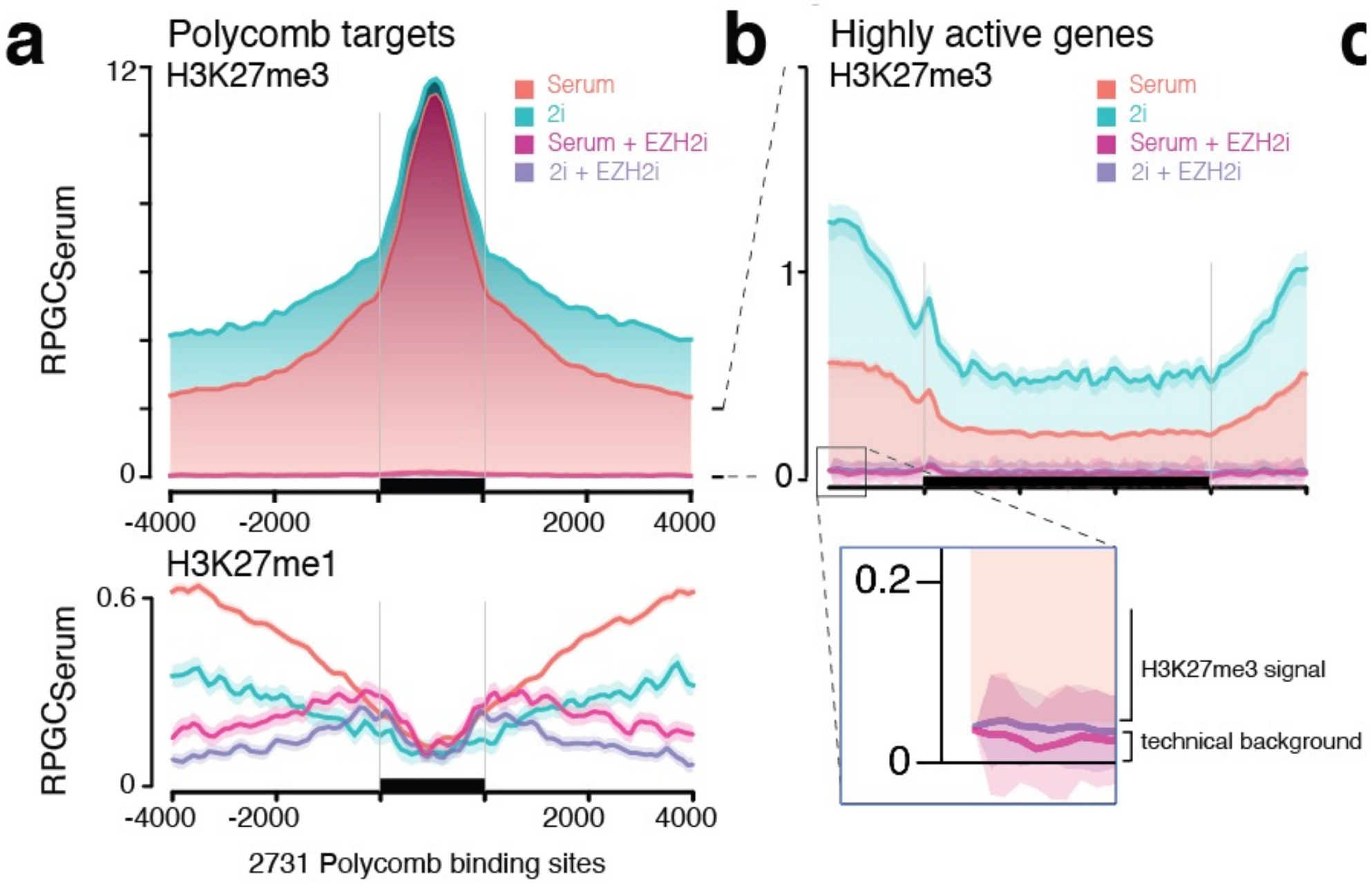
https://doi.org/10.1038/s41596-024-01058-z

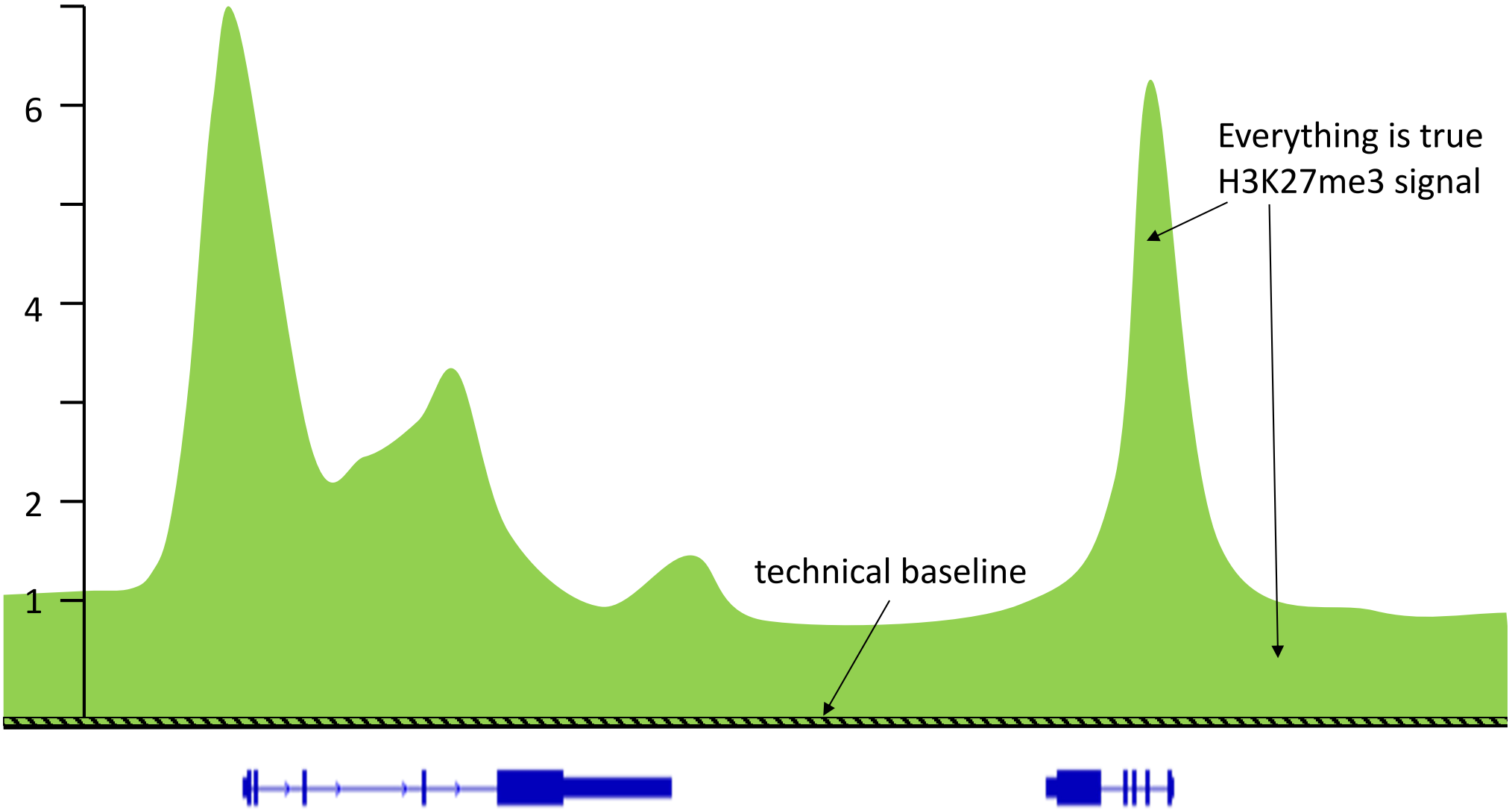
Comparison normal and quantitative ChIP

Comparing naïve and ‘primed’ mouse embryonic stem cells

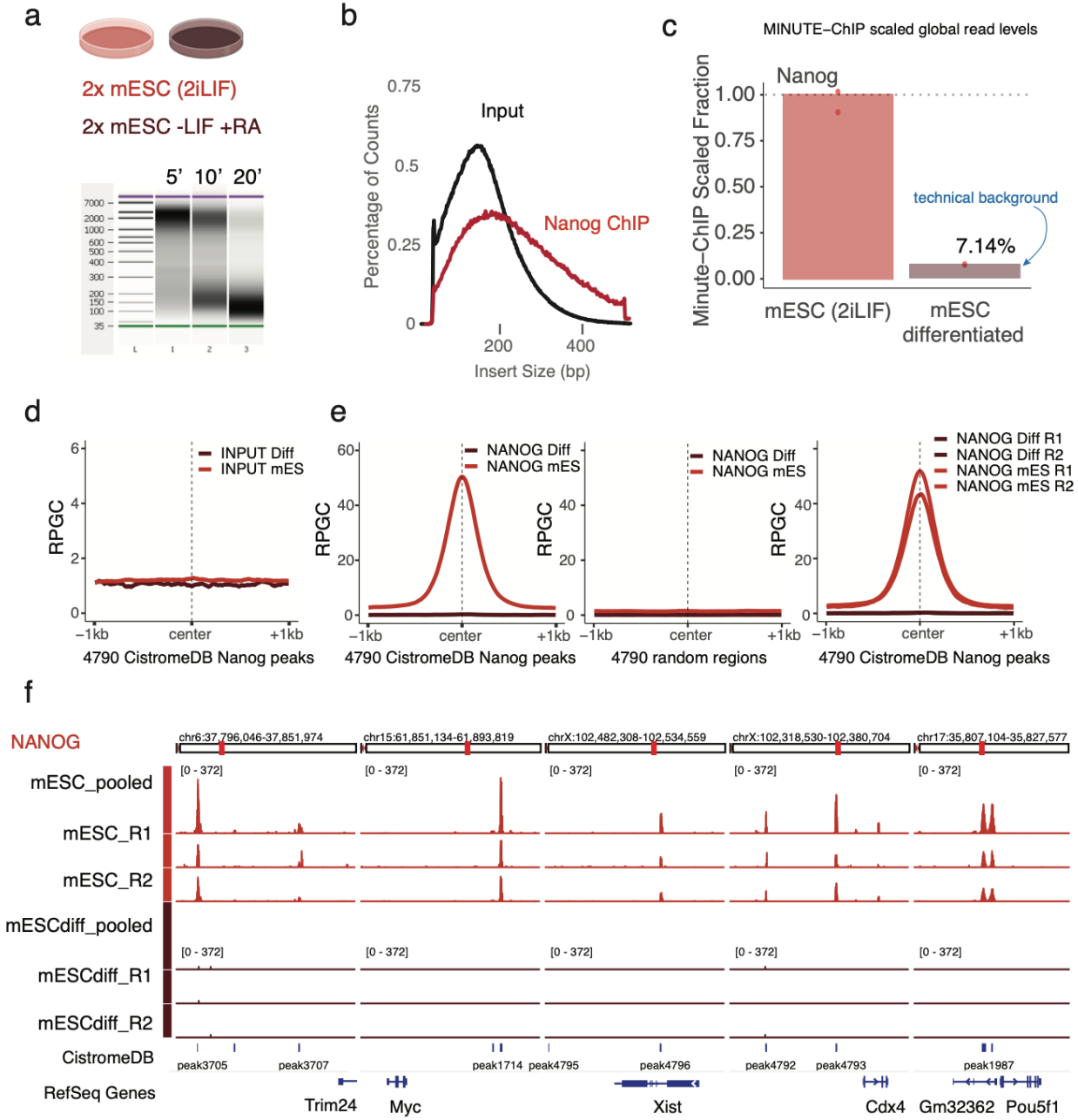


Multiplexed ChIP has very little technical background




6
4
2
1
Everything is true
H3K27me3 signal
technical baseline

MINUTE-ChIP for transcription factors



Kumar et. al., Nature Protocols, 2024

Excercise – Reanalysis of MINUTE-ChIP data

MINUTE-ChIP

- Background
- Primary analysis
  - Conda environment
  - Files
  - Running Minute
  - Scaling info
  - IGV tracks
- Downstream analysis
  - Files
  - Looking at bivalent genes
  - Genome-wide bin distribution

Article | Open Access | Published: 30 May 2022

Polycomb repressive complex 2 shields naïve human pluripotent cells from trophectoderm differentiation

Banushree Kumar, Carmen Navarro, Nerges Winblad, John P. Schell, Cheng Zhao, Jere Weltner, Laura Baqué-Vidal, Angelo Salazar Mantero, Sophie Petropoulos, Fredrik Lanner ✉ & Simon J. Elsässer ✉

Nature Cell Biology **24**, 845–857 (2022) | Cite this article

Questions?

simon.elsässer@scilifelab.se