

“Advanced” ChIP-Seq

- QC
- Applications of ChIP-Seq and related methods
- Quantitative methods
- ChIP-Seq alternatives

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

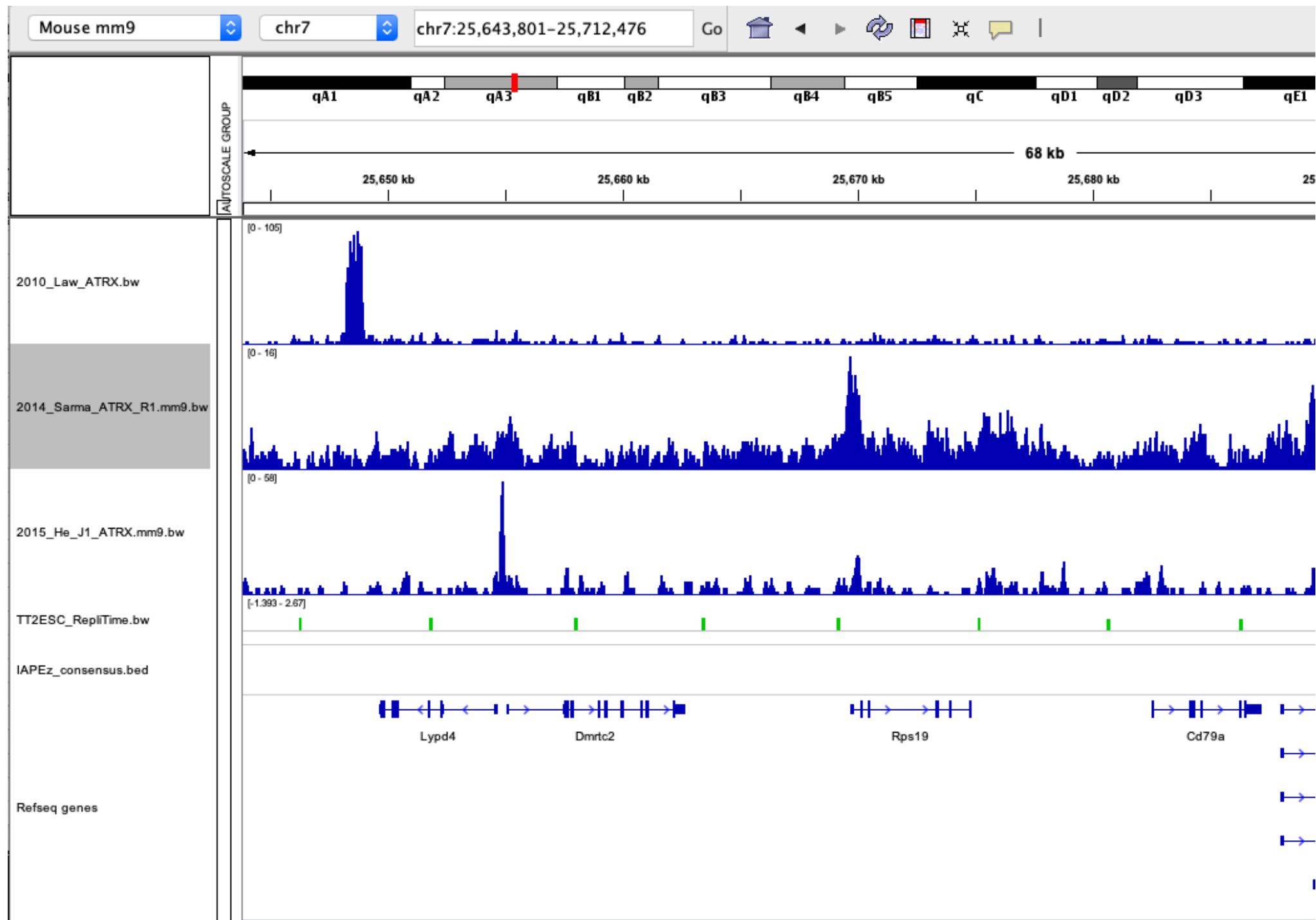
Cell

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,*}



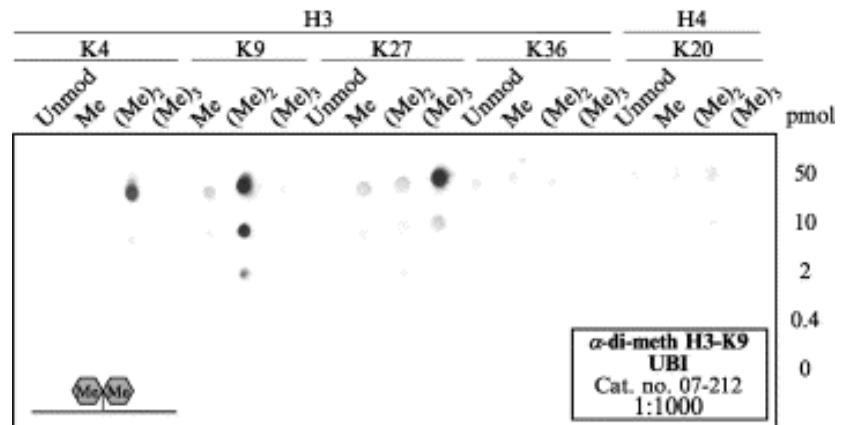


Good ChIP requires good Antibody

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 Transcription Factor 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

All

Homo sapiens
Mus musculus

Biological Sources

All

1-cell pronuclei
1015c
10326
1064Sk
106A

Factors

All

AATF
ABCC9
ACSS2
ACTB
ADNP

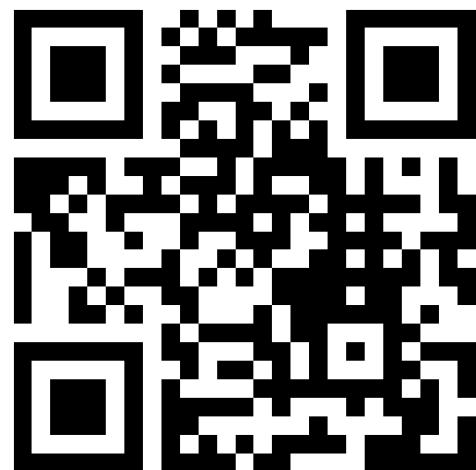
Results

| Batch | Species | Biological Source | Factor | Publication | Quality Control |
|--------------------------|--------------|--------------------------|--------|--|-----------------|
| <input type="checkbox"/> | Homo sapiens | HeLa; Epithelium; Cervix | BTAF1 | Johannes F, et al. Bioinformatics 2010 | |
| <input type="checkbox"/> | Homo sapiens | HeLa; Epithelium; Cervix | GAPDH | Johannes F, et al. Bioinformatics 2010 | |

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

Applications of ChIP-Seq and related methods

www.menti.com ----> use the code 76 77 39 9



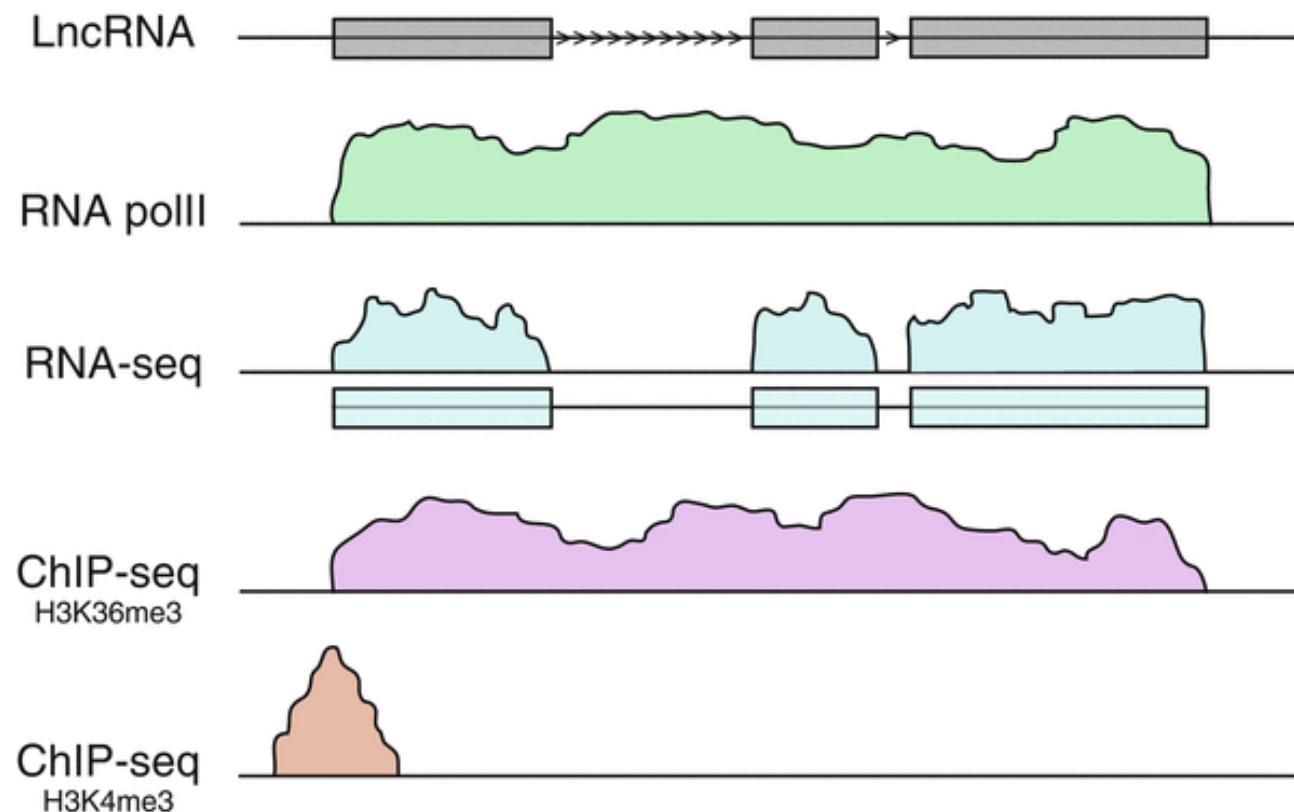
Applications of ChIP-Seq and related methods

- Map features to genome (--> knowing where a feature 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?

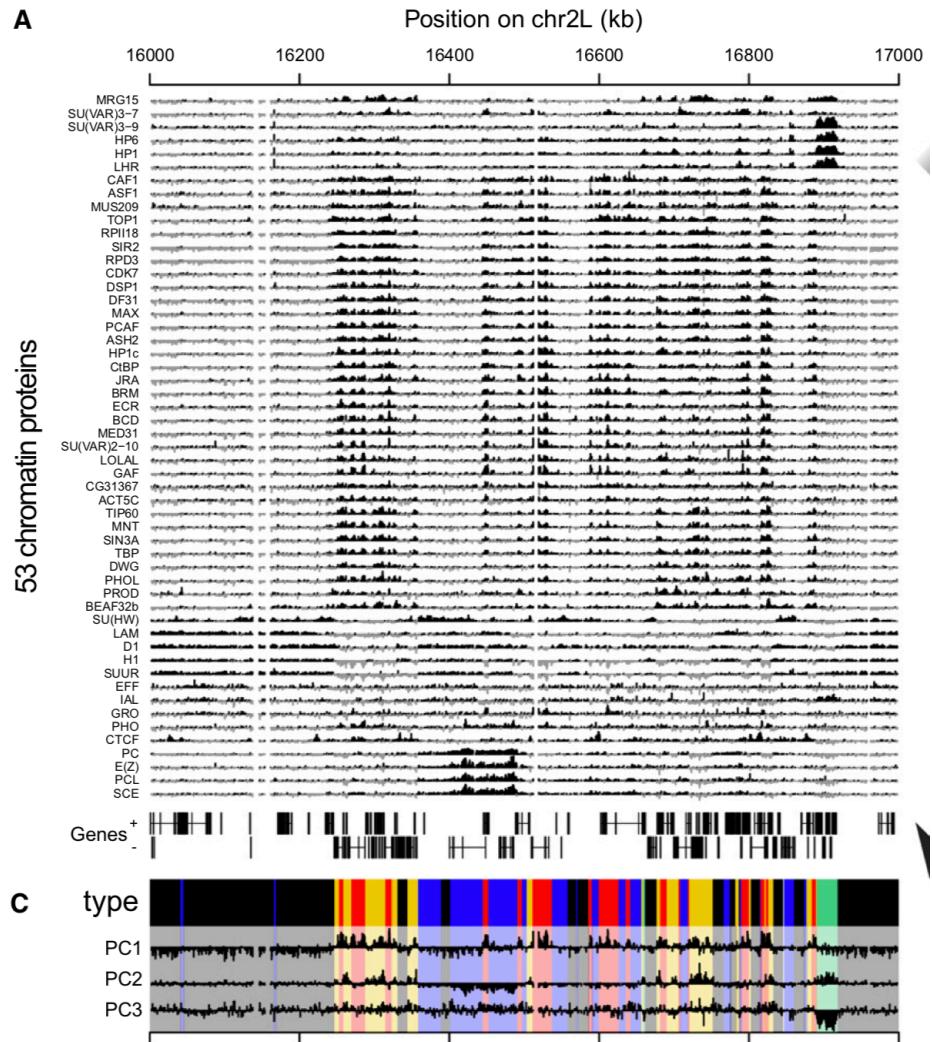
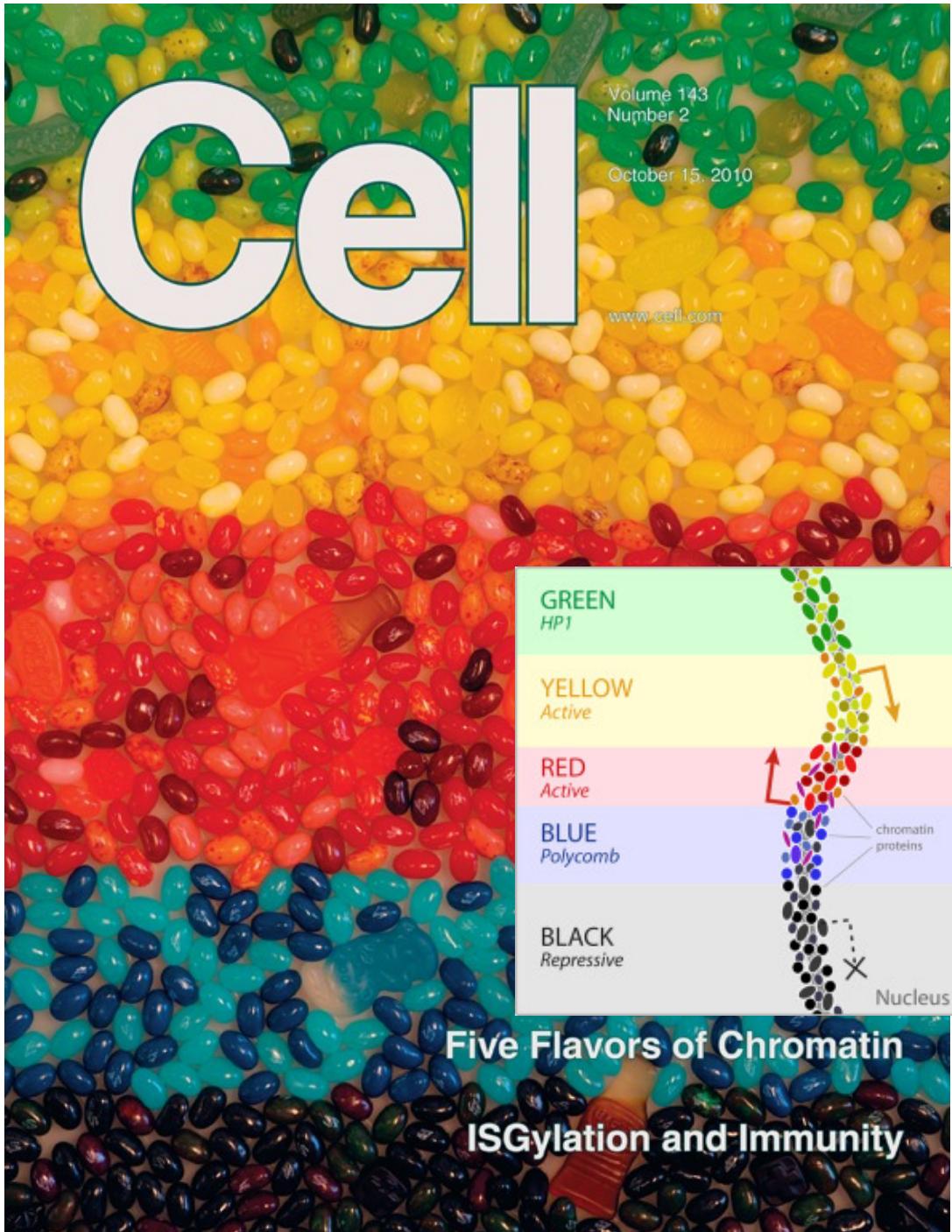
Using histone modifications to predict functional regions in the genome

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

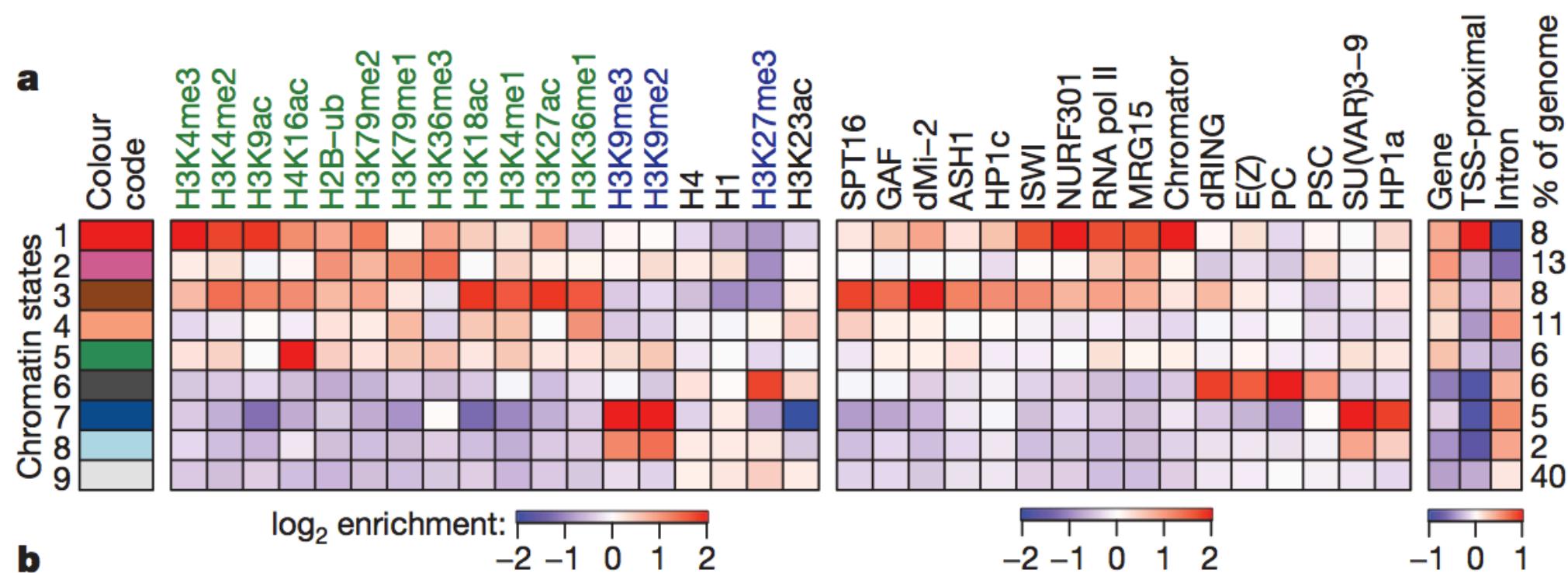
Mitchell Guttman^{1,2}, Ido Amit¹, Manuel Garber¹, Courtney French¹, Michael F. Lin¹, David Feldser³, Maite Huarte^{1,6},



Chromatin 'states' define functional regions



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



Applications of ChIP-Seq and related methods

- Map features to genome (--> knowing where a feature 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!

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

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)

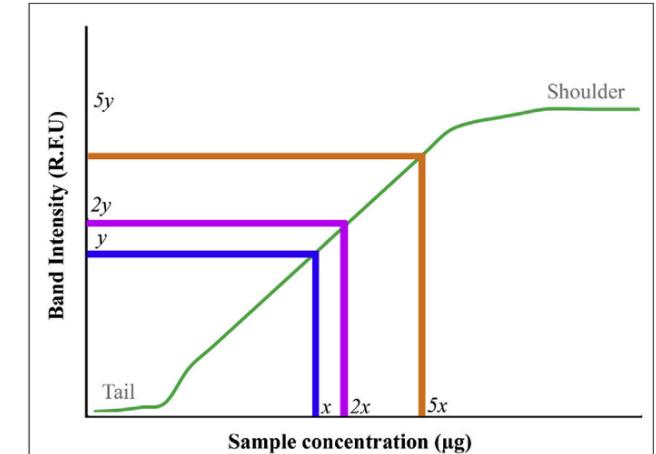
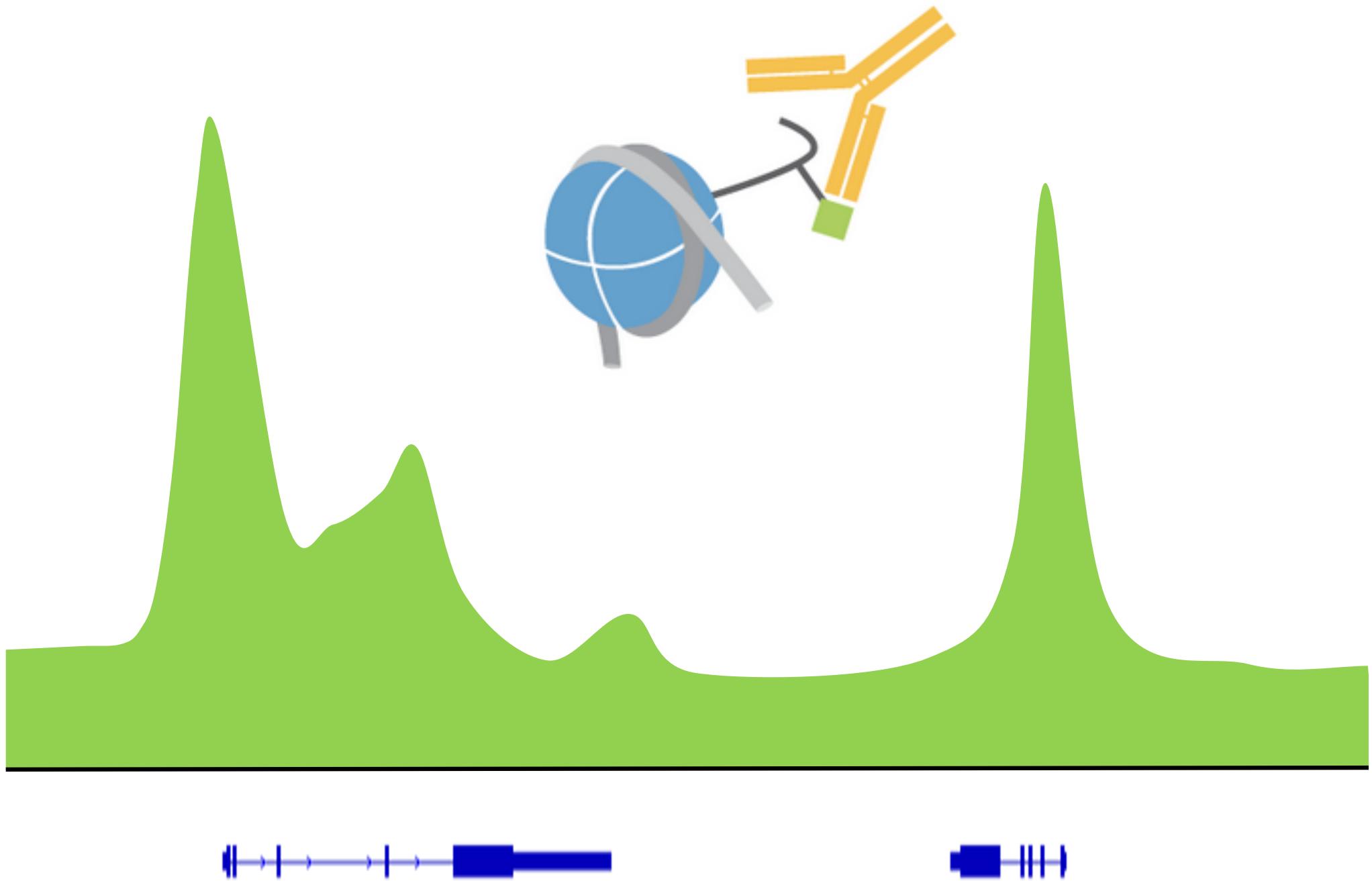
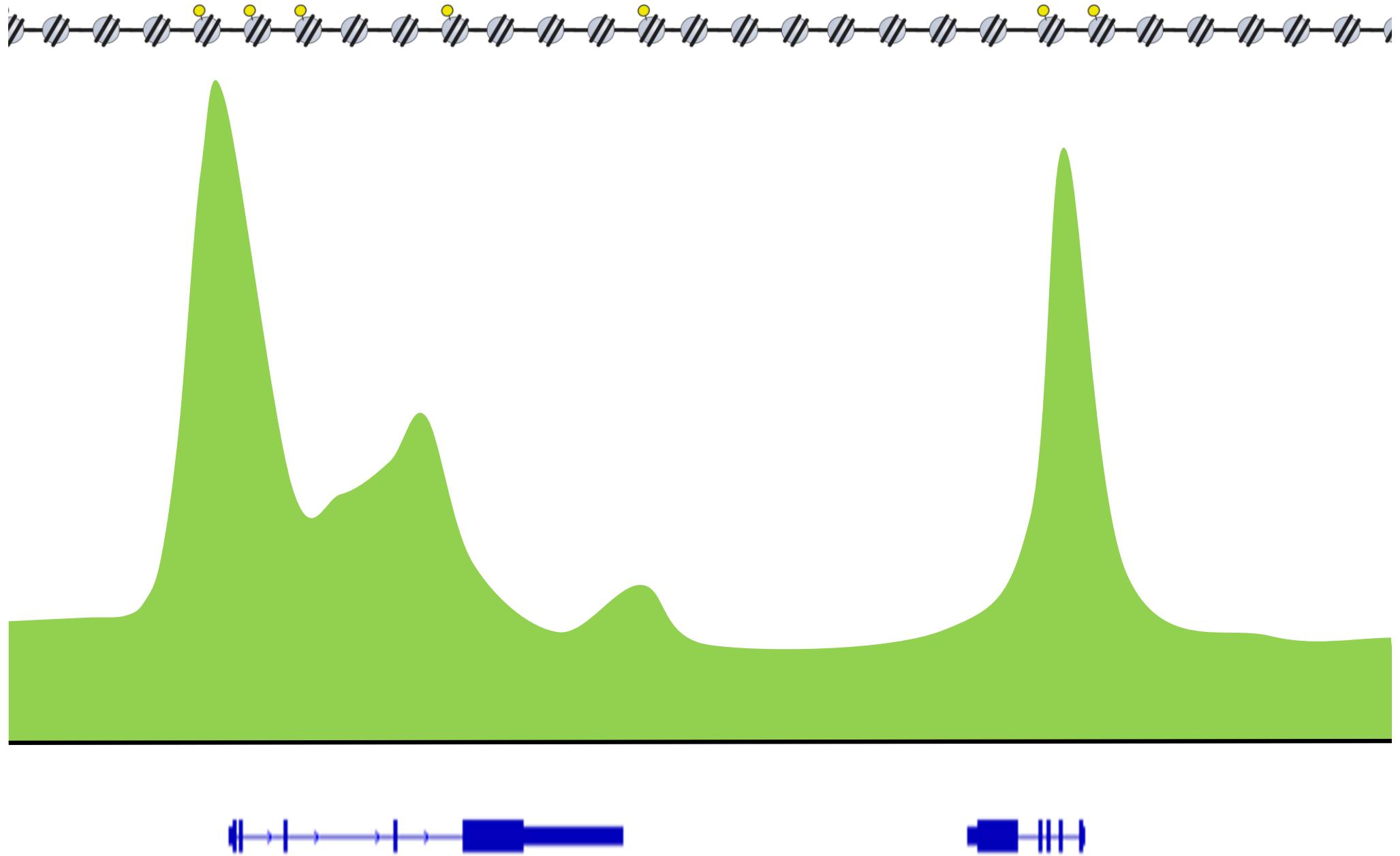


Fig. 2. Linear relationship between sample concentration and band intensity. The signal derived from the protein bands on a Western blot varies with the amount of sample extract loaded onto the protein gel. The illustrated graph depicts a linear and proportional relationship between amount of sample 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.

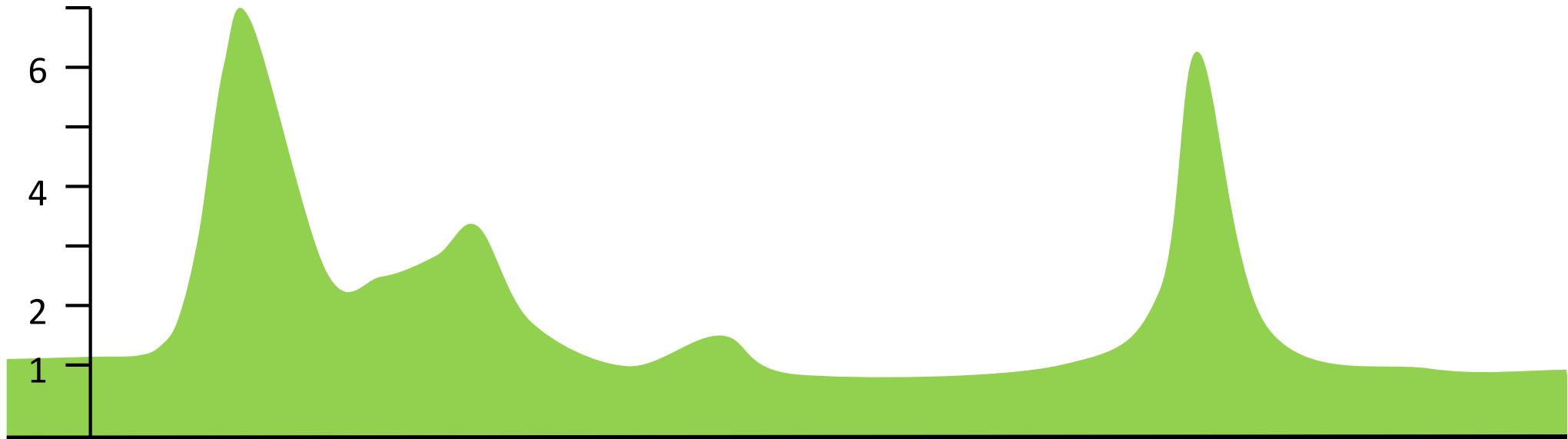
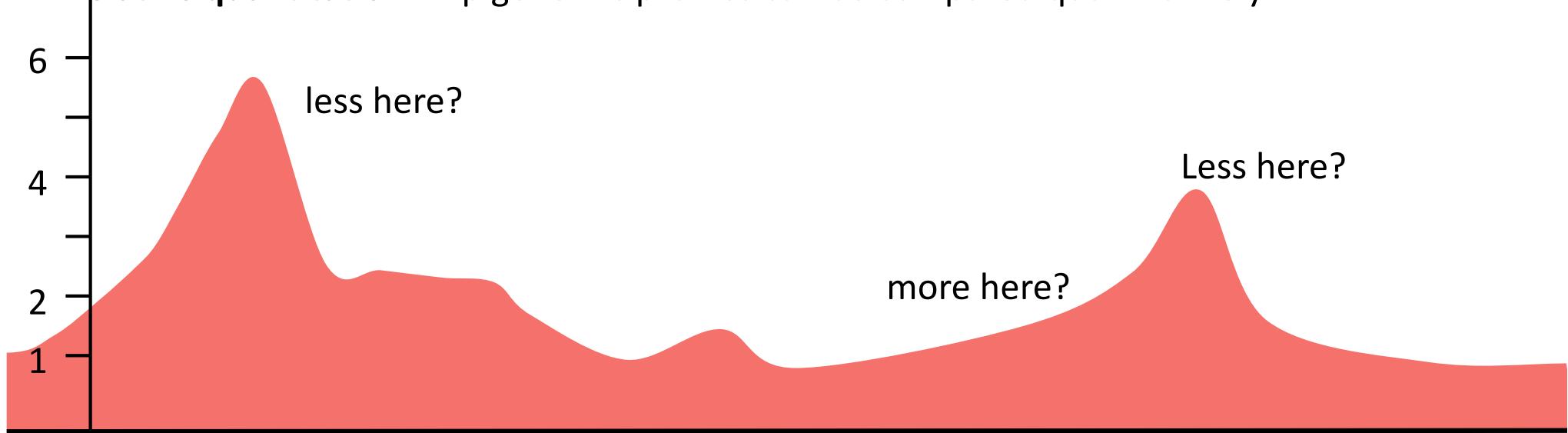
ChIP-Seq signal (histone PTM or Binding Protein occupancy)



Interpreting ChIP-Seq signal



Relative quantitation – Epigenomic profiles can be compared quantitatively



Relative quantitation

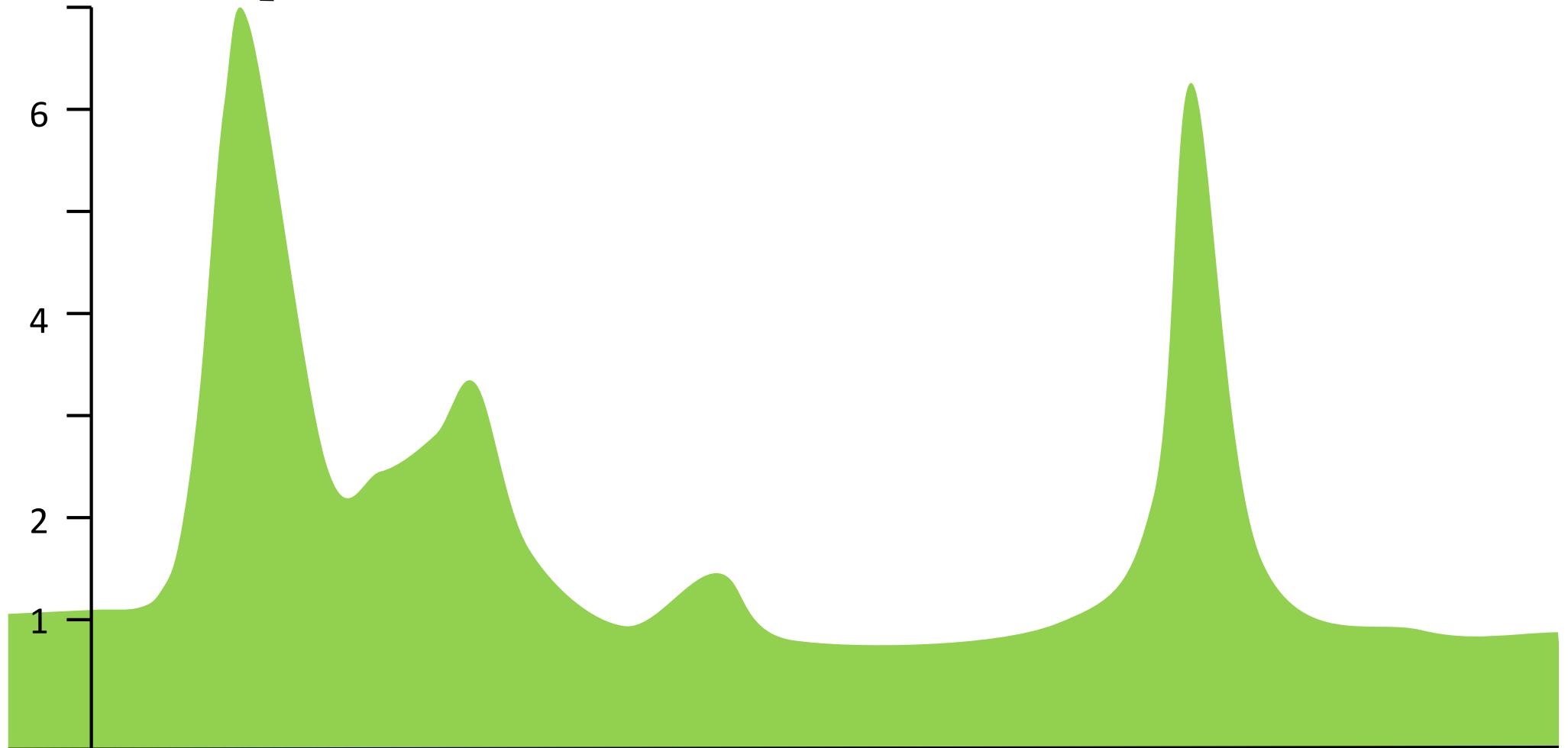
Epigenomic profiles can be compared

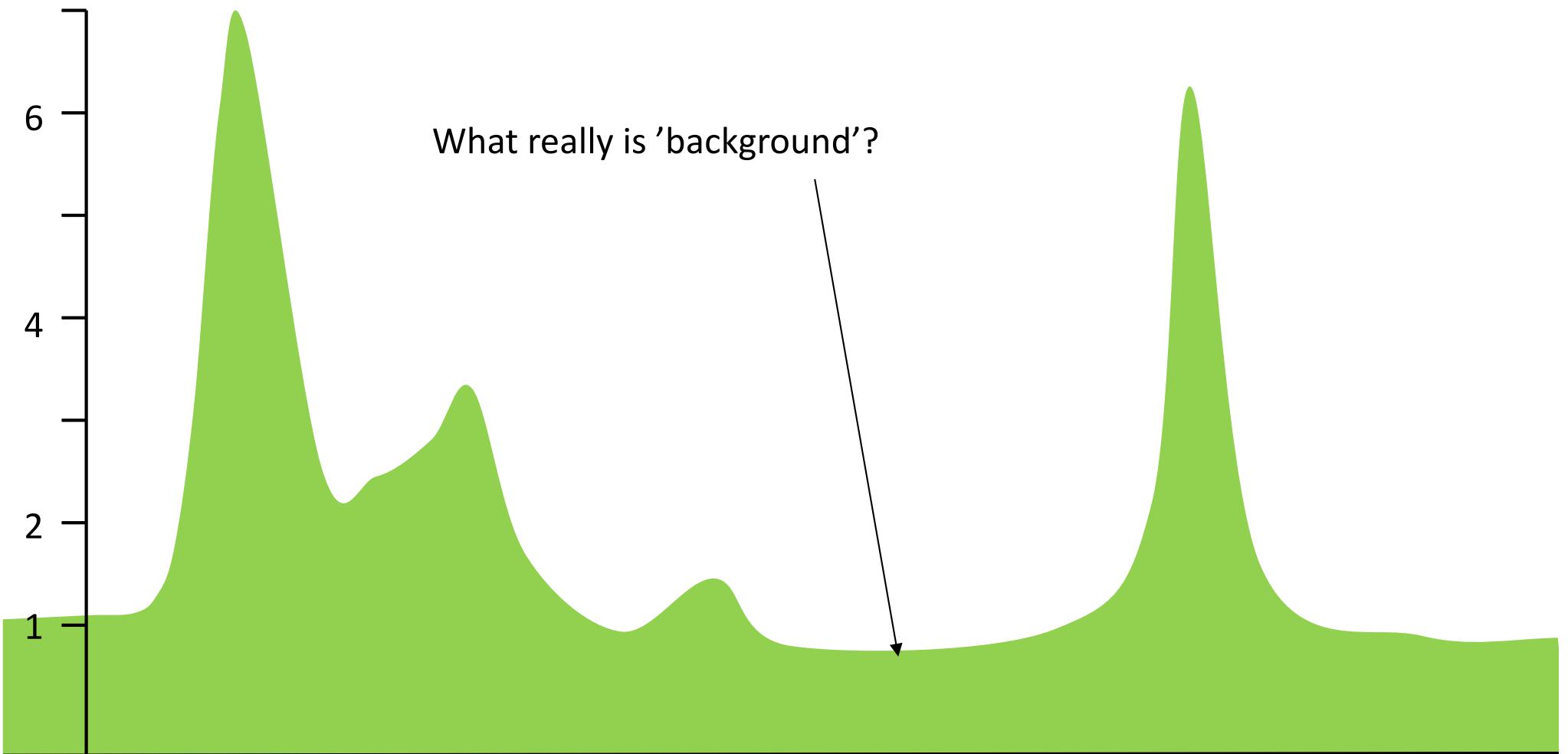
Before comparing, we need to ensure that samples are normalized. Traditional normalization brings all samples to the same effective sequencing depth, and it is assumed that then the samples can be compared quantitatively

Normalization methods used

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

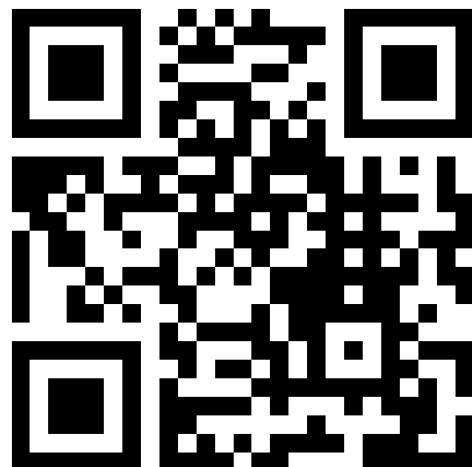
What does the peak height mean?

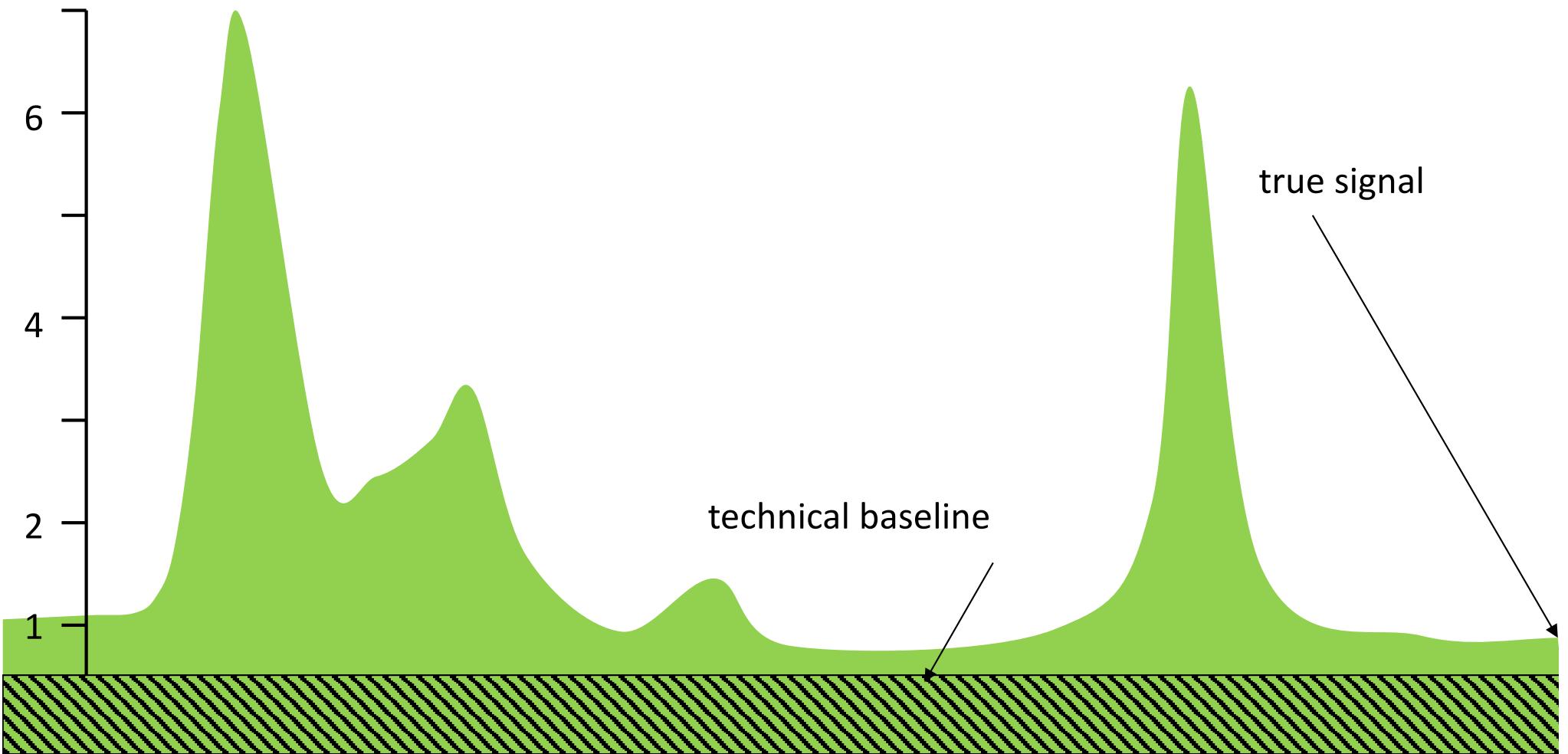




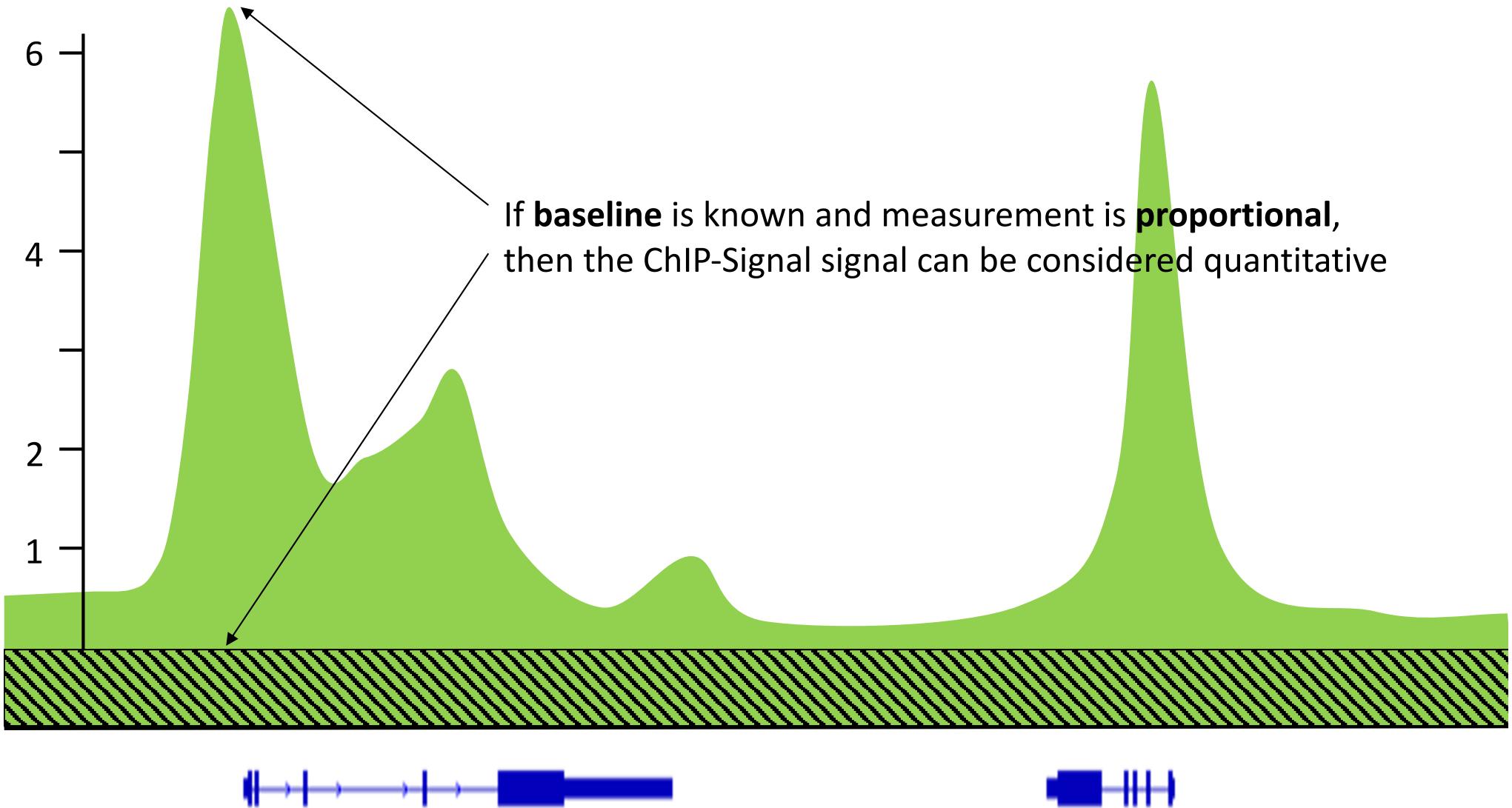
What really is 'background'?

www.menti.com ----> use the code 76 77 39 9



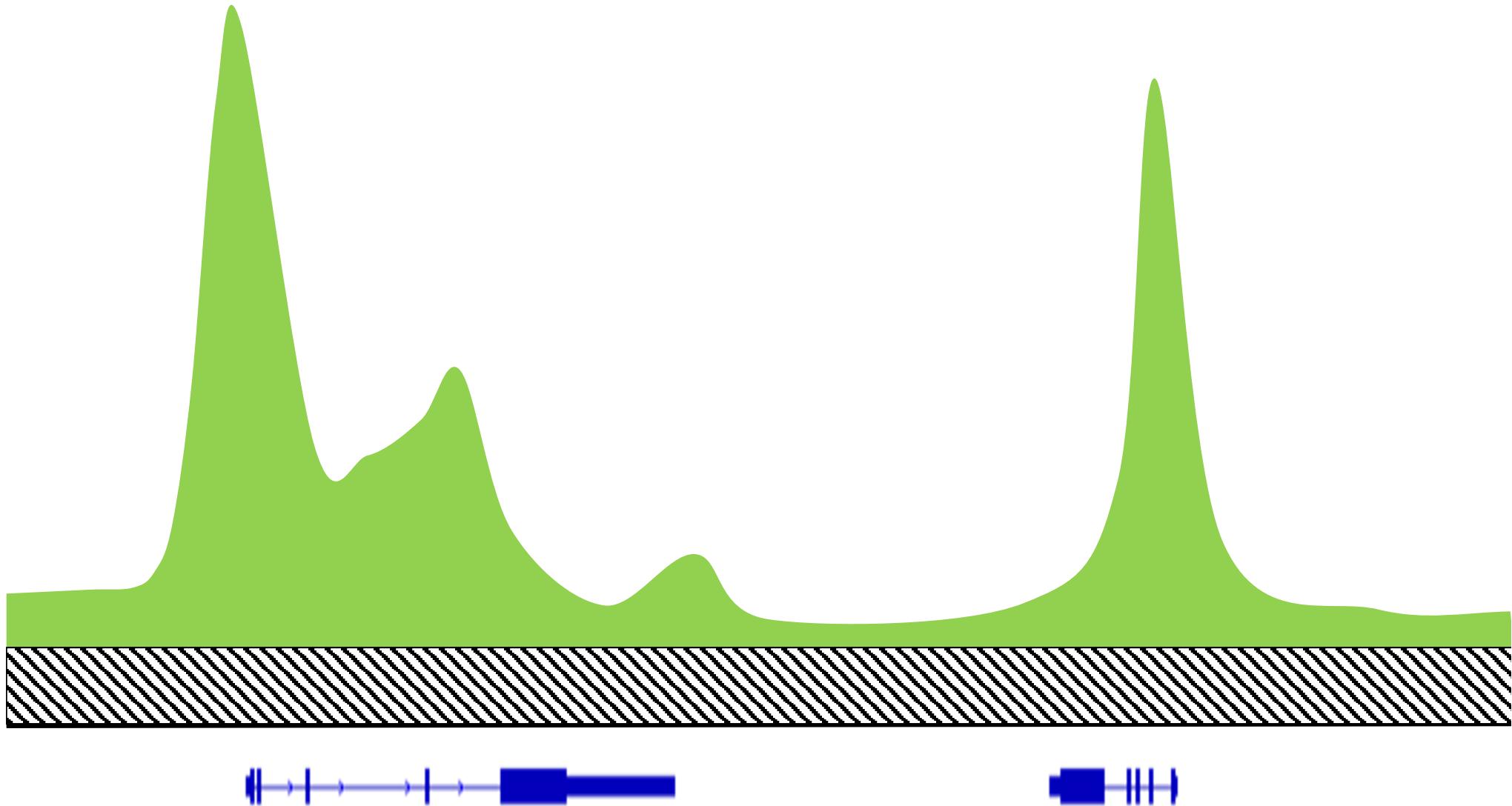


Relative quantitation – accurate comparison across regions in the genome

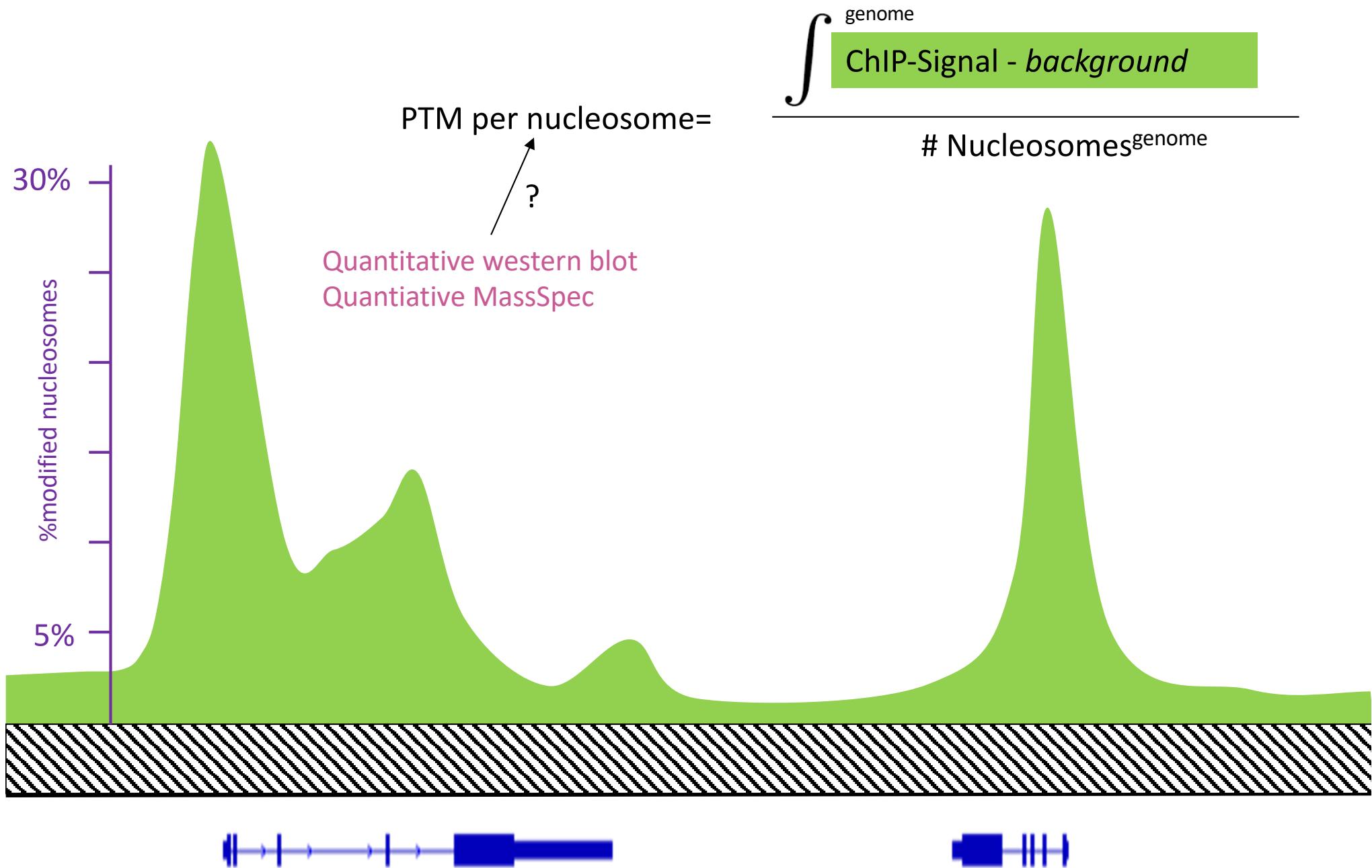


Absolute quantitation – relating signal to true abundance of histone PTM

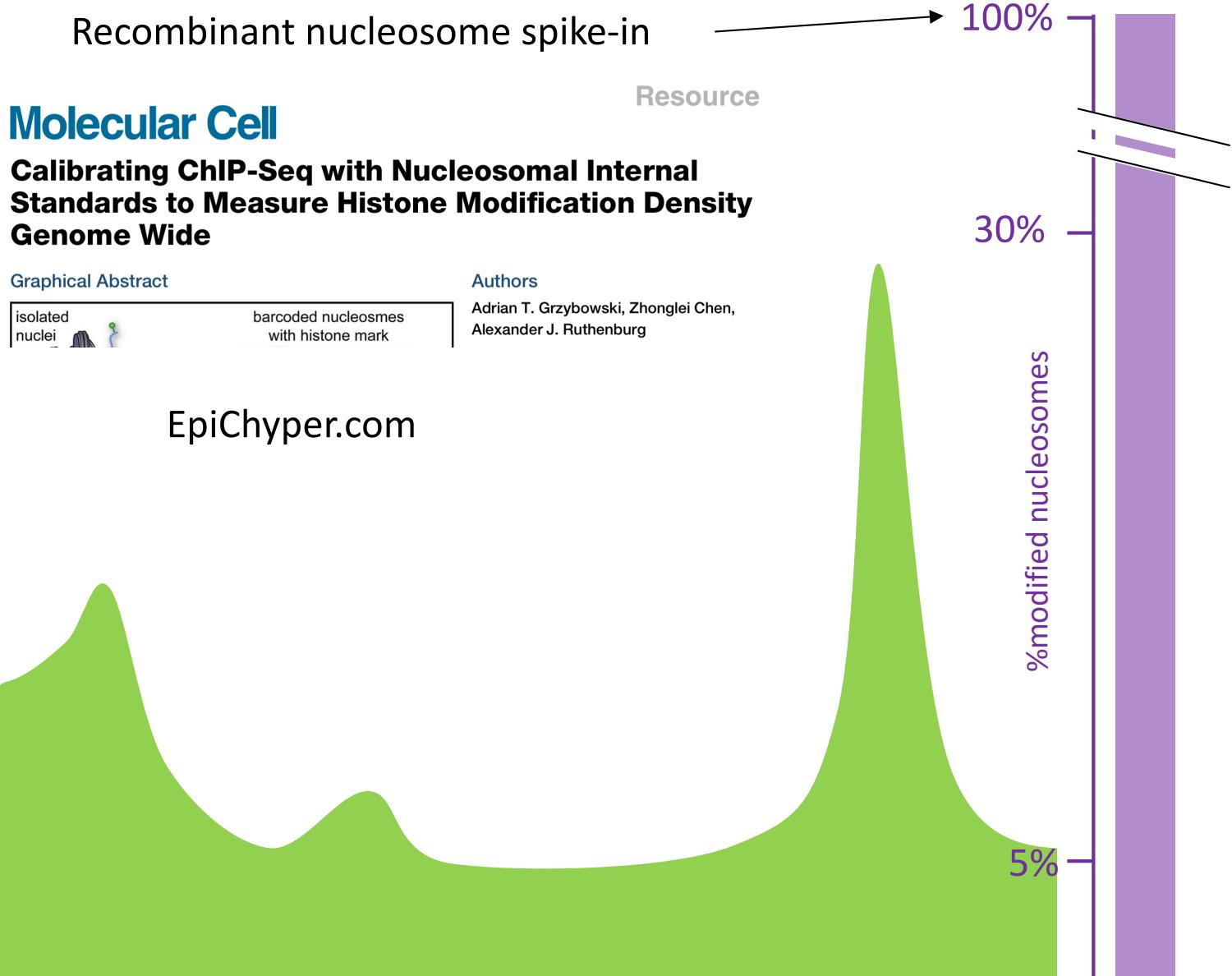
$$\text{Total PTM amount} = \int_{\text{genome}} \text{ChIP-Signal} - \text{background}$$



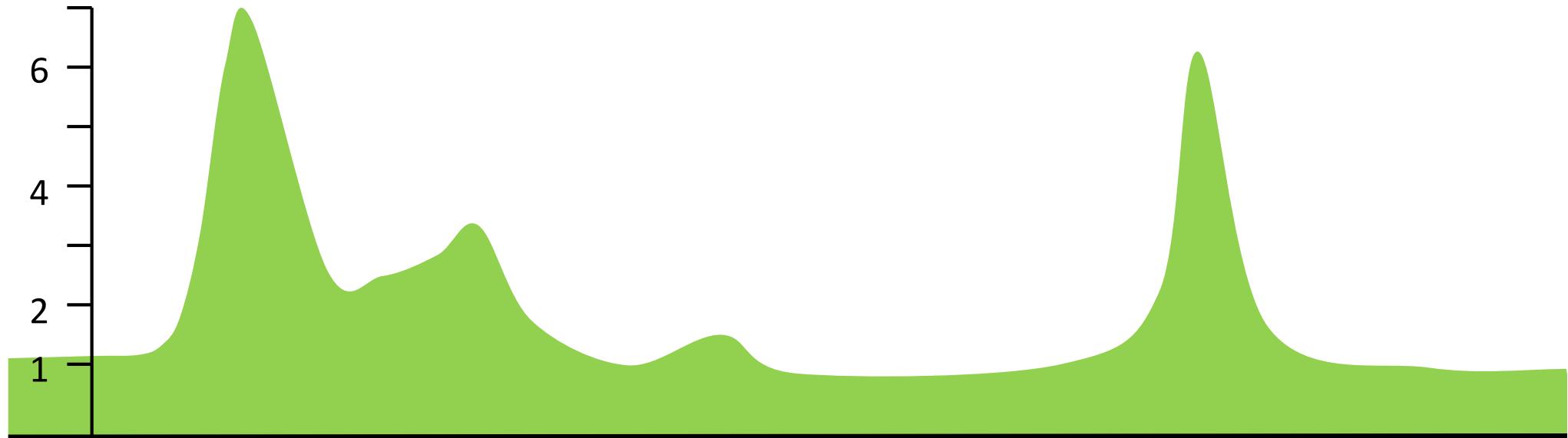
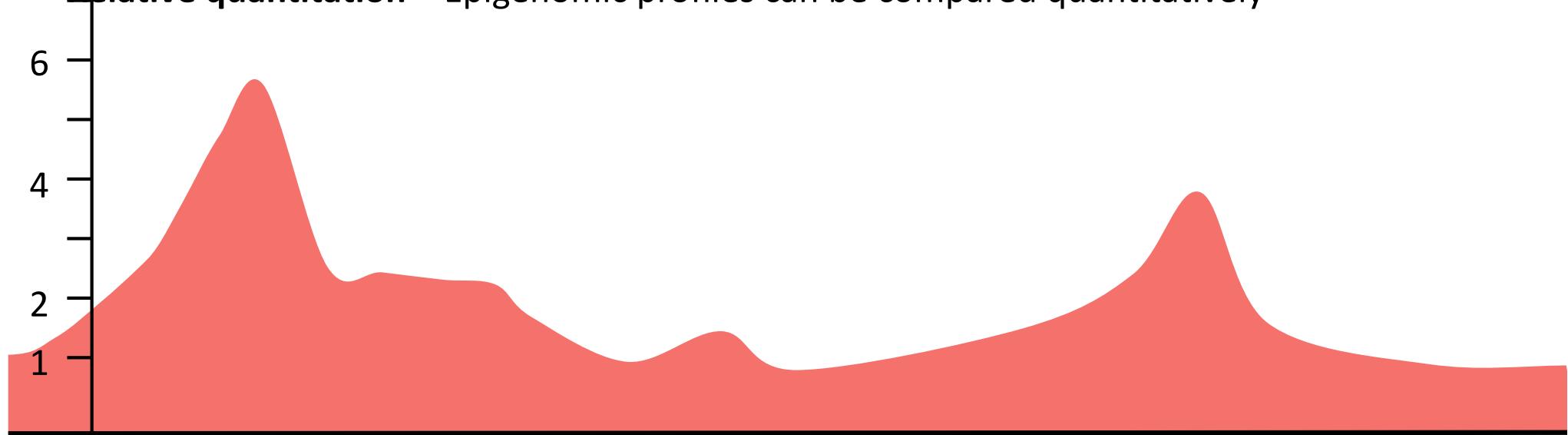
Absolute quantitation – relating signal to known global abundance of histone PTM



Absolute quantitation – relating signal to reference measurement



Relative quantitation – Epigenomic profiles can be compared quantitatively



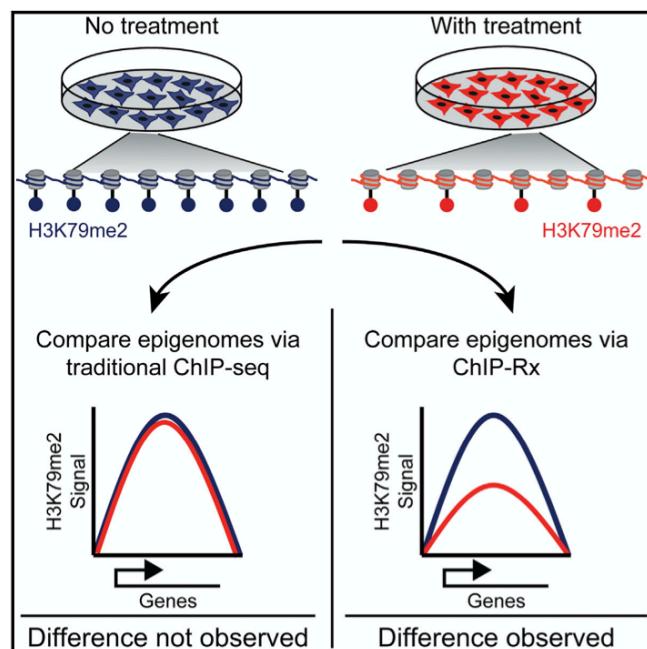
Quantitative ChIP with *Drosophila* Spike-in

Resource

Cell Reports

Quantitative ChIP-Seq Normalization Reveals Global Modulation of the Epigenome

Graphical Abstract



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

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

Descotes N, Tsirigos A, Reinberg D

Preprint from bioRxiv, 22 Feb 2018

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

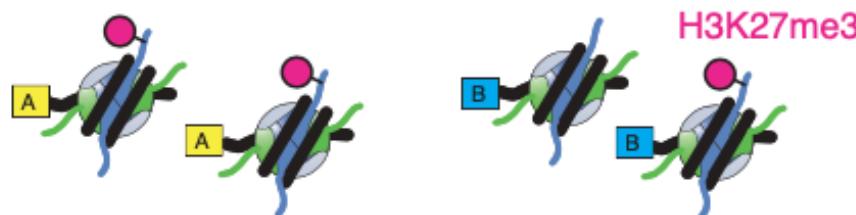
ChIP-Rx allows genome-wide quantitative comparisons of histone modification status

condition A : condition B

modified nucleosomes:

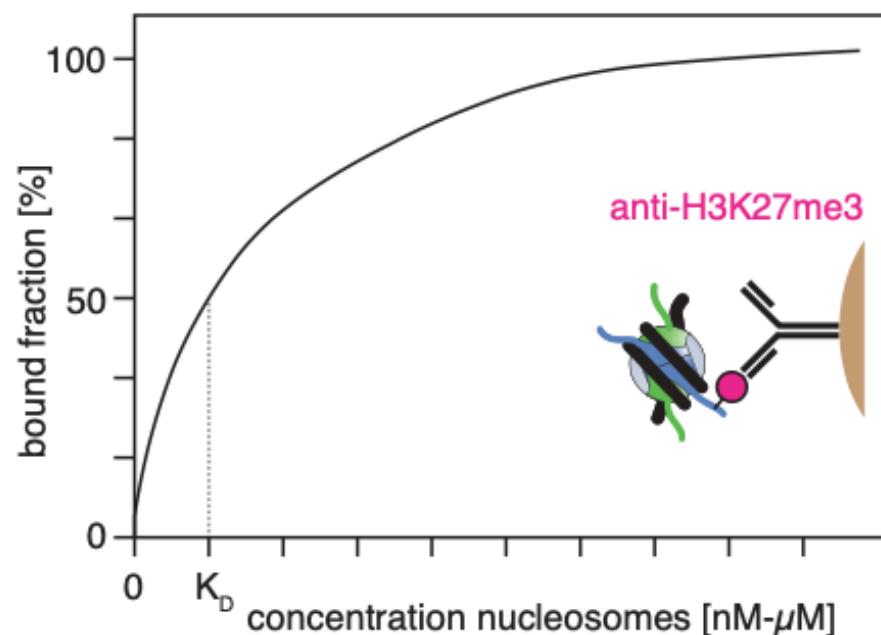
2

1



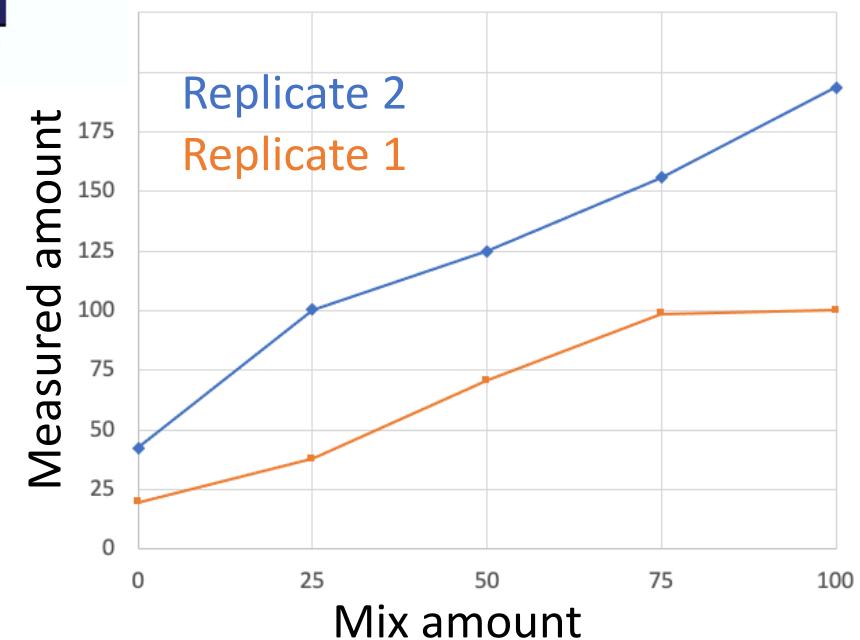
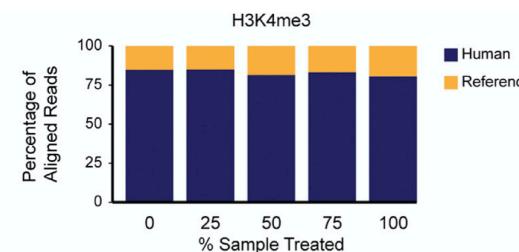
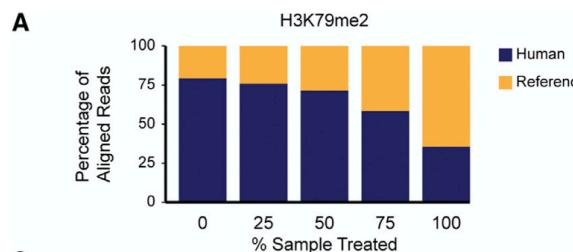
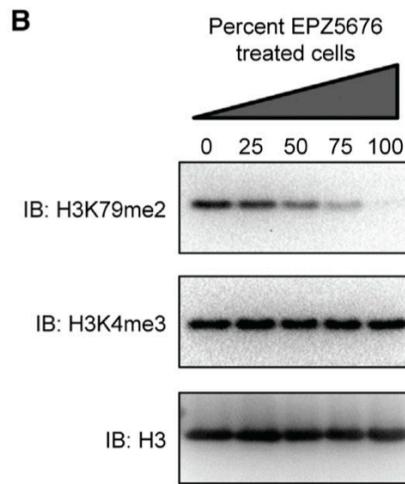
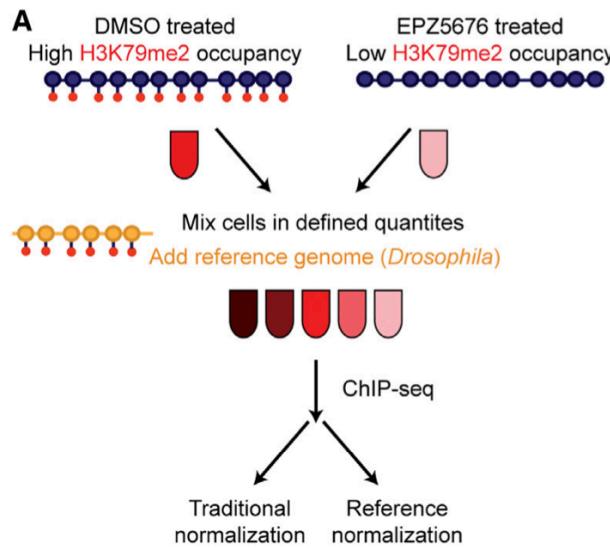
all modified nucleosomes compete with equal affinity

quantification holds true irrespective of the binding curve



Barcodes sequenced: 2:1

[A] : [B]

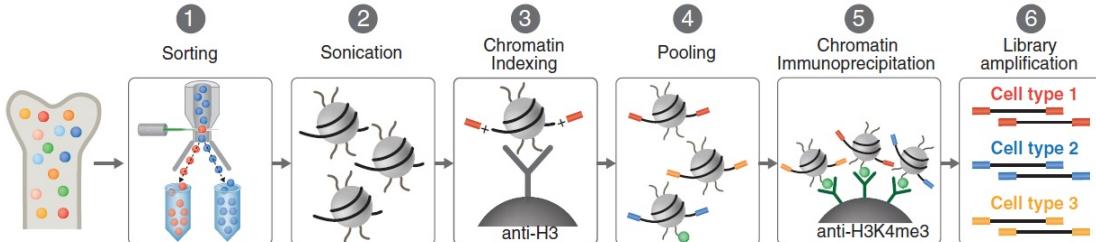


Limitations of Spike-in ChIP

- 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.

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

Molecular Cell

All Content • Molecular Cell

Explore Online Now Current Issue Archive Journal Information For Authors

< Previous Article Volume 61, Issue 1, p170–180, 7 January 2016

TECHNOLOGY

A Multiplexed System for Quantitative Comparisons of Chromatin Landscapes

Peter van Galen, Aaron D. Viny, Oren Ram, Russell J.H. Ryan, Matthew J. Cotton, Laura Donohue, Cem Sievers, Yotam Drier, Brian B. Laiu, Shawn M. Gillespie, Kaitlin M. Carroll, Michael B. Cross, Ross L. Levine, Bradley E. Bernstein

Published Online: December 10, 2015

Open Archive | PlumX Metrics

DOI: <http://dx.doi.org/10.1016/j.molcel.2015.11.003> | CrossMark

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

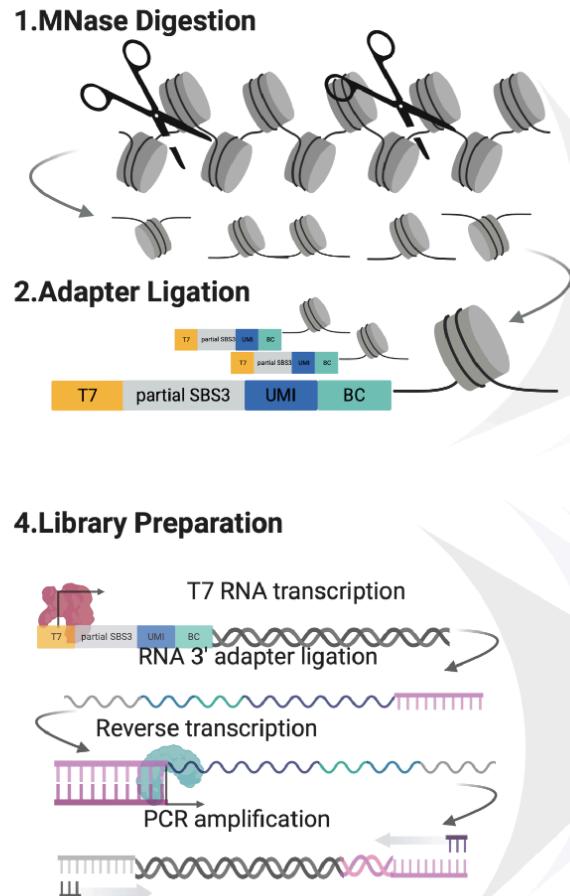
Authors

Banushree Kumar, Simon J. Elsässer

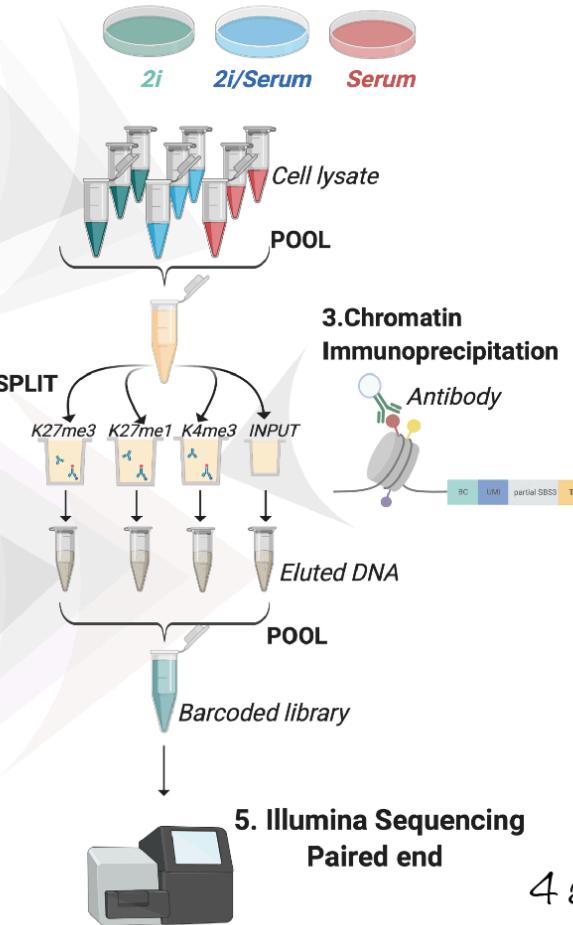
Resource

Multiplexed ChIP (MINUTE-ChIP)

A

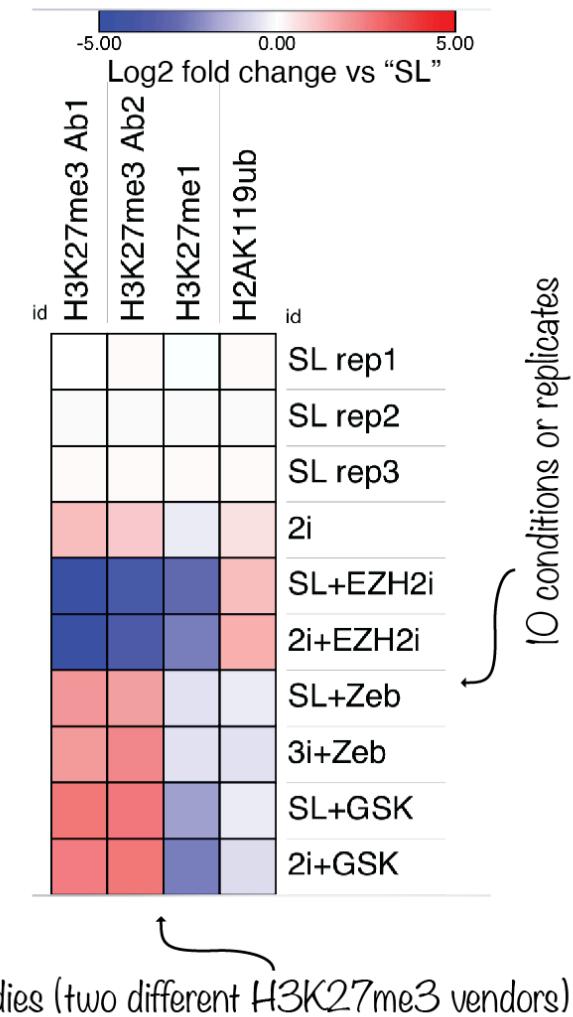


conditions and/or replicates



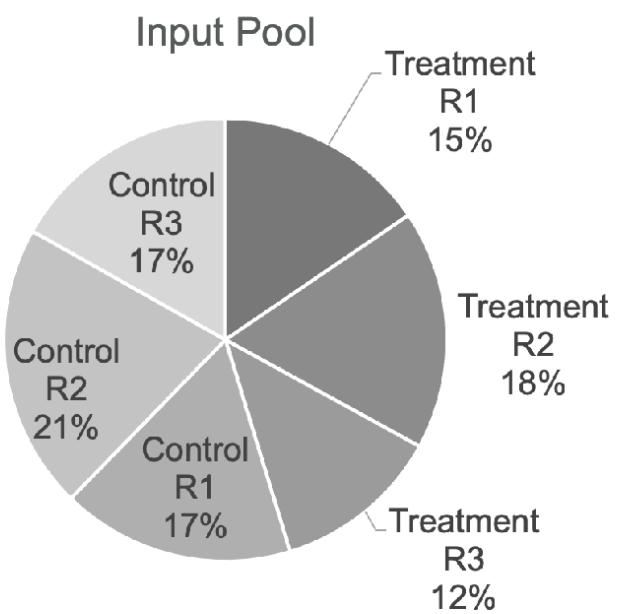
B

Example multiplexed ChIP

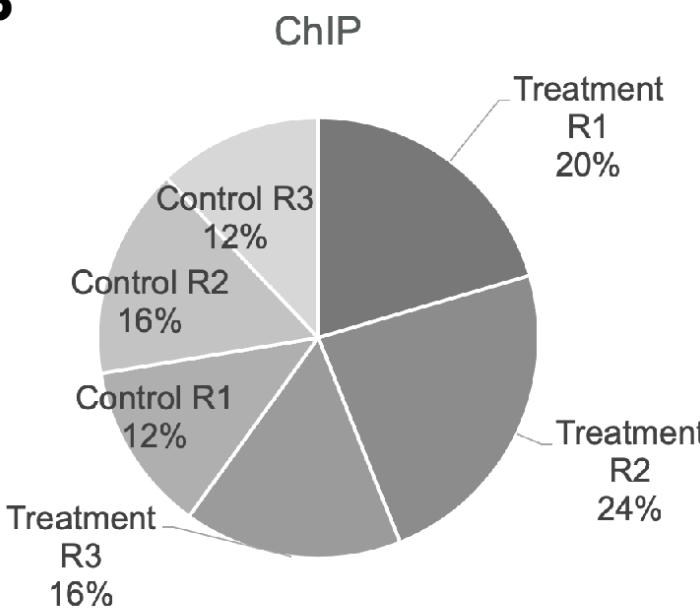


MINUTE-ChIP Quantification

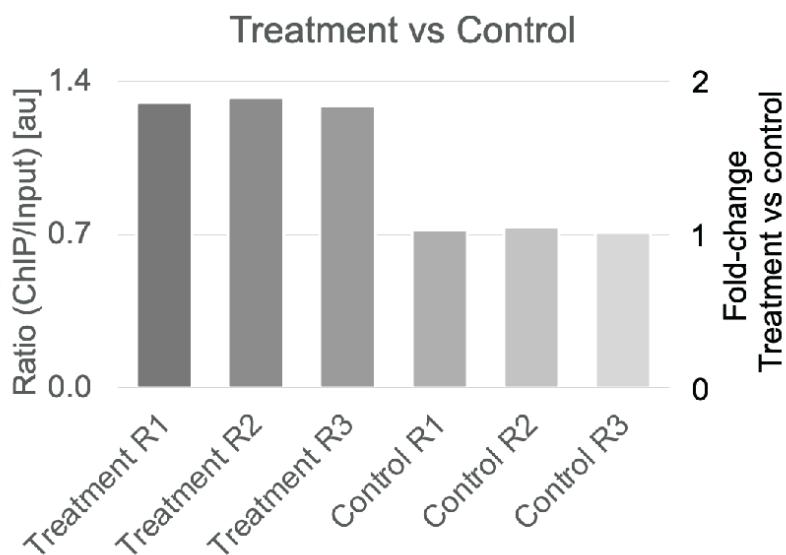
A



B



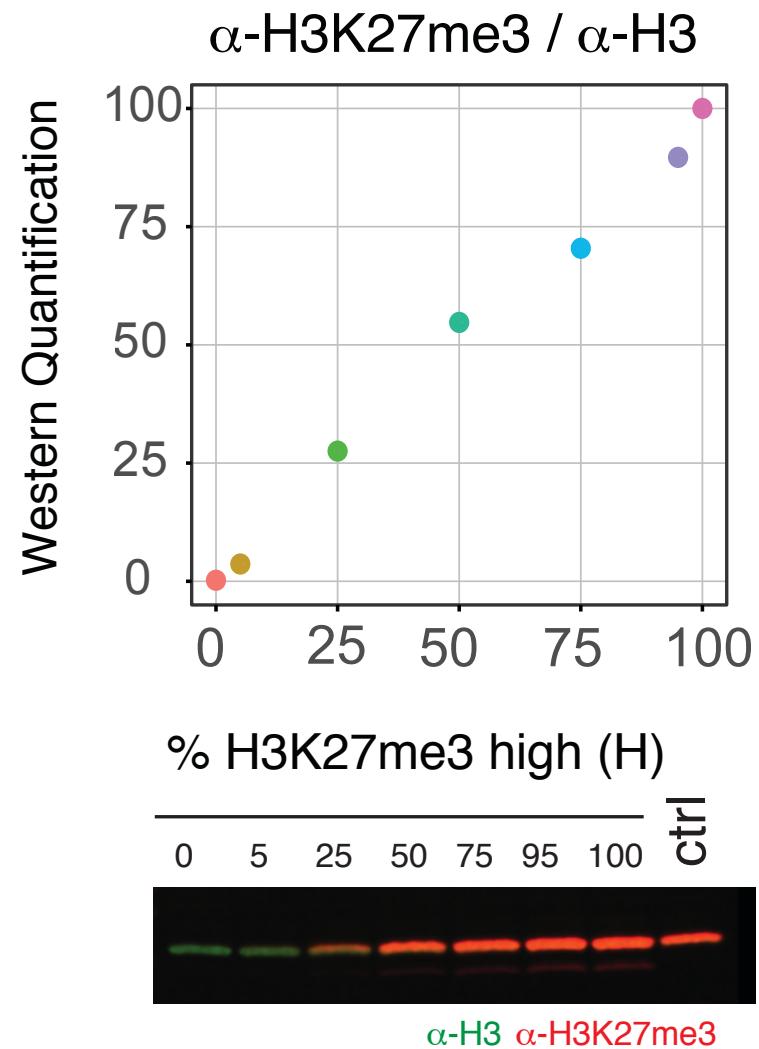
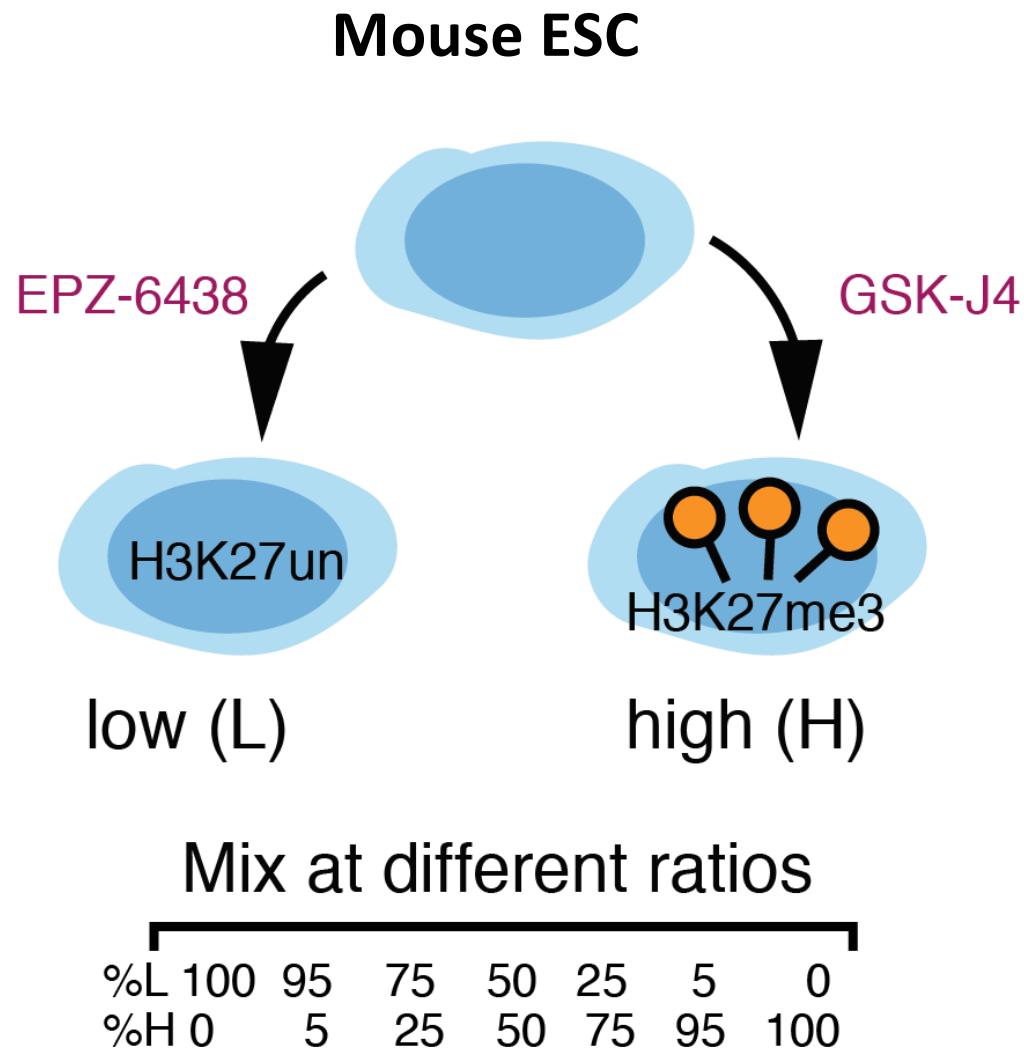
C



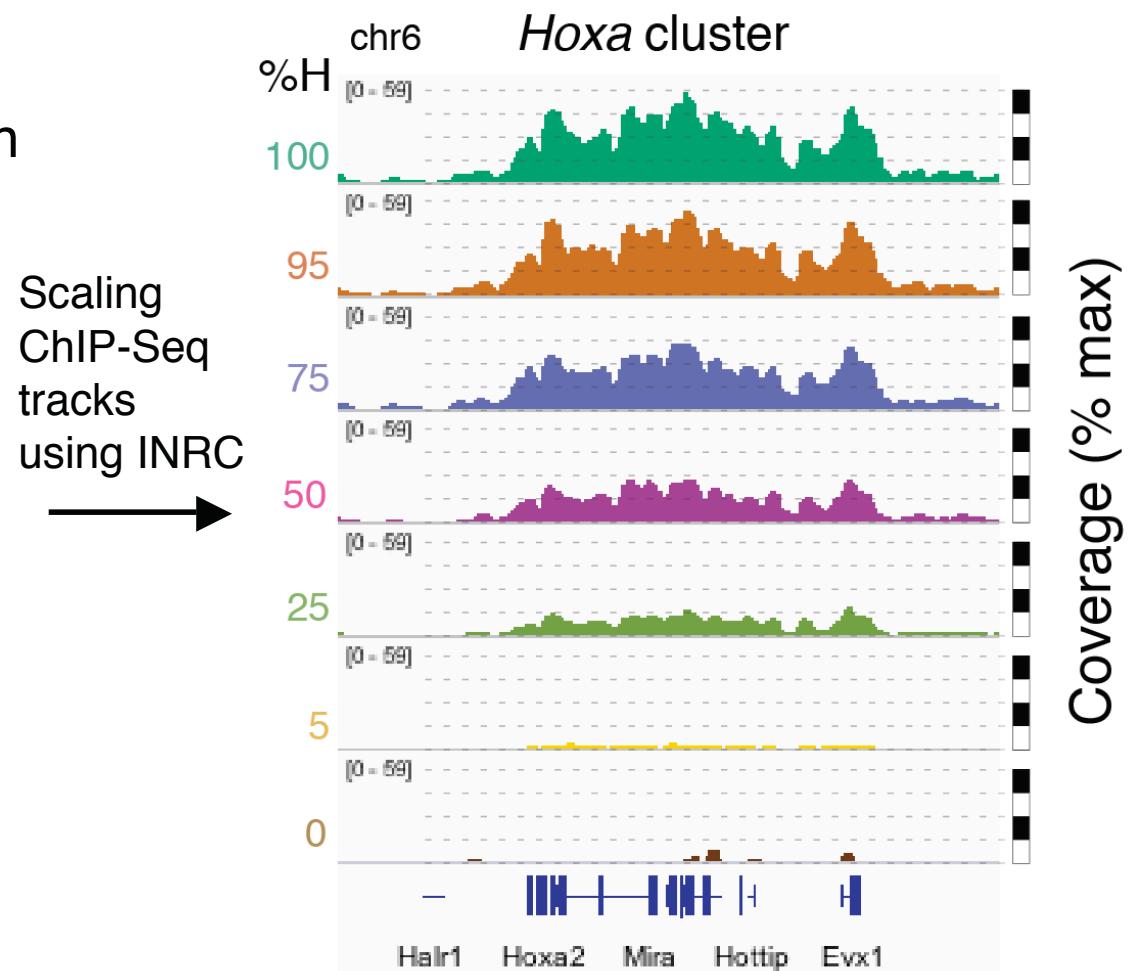
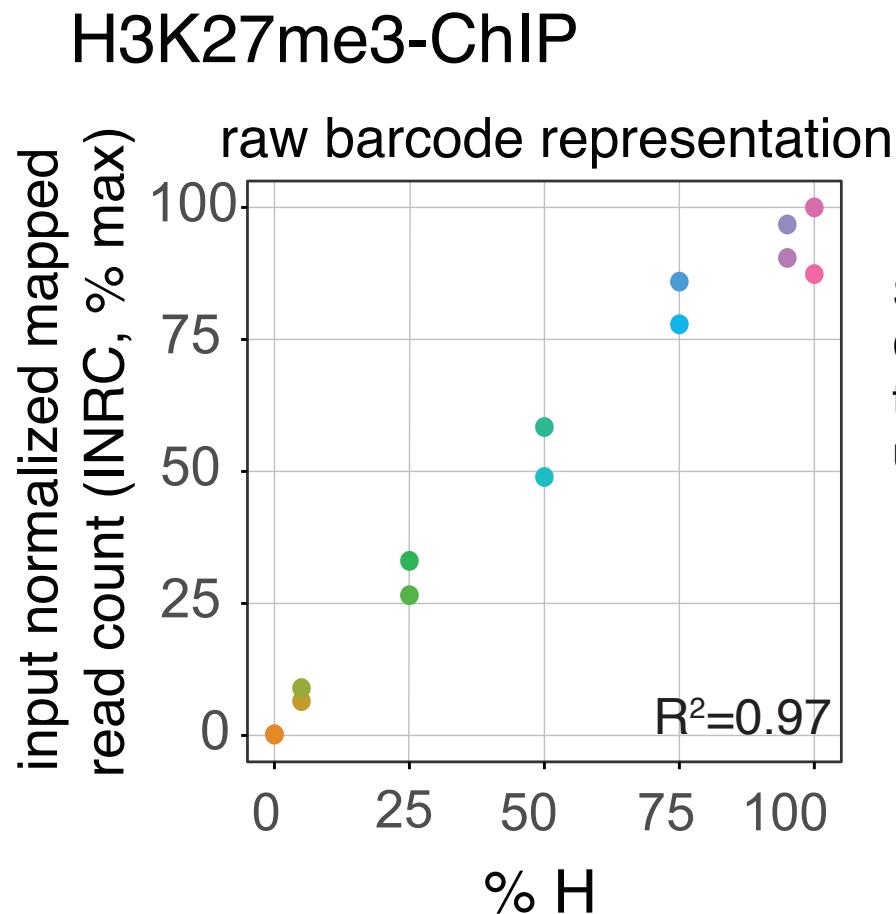
D

$$\text{Fold-change} \left(\frac{\text{Treatment}}{\text{Control}} \right) = \frac{\text{ChIP(Treatment)}}{\text{Input(Treatment)}} / \frac{\text{ChIP(Control)}}{\text{Input(Control)}}$$

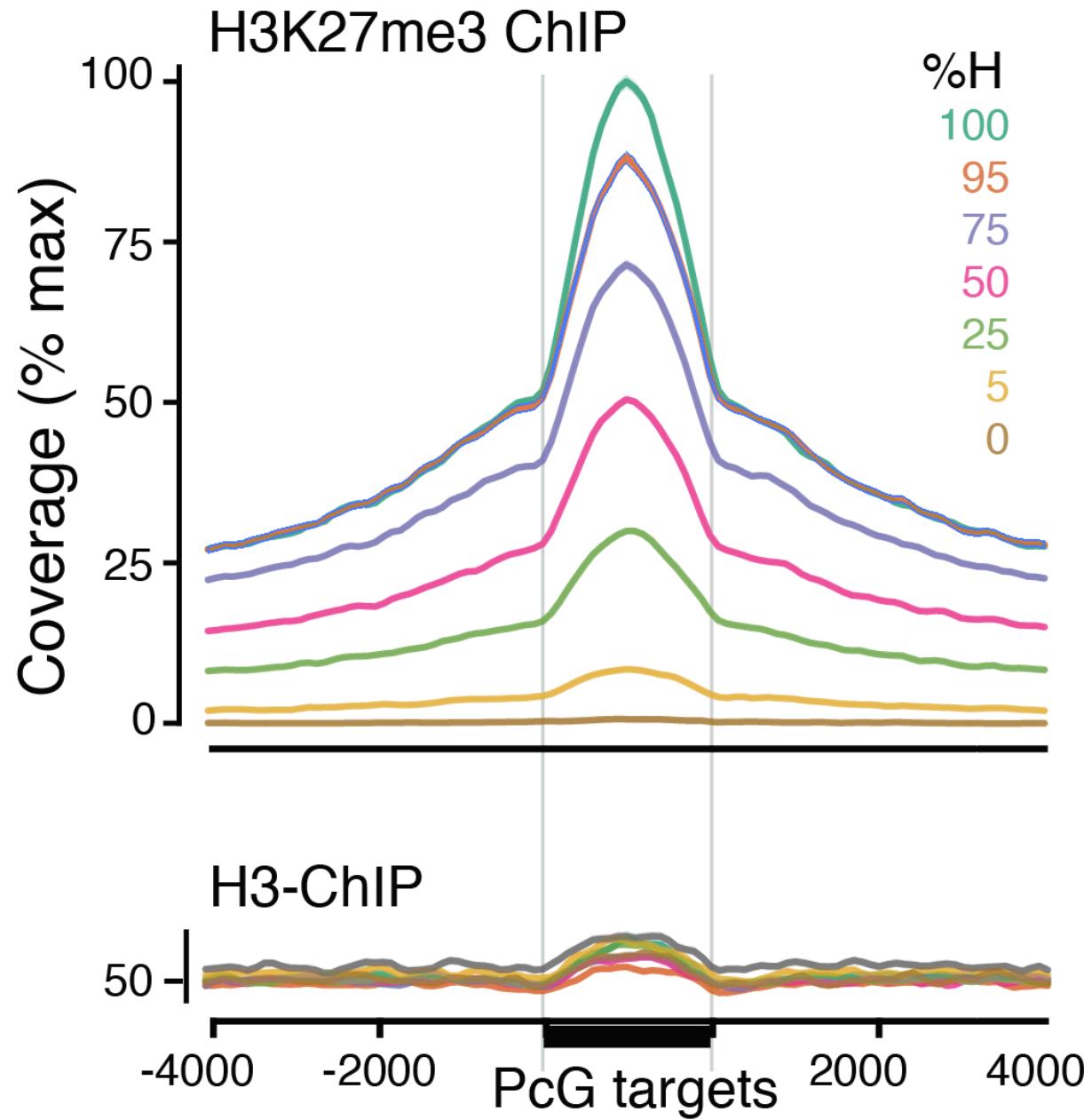
MINUTE-ChIP Calibration Experiment Setup

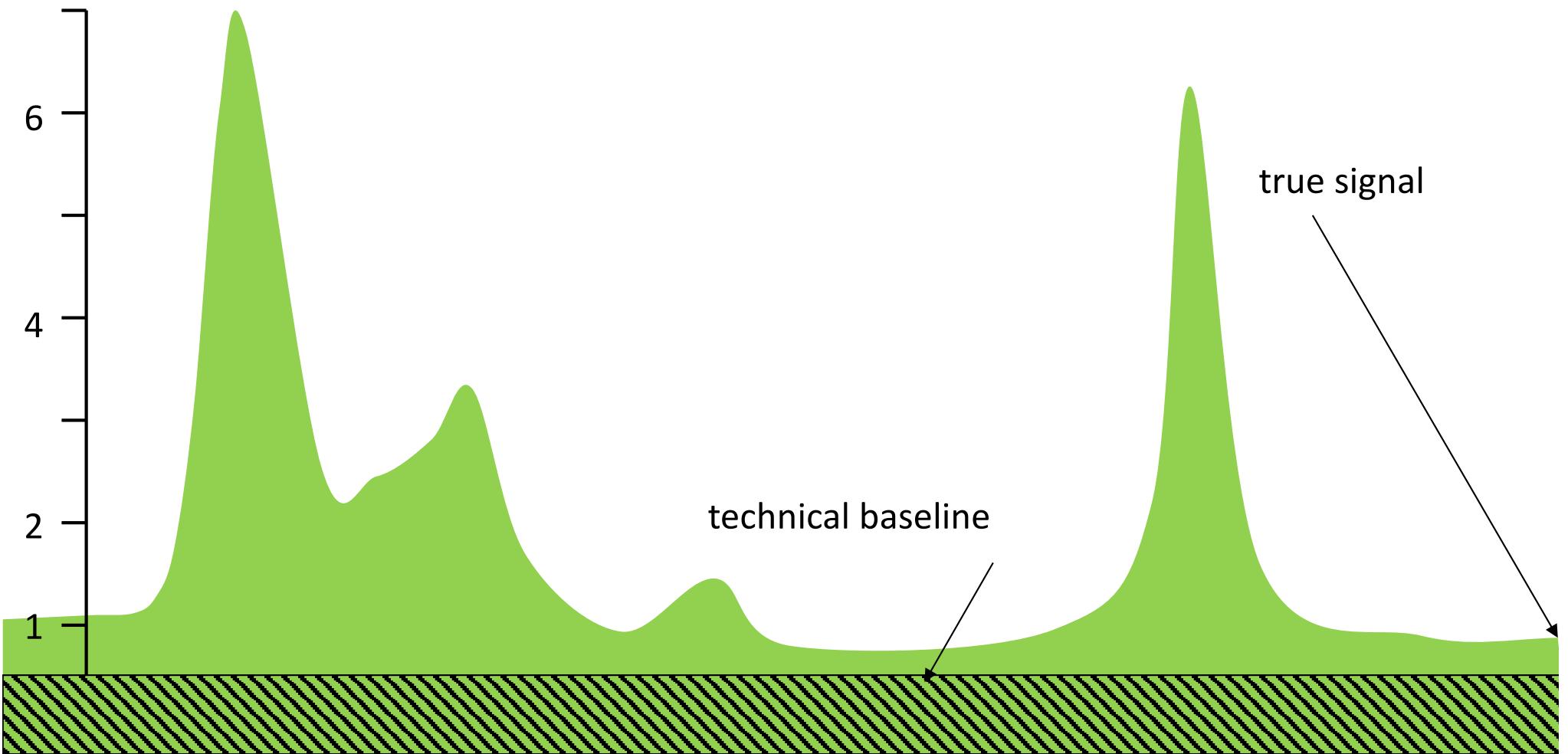


MINUTE-ChIP Calibration Experiment

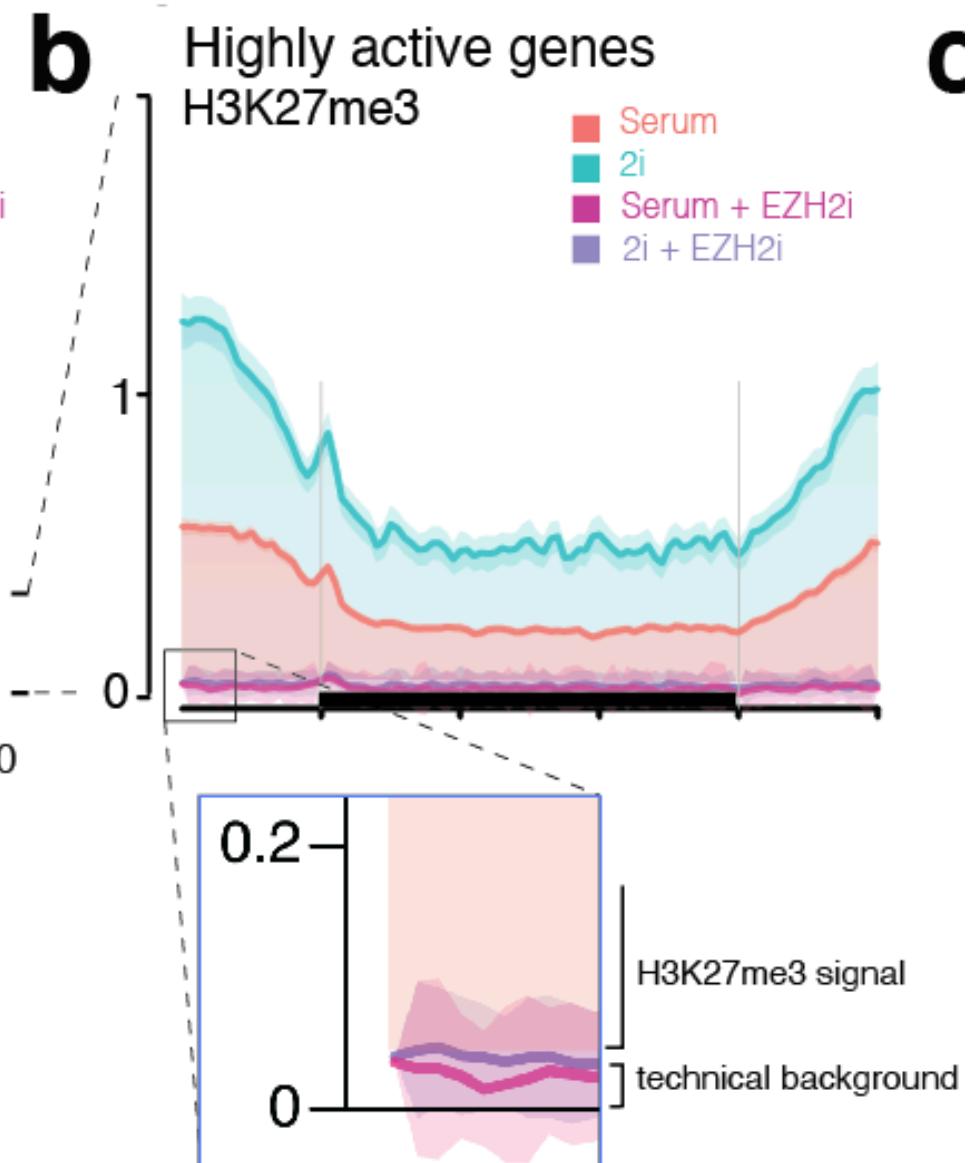
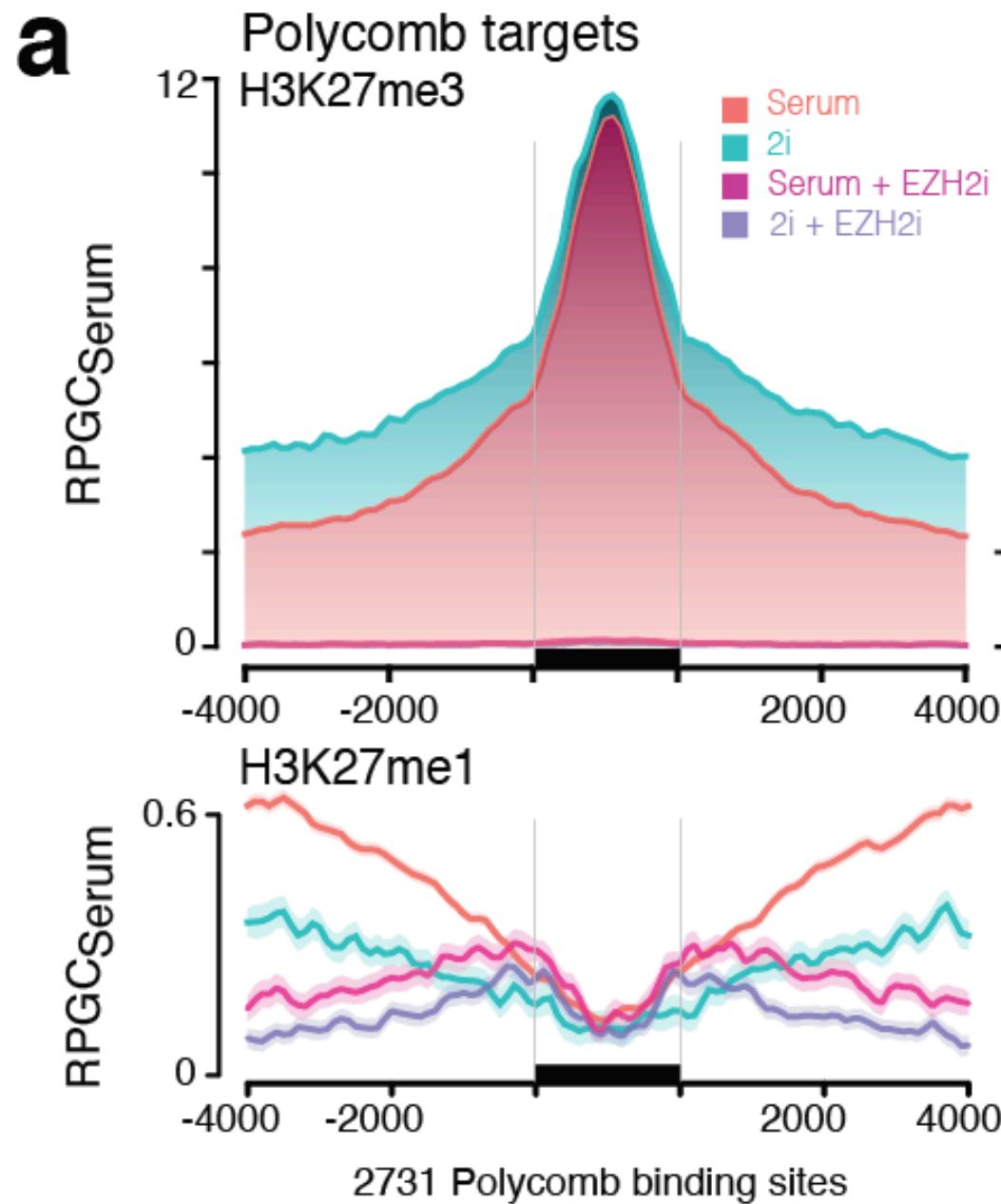


MINUTE-ChIP Calibration Experiment

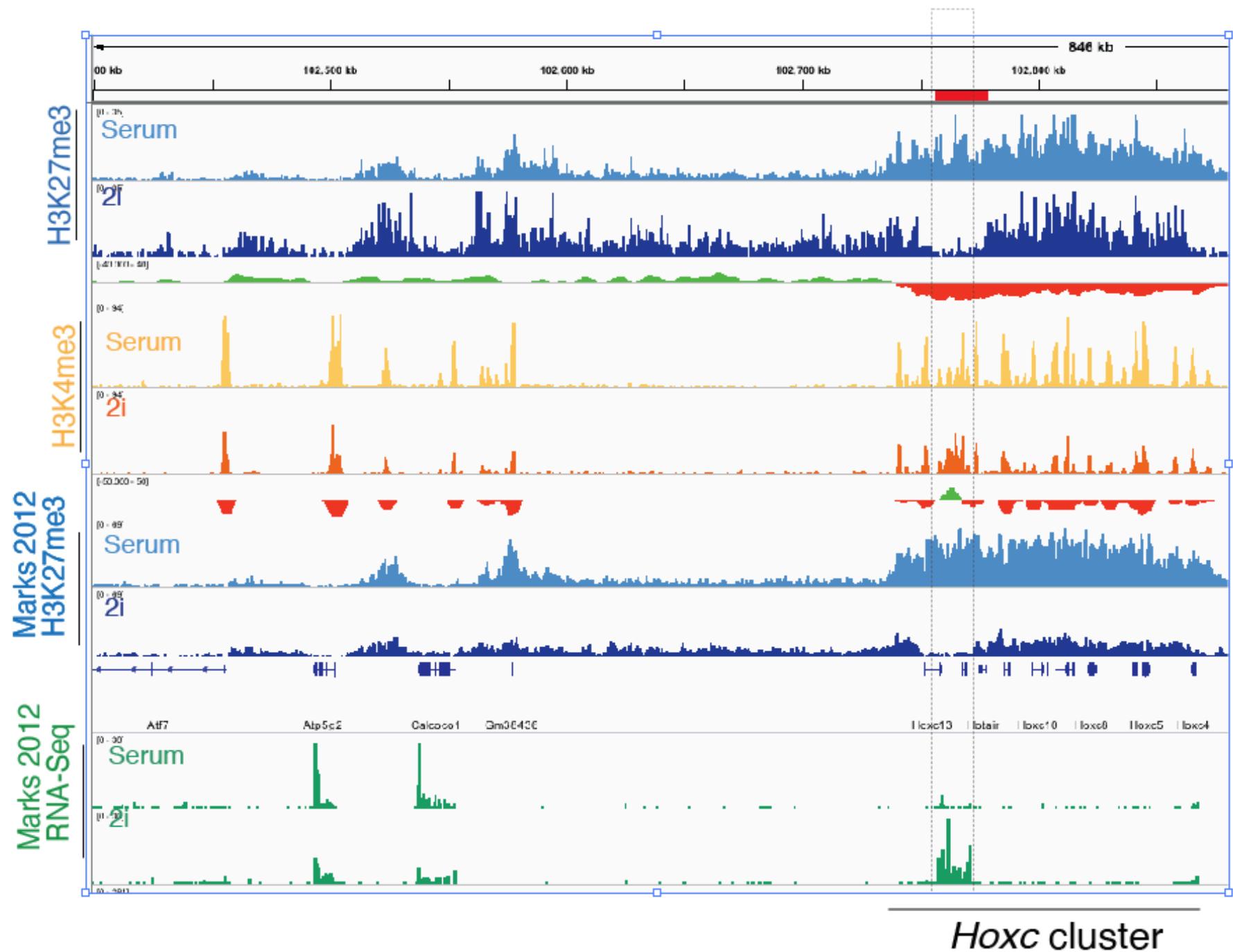




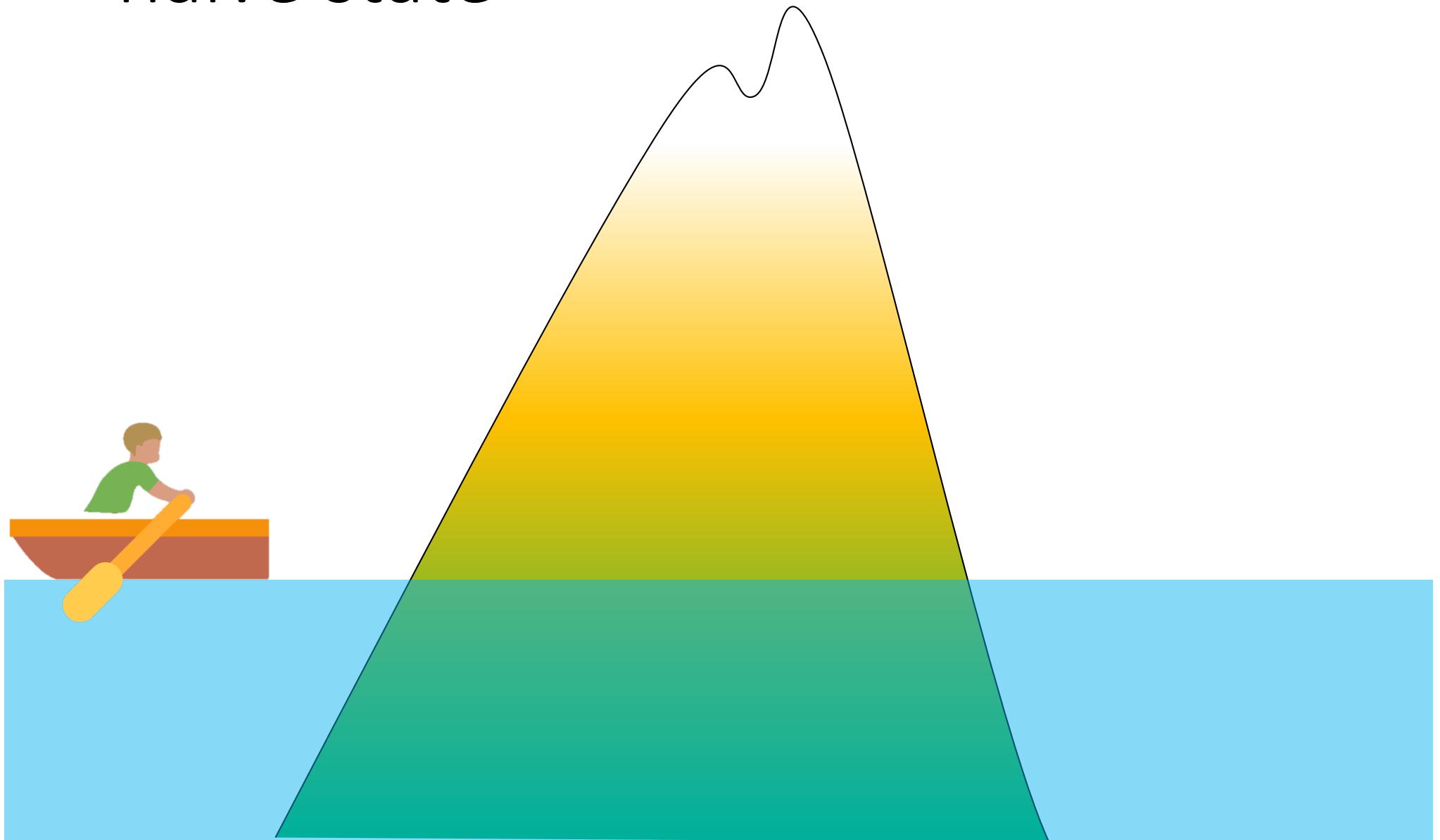
Multiplexed ChIP has very little technical background

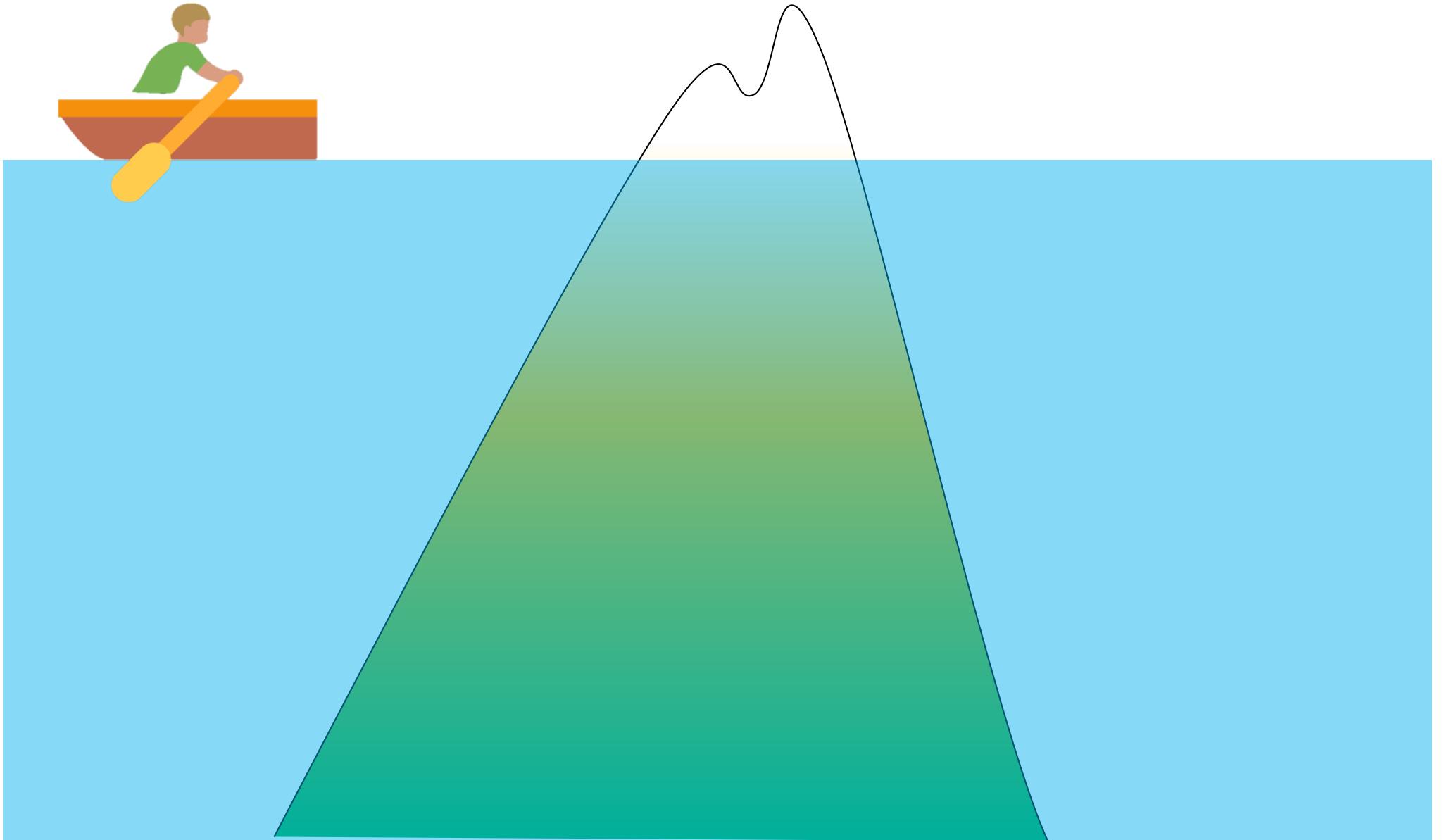


Comparison normal and quantitative ChIP



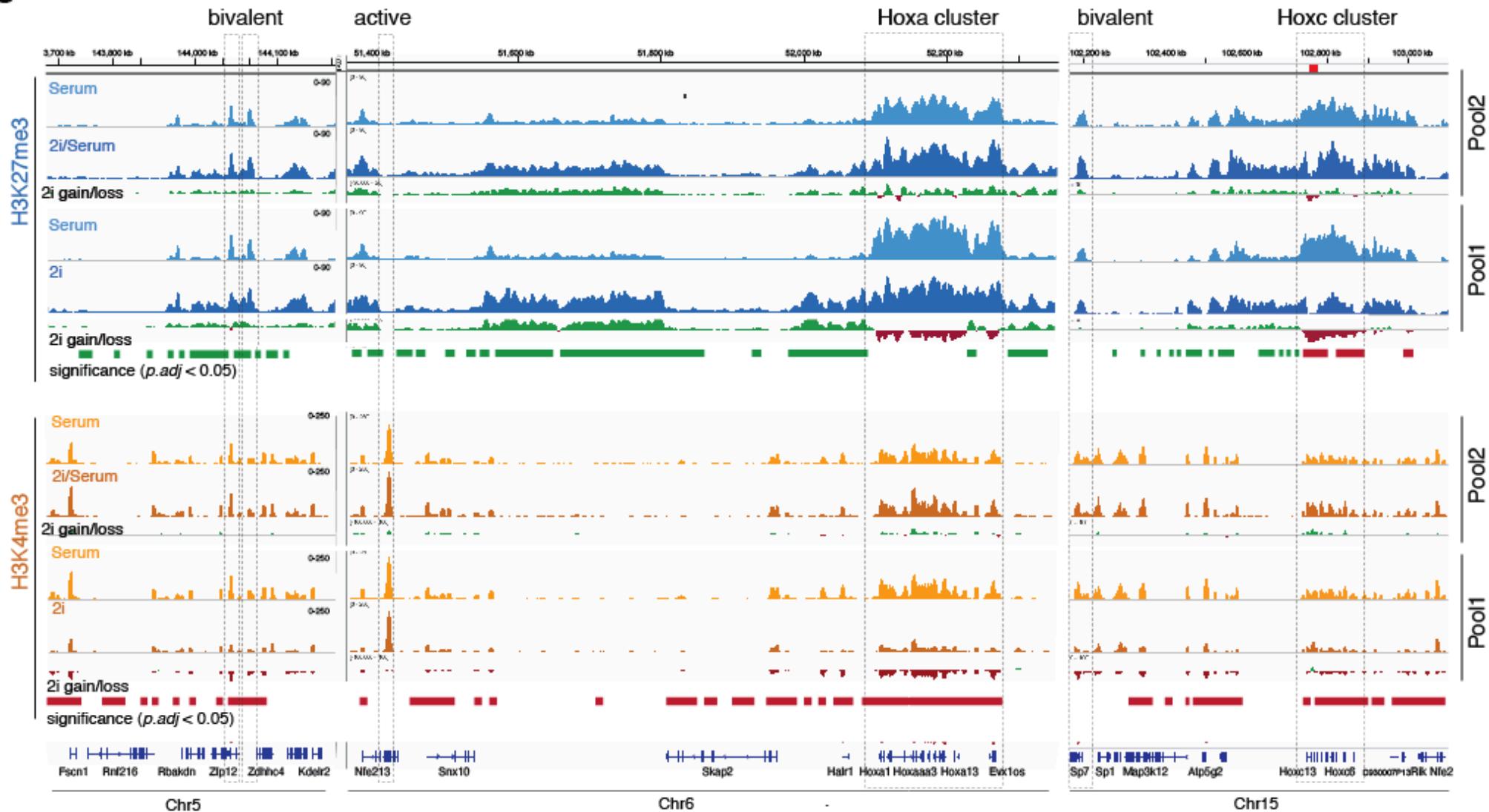
H3K27me3 ‘sea’ levels rise in naïve state





Differential occupancy with DESeq

Proportionality of measurement and low background qualify
MINUTE-ChIP data for statistical analysis (using parametric test)

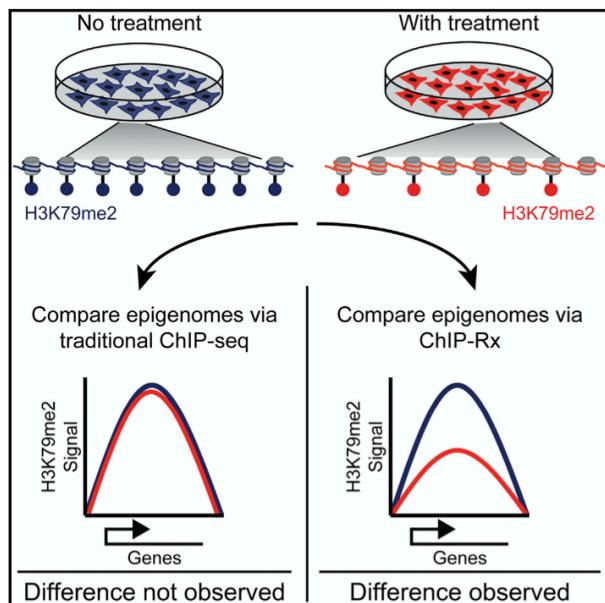


Tutorial – reanalysis of Orlando data using Bioconductor package

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

Resource

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

Descotes N, Tsirigos A, Reinberg D

Preprint from bioRxiv, 22 Feb 2018

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.

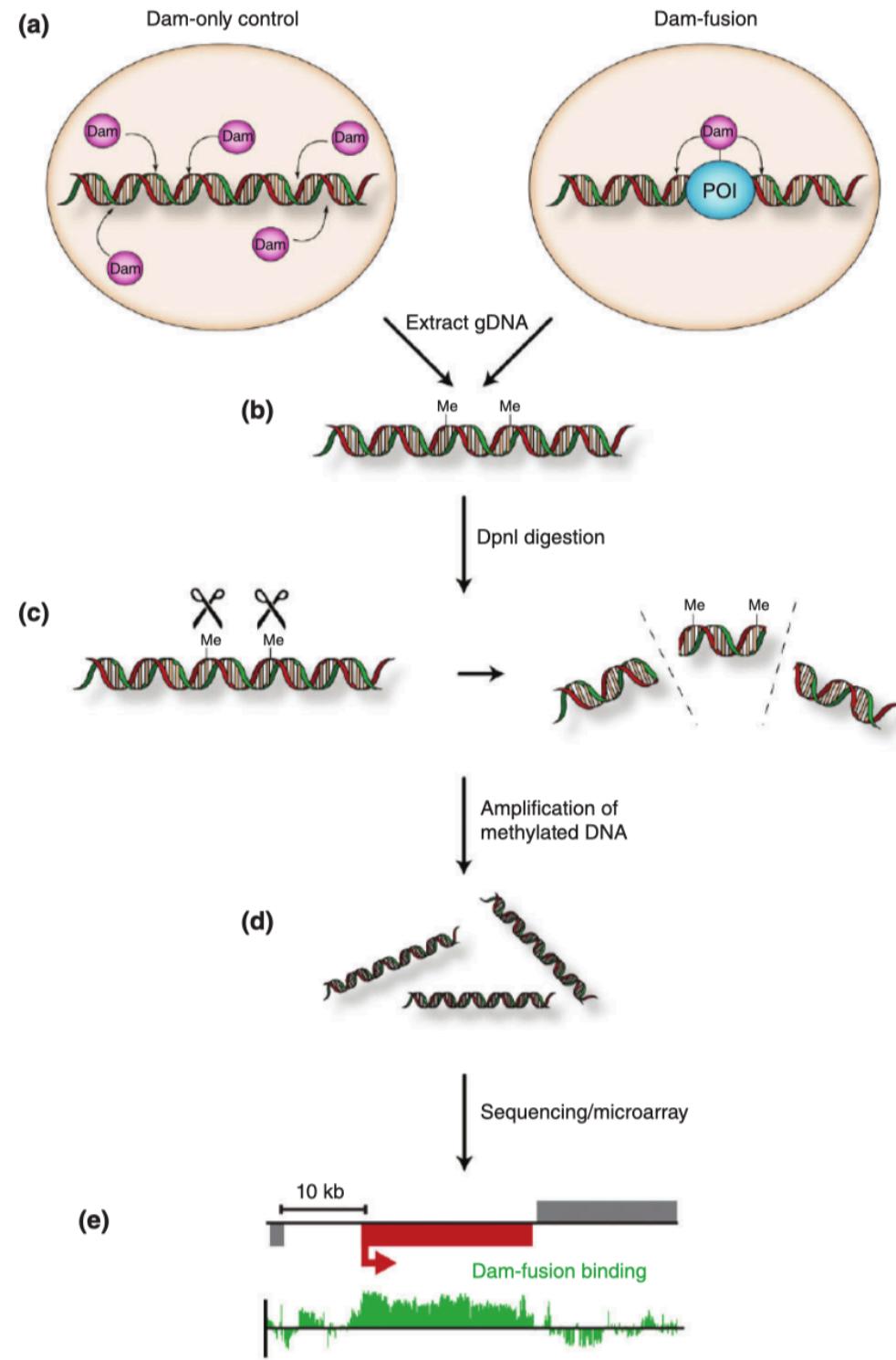
Accession Numbers

GSE60104

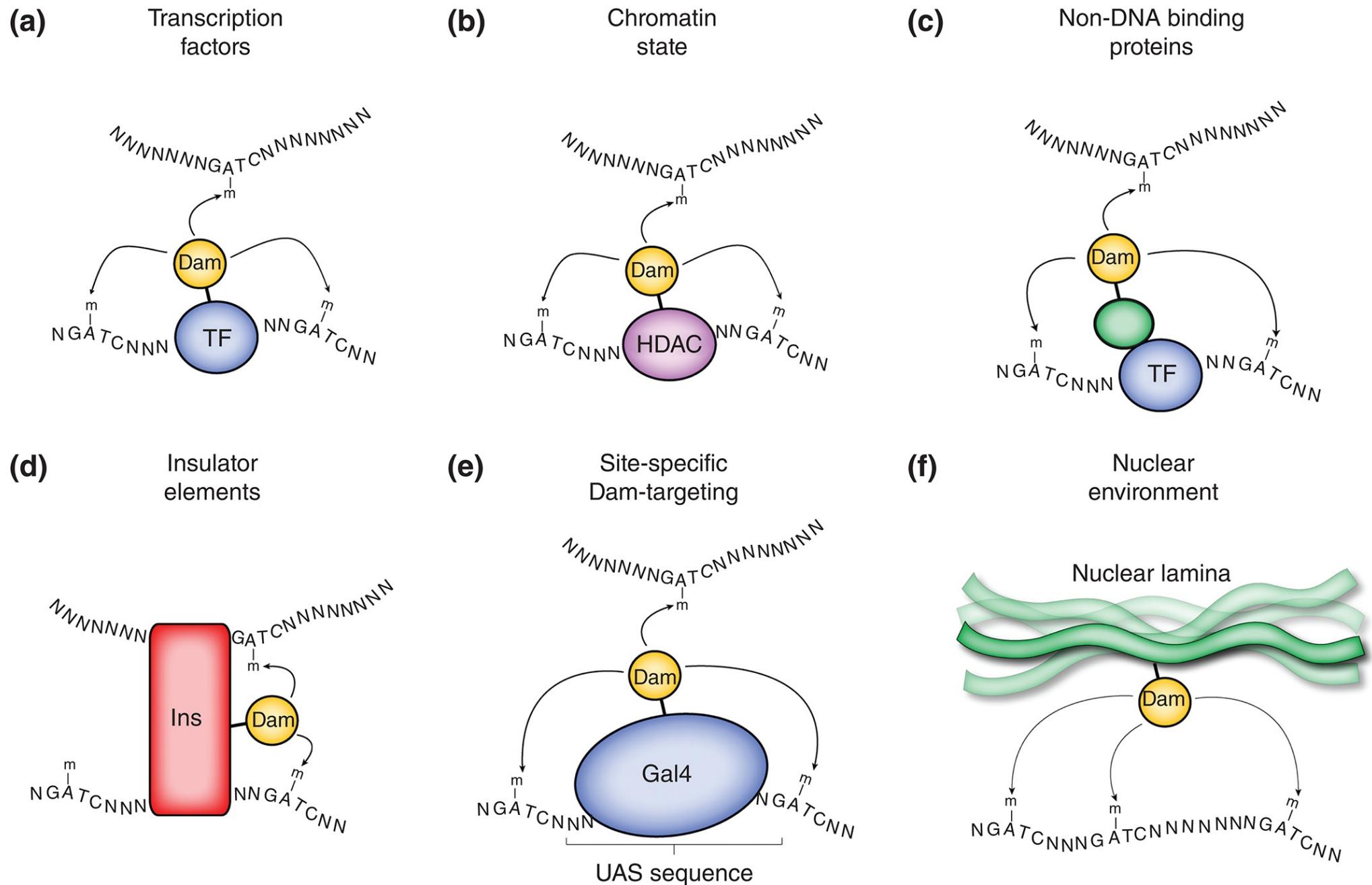
ChIP-Seq alternatives

- Dam-ID
- CUT&RUN
- CUT&Tag

Dam-ID



Dam-ID



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

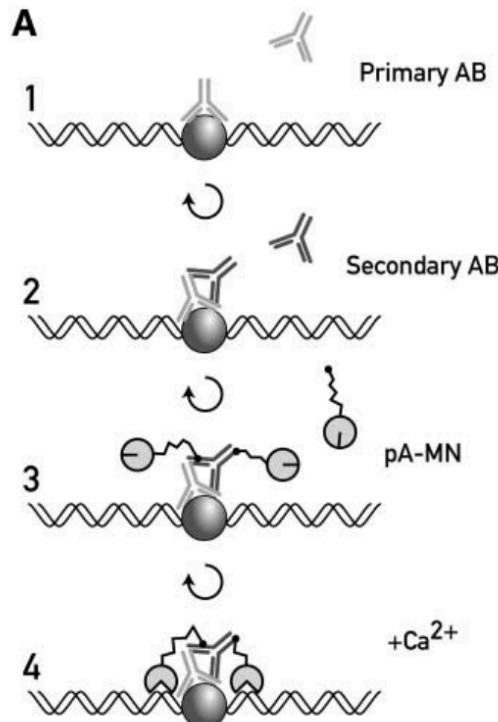
Manfred Schmid, Thérèse Durussel,
and Ulrich K. Laemmli^{*}
Departments of Biochemistry and Molecular Biology
NCCR Frontiers in Genetics
University of Geneva
30, Quai Ernest-Ansermet
CH1211, Geneva 4
Switzerland

Technique

ChIC: Chromatin-immuno cleavage

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 insoluble-type proteins appear afflicted with increased back-



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

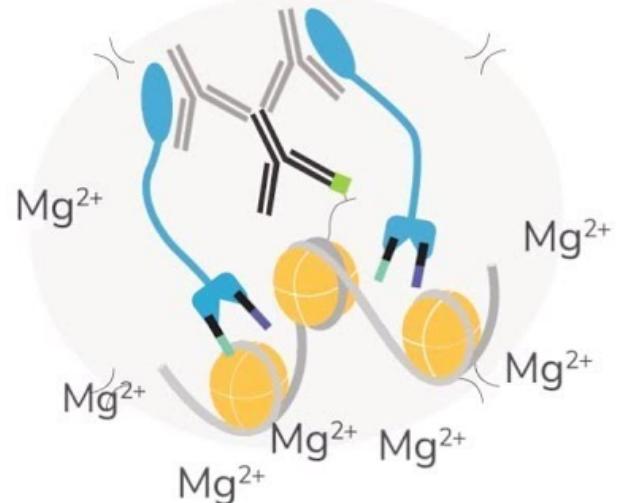
Single-cell chromatin immunocleavage sequencing (scChIC-seq) to profile histone modification

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

CUT&Run, CUT&Tag

Solid support



CUT&Tag

Solid support



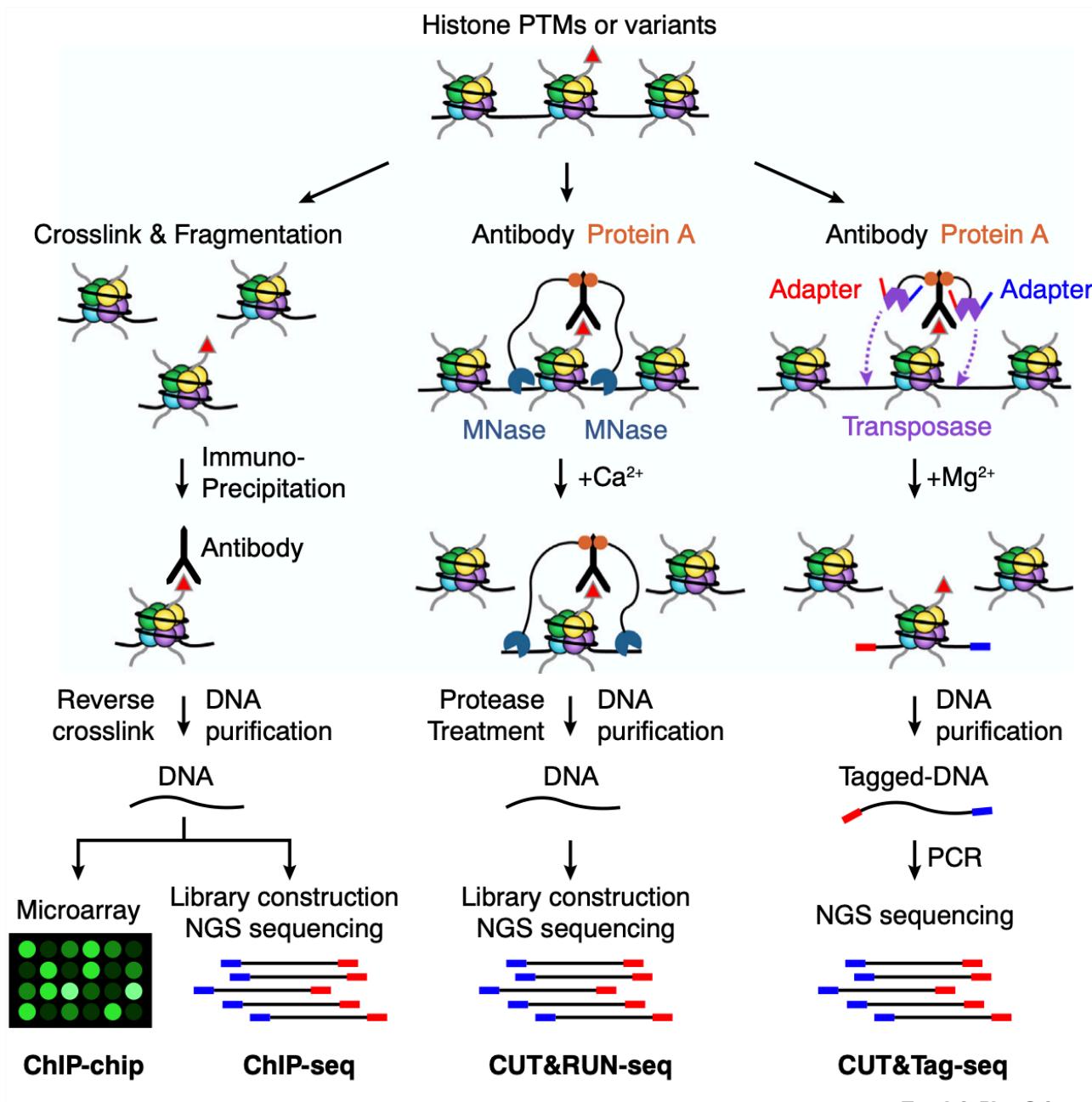
CUT&RUN



EpiCypher®

Bringing Epigenetics to Life

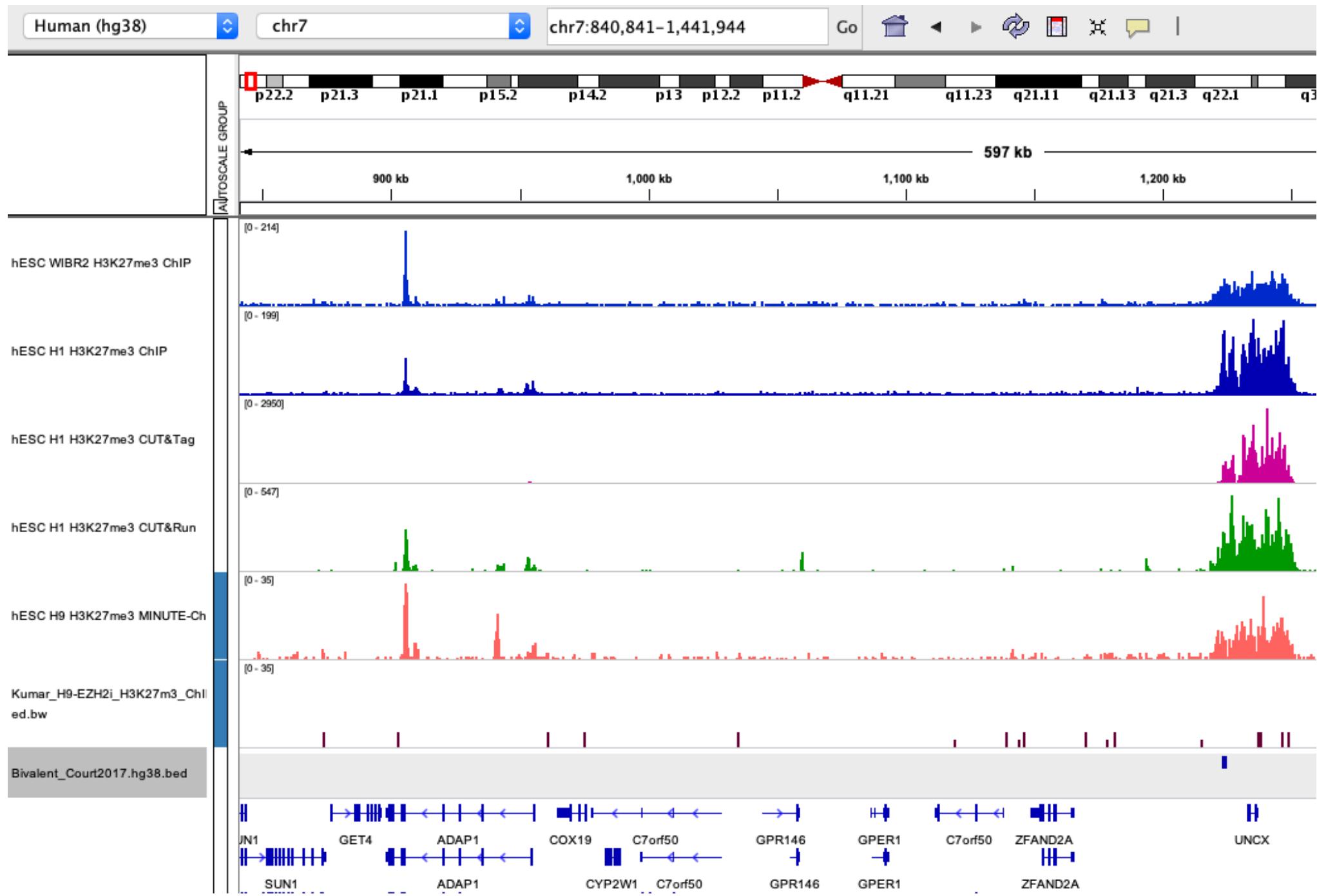
CUT&Run, CUT&Tag



CUT&Run, CUT&Tag

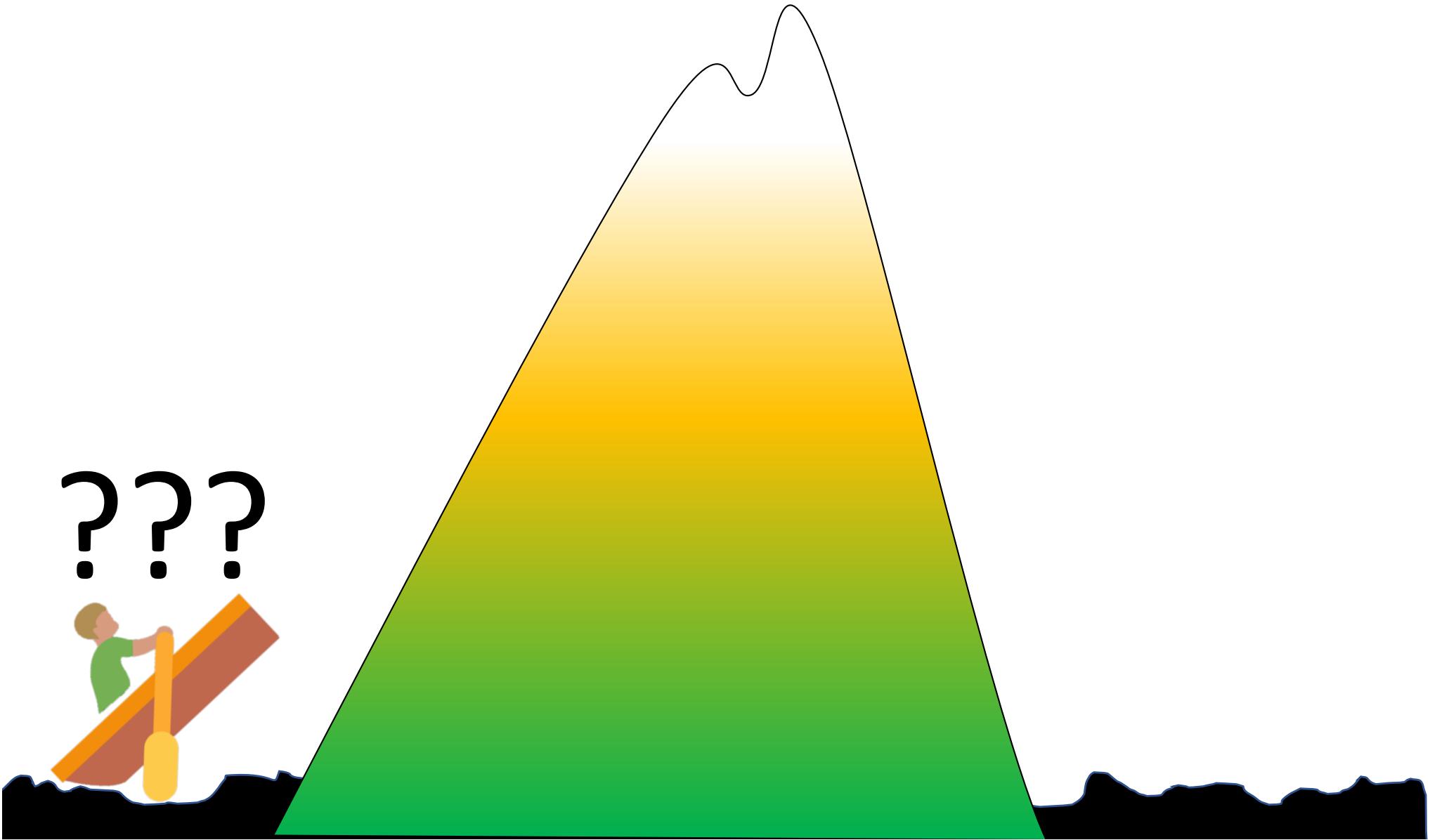
| Features | ChIP-Seq | CUTANA™ CUT&RUN | CUTANA™ CUT&Tag |
|--|---|---|---|
| Sample Input | Sheared Chromatin | Cells OR nuclei | Nuclei (recommended) |
| Typical Required Cell # | > 1 Million | 500K | 100K |
| Ideal Targets | Histone PTMs & chromatin-interacting proteins | Histone PTMs & chromatin-interacting proteins, including remodelers | Histone PTMs & select validated targets |
| Secondary Antibody | No | No | Yes |
| Library Preparation | Yes | Yes | No (Direct to PCR) |
| Protocol Time (Cells → NGS libraries) | ~ 1 week | 2 days (can be automated) | 2 days (can be automated) |
| Sequencing Depth | > 30 million | 3-5 million | 3-5 million |
| Signal : Noise | Low | High | High |
| Experimental Throughput | Low | High | High |





Weighing pros and cons

- Cell number needed
- Time aspect
- Signal-to-noise
- Background (think technical versus biological!)
- Reproducibility
- QOability (e.g. no input)



Single-cell revolution

