invitrogen

SNAP-ChIP™ Panels

USER GUIDE

for use with:

K-AcylStat Panel, SNAP-ChIP™ spike-in(Cat. No. A47358 and A47359)

K-MetStat Panel, SNAP-ChIP™ spike-in (Cat. No. A47356 and A47357)

OncoStat Panel, SNAP-ChIP™ spike-in (Cat. No. A47343 and A47355)

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Product information

Product description

SNAP-ChIP™ (**S**ample **N**ormalization and **A**ntibody **P**rofiling **Ch**romatin **I**mmuno**P**recipitation) spike-in is a proprietary method developed by EpiCypher™ that uses DNA-barcoded designer nucleosomes (dNucs) bearing distinct post-translational modifications (PTMs) as next-generation spike-in controls for ChIP. SNAP-ChIP™ panels are directly compatible with your current ChIP workflow, with semi-synthetic nucleosomes bearing the PTM of interest immunoprecipitated and processed alongside sample chromatin (see **Figure 1**). Recovery of the associated DNA barcodes is deciphered by quantitative PCR (ChIP-qPCR) or Next-Generation Sequencing (ChIP-seq). SNAP-ChIP™ provides defined standards to evaluate antibody performance and monitor technical variability in ChIP, setting it apart from other spike-in controls.

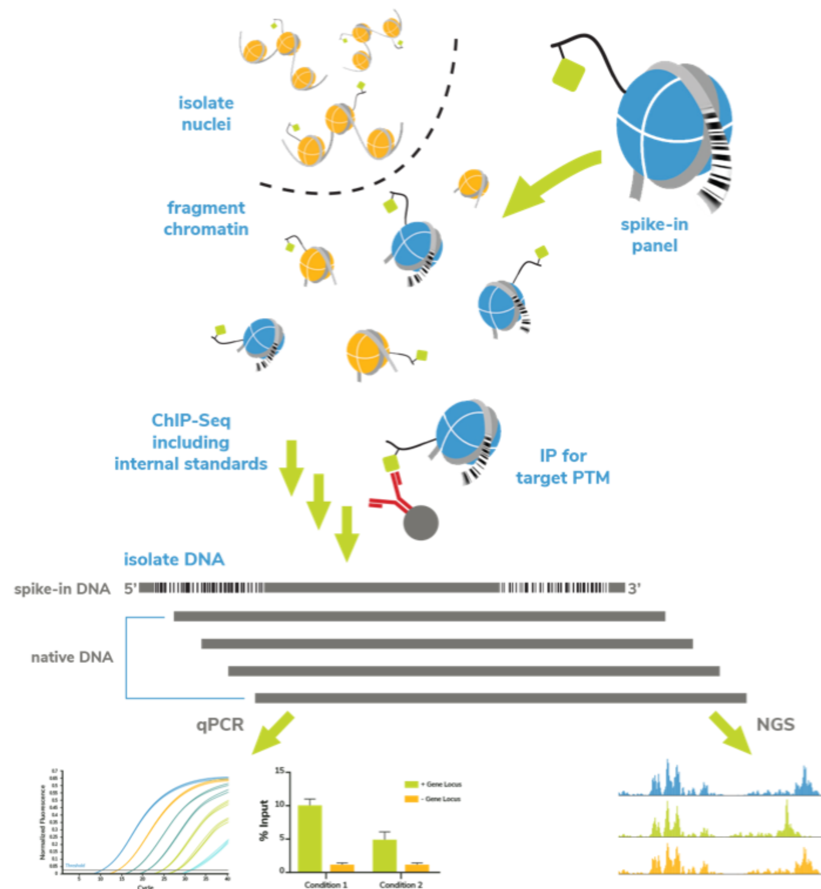

Figure 1 **Overview of SNAP-ChIP™ (adapted from proprietary EpiCypher™ ICeChIP technology[1]). A pool of recombinant dNucs with defined PTMs identified by unique DNA barcodes is added to sample chromatin prior to immunoprecipitation (IP). Capture of the barcoded nucleosomes (on- and off-target) allows the user to assess antibody performance (specificity and target enrichment) and monitor technical variability.**

The K-MetStat panel consists of a pool of nucleosomes carrying 15 well-studied, disease relevant lysine methyl marks on histones H3 and H4 plus an unmodified control (see **Figure 2**). The K-AcylStat panel consists of a pool of nucleosomes carrying 22 well-studied, disease-relevant lysine acylation marks on histones H2A, H3, and H4 plus an unmodified control (see **Figure 3**). The OncoStat panel consists of a pool of nucleosomes harboring 7 well-studied histone H3.3 mutations that have been implicated in cancer plus a wild-type H3.3 control (see **Figure 4**). A single spike-in of the panel allows users to check antibody specificity by examining the post-IP recovery of on- versus off-target SNAP-ChIP nucleosomes. Of note, the OncoStat SNAP-ChIP™ Panel is fully compatible with K-MetStat or K-AcylStat in the same ChIP experiment.

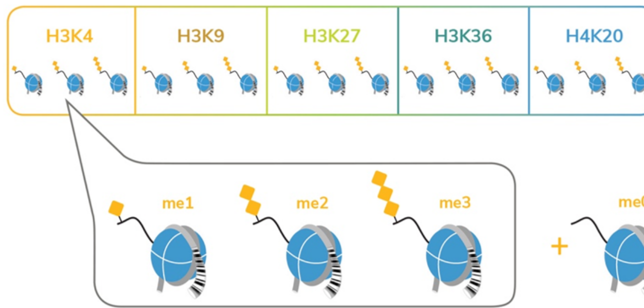

Figure 2 Schematic depicting the 15 dNucs plus unmodified control included in the K-MetStat panel. Each nucleosome is wrapped by two unique DNA barcodes, providing an internal technical replicate for each PTM of interest.

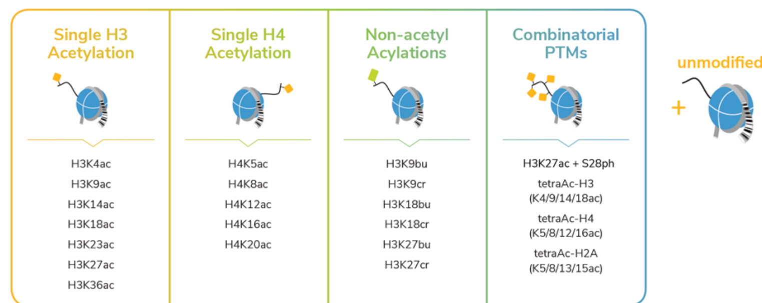

Figure 3 Schematic depicting the 22 dNucs plus an unmodified control included in the K-AcylStat panel. Each nucleosome is wrapped by two unique DNA barcodes, providing an internal technical replicate for each PTM of interest.

Figure 4 Schematic depicting the 7 oncogenic mutations plus wild type control included in the OncoStat panel. Each nucleosome is wrapped by two unique DNA barcodes, providing an internal technical replicate for each mutation of interest.

Advantages of SNAP-ChIP™:

- Homogenous, fully-defined standards that faithfully represent target mononucleosomes in the experimental sample.
- dNucs are subjected to rigorous quality control for lot-to-lot consistency.
- Unique DNA barcodes can be distinguished from experimental sample genomes (confirmed human, mouse, *Drosophila*, and *Saccharomyces cerevisiae*).
- Spike-ins provide a direct readout of antibody performance:
  - Panels contain a pool of related PTMs that allow users to determine antibody specificity (recovery of on- vs. off-target PTMs in the panel).
  - Determination of antibody enrichment (amount of target PTM immunoprecipitated relative to input).

- Ability to monitor technical variability between samples.
- Analysis of DNA barcodes (via qPCR) provides useful STOP/GO capability before advancing to NGS.

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Methods

Procedure overview

Incorporate SNAP-ChIP™ spike-in into your ChIP experiments in 3 simple steps:

1. Spike the SNAP-ChIP™ panel into samples at the earliest appropriate step in your ChIP protocol (see **Figure 5** or Step 1 in “Procedure” on page 9).
2. After immunoprecipitation and DNA isolation, use qPCR to assess whether ChIP has successfully (and specifically) enriched the target PTM. Use this information to decide whether to invest in NGS (STOP/GO capability). For example, does your anti-H3K4me3 cross-react with related methyl species (H3K4me0/1/2)?
3. NGS data will give further information regarding antibody specificity for all PTMs in the panel (*e.g.,* me1-2-3 for H3K4/9/27/36 and H4K20), identifying additional potential cross-reactivities. If all is as expected, users can feel confident to proceed with data analysis.

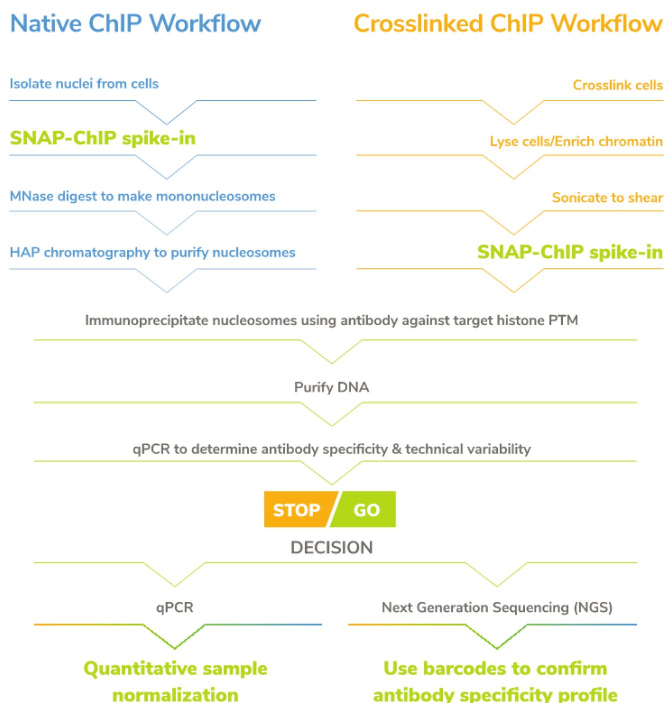

Figure 5 SNAP-ChIP™ spike-in is compatible with both native and crosslinked ChIP protocols, where a simple spike-in of the panel enables the assessment of antibody specificity and technical variability prior to investment in NGS (STOP/GO decision). SNAP-ChIP™ spike-in can also be used to normalize experimental data, improving quantitative comparisons in qPCR.

Procedure

1. Prepare samples using desired protocol (crosslinked or native).

   Note: If using a crosslinked protocol, spike in SNAP-ChIP™ post-sonication. If using a native protocol, spike in prior to micrococcal nuclease digestion (see Figure 5).

   Note: SNAP-ChIP spike-in nucleosomes are compatible with standard crosslinked protocols containing up to 0.1% SDS in the ChIP buffer. For protocols with higher concentrations of ionic detergent during sample preparation, immunoprecipitation, and/or washing steps, users should evaluate compatibility with SNAP-ChIP™ spike-in.

2. Spike-in 2 µL of SNAP-ChIP™ spike-in per 10 µg sample chromatin (~10^6 cell equivalents per ChIP). If more or less chromatin is used, scale the spike-in volume accordingly.

   Note: See "Quantify sample chromatin" on page 15 for details.

3. Proceed with IP using an antibody to the desired PTM.

   Note: Be sure to saturate the IP resin (e.g., magnetic beads) with antibody. The lot-specific datasheet should contain bead capacity.

4. Purify immunoprecipitated DNA (e.g., PureLink™ PCR Purification Kit or equivalent).

5. Run qPCR to evaluate antibody performance and technical variability:

qPCR Reaction Mix (Per 10 µL Reaction)		
Reagent	**Final Concentration**	**Volume to Add**
1:10 diluted ChIP or Input DNA	User-specific	4.0 µL
2X qPCR Master Mix	1X	5.0 µL
20X SNAP-ChIP™ Primer	1X (250 nM for each primer)	0.5 µL
20X SNAP-ChIP™ Probe	1X (250 nM)	0.5 µL

6. Run qPCR using appropriate reaction conditions (e.g., PowerUp™ SYBR™ Green Master Mix = 95°C for 3 min followed by 40 cycles of 95°C for 5 sec plus 60°C for 30 sec).

7. Analyze data using standard ΔΔCt calculation (see "How to assess antibody specificity using the SNAP-ChIP spike-in" on page 11).

8. Evaluate whether IP was specific, and the experiment is thus of sufficient quality to invest in NGS. Proceed with library preparation.

9. Use the spike-in DNA barcode sequences (see product page at thermofisher.com) to align read counts to SNAP-ChIP™ spike-in.

Data analysis and quantification

Why do I need to assess antibody specificity in ChIP?

- Antibody cross-reactivity can lead to gross data misinterpretation.
- It is essential to determine antibody capability in the application of interest. Commonly used methods for antibody validation (e.g., peptide arrays) are biophysically very different from ChIP; therefore, any cross-reactivity (or lack thereof) detected in such an approach may not be applicable in a ChIP context[2].
- Exclusive use of positive / negative genomic loci controls to monitor IP enrichment is invariably a lower resolution approach (and may be further compromised if the initial control loci were identified with cross-reactive reagents).
- SNAP-ChIP™ spike-in addresses these limitations by enabling platform-specific validation of antibody specificity within every ChIP experiment (**Figure 6**).

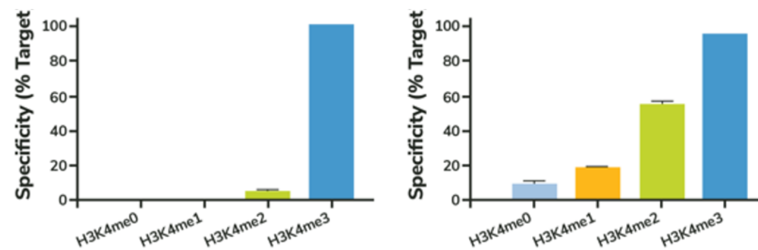

Figure 6 A SNAP-ChIP™ spike-in experiment using two commercially available H3K4me3 antibodies. qPCR for the barcodes corresponding to alternate H3K4 methyl states shows that Antibody #1 (left) is highly specific for H3K4me3, exhibiting <3% cross-reactivity with H3K4me2. In contrast, Antibody #2 (right) shows ~60% cross-reactivity with H3K4me2, compromising any corresponding ChIP data.

How to assess antibody specificity using the SNAP-ChIP spike-in

1. Perform SNAP-ChIP™ spike-in as in the Procedure (see "Procedure" on page 9).
2. Run qPCR to check recovery of the barcodes corresponding to the on-target PTM compared to off-target PTMs.

   Note: It is recommended at minimum to check antibody specificity against PTMs that present the most likely source of cross-reactivity. For example, for an H3K4me3 ChIP, run qPCR for the barcodes corresponding to SNAP-ChIP™ unmodified, H3K4me1, H3K4me2, and H3K4me3 dNucs. If an antibody exhibits specificity at this stage, the remaining PTMs in the panel (H3K9, H3K27, H3K36,

and H4K20 methyl states) can be actively checked by qPCR or will be passively captured in ChIP-seq.

3. Analyze data to calculate antibody specificity (off-target relative to on-target) and enrichment (% on-target Input recovered) (see Table 1).

   Note: The enrichment score provides a measure of the amount of target recovered after IP. An antibody can exhibit high specificity for the target but have low enrichment, which could become problematic for experimental normalization due to noise in the data. Antibodies with enrichment >10% generally yield reproducible results. Antibodies with reduced enrichment (1-10%) may be usable if they exhibit high specificity. Antibodies with enrichment scores <1% often result in excessively variable data.

$$\% \, \boldsymbol{Enrichment} = \left(\frac{\mathbf{2}^{\boldsymbol{Ct_{Input}} - \boldsymbol{Ct_{IP}}}}{\boldsymbol{x}} \right) \times \mathbf{100\%}$$

where $\boldsymbol{Ct_{Input}}$ = *qPCR cycle threshold for SNAP-ChIP on-target Input*
$\boldsymbol{Ct_{IP}}$ = *qPCR cycle threshold for SNAP-ChIP on-target IP*
$\boldsymbol{x}$ = *percent of Input sample that was set aside (e.g. 10%)*

4. In ChIP-seq, all barcodes will be captured in the NGS data. Align the read counts to the barcodes. A SNAP-ChIP™ spike-in alignment algorithm for paired-end sequencing is available at basepairtech.com.

Table 1 Calculations for determining antibody specificity using the SNAP-ChIP™ on- and off-target barcodes, where "on-target" refers to the SNAP-ChIP™ dNuc immunoprecipitated by the ChIP antibody and "off-target" refers to any other dNuc in the SNAP-ChIP™ panel.

Ct Mean	ΔCt	ΔΔCt	RQ (Relative Quantification)	Specificity (% Target)
= Average of qPCR technical replicates	= $Ct_{IP} - Ct_{input}$	= $\Delta Ct_{off\text{-}target}$ = $\Delta Ct_{on\text{-}target}$	= $2^{-\Delta\Delta Ct}$	= RQ x 100%

Normalize ChIP data using the SNAP-ChIP™ spike-in

SNAP-ChIP™ spike-in provides a homogenous, defined spike-in control that can be used as a normalization factor to standardize experiments. This controls for unanticipated technical variability (see Figure 6) and enables trans-experiment comparisons. The use of SNAP-ChIP™ spike-in to account for technical variation in the ChIP procedure reduces noise in the data, thereby improving detection of true biological changes.

1. Perform SNAP-ChIP™-qPCR as described in the "Procedure" on page 9, keeping the ratio of SNAP-ChIP™ to sample chromatin consistent across samples.

   Note: It is essential to run an Input (pre-IP chromatin) control for every ChIP sample.

2. Check that the antibody did not cross-react with unintended targets in the SNAP-ChIP™ panel (see the left panel in Figure 6).

   Note: Cross-reactivity prohibits normalization because any recovery of off-target PTMs contributes sample data, which cannot be accounted in the normalization. As an example: the biological perturbation under study differentially impacts the antibody on- (H3K4me3) and off-target (H3K4me2) signals in the sample chromatin. This cannot be corrected by the on-target spike-in normalization.

3. Calculate the % Input for each gene locus of interest (or genome-wide for ChIP-seq).

4. Calculate the % Input for the on-target SNAP-ChIP™ spike-in (*e.g.* in a H3K4me3 ChIP experiment, use the barcodes corresponding to the H3K4me3 dNuc in the SNAP-ChIP™ panel).

   Note: See the % Enrichment calculation ("How to assess antibody specificity using the SNAP-ChIP spike-in" on page 11) to calculate the % Input for ChIP-qPCR. For ChIP-seq, simply divide the read counts for IP/Input.

5. Apply the formula:

$$\boldsymbol{Normalized\ signal} = \frac{(\%\ \boldsymbol{Input\ of\ Gene\ Locus})}{(\%\ \boldsymbol{Input\ of\ SNAP\ ChIP})}$$

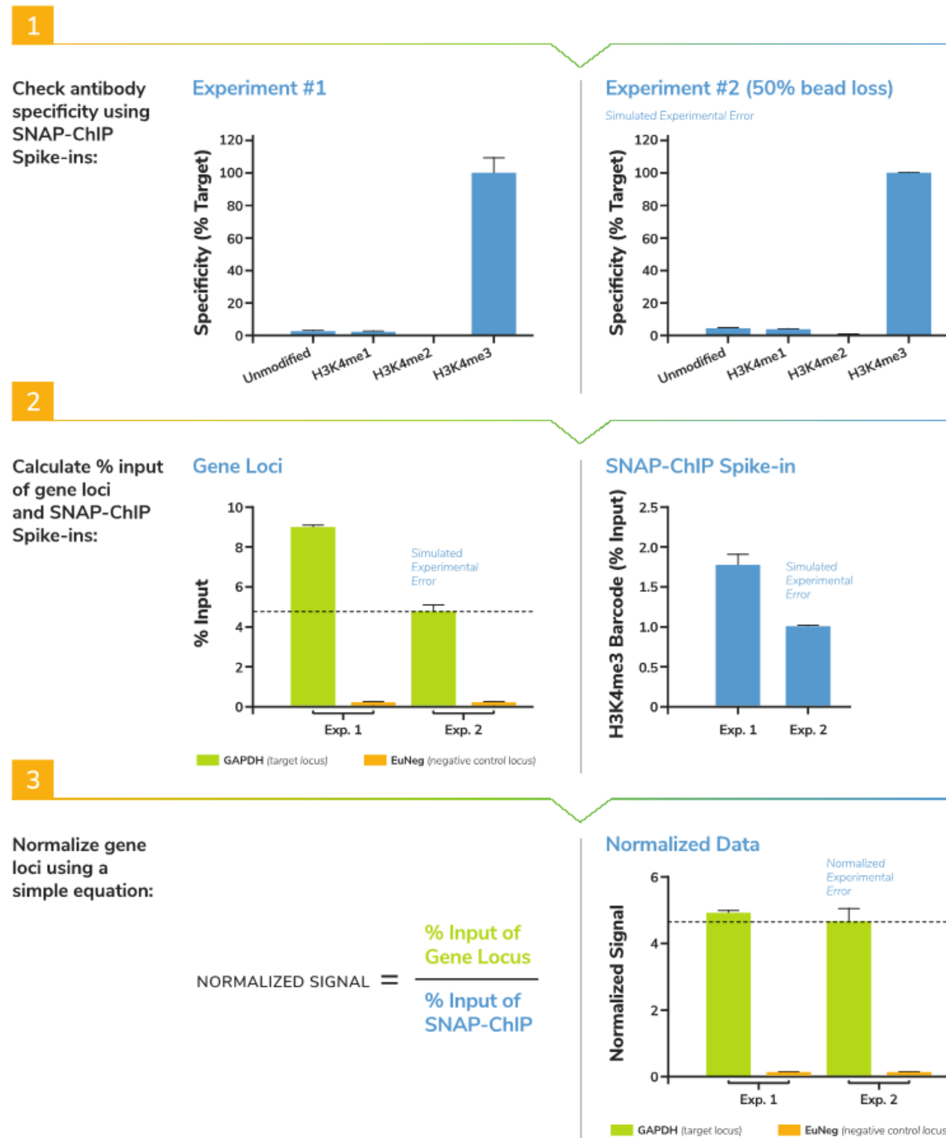

Figure 7 Overview of data normalization using the SNAP-ChIP™ spike-in approach. Technical variability was simulated by the loss of bead-antibody conjugates (Experiment #2). (1) A check of antibody specificity using the SNAP-ChIP™ spike-ins (critical before proceeding to normalization) confirmed that the antibody enriched for the intended target (H3K4me3), but not related species (H3K4me0, H3K4me1, or H3K4me2). (2) Examination of genomic loci (GAPDH and euchromatin negative control region, EuNeg: left panel) revealed a difference between these two experiments that could indicate biological perturbation. However, the SNAP-ChIP™ spike-in (right panel) also shows this discrepancy, indicating that the observed difference is due to technical variability in the ChIP procedure. (3) A simple equation can be applied to resolve this issue and elucidate true biological change(s).

Quantify sample chromatin

Note: The guidelines below are for UV absorbance measurement of DNA. When working with small numbers of cells (<10^6) below the sensitivity of standard spectrophotometers (~2 ng/mL), a more sensitive DNA quantitation tool (e.g., fluorometer) may be needed.

Native ChIP

1. After nuclei purification, remove a small aliquot of sample. Dilute 1:10 in 2 M NaCl to extract proteins (e.g., dilute 2 mL nuclei in 18 mL NaCl).
2. Vortex for 10 minutes or sonicate in a water bath to solubilize DNA.
3. Use a spectrophotometer to determine nucleic acid content by measuring the absorbance at 260 nm (OD_{260}).

   Note: For most accurate measurement, perform triplicate readings. If sample is too viscous, readings will not be consistent. Try higher dilutions (e.g., 1:100) or more vigorous vortexing / sonication.
4. Use molar extinction coefficient for DNA to calculate concentration:

   DNA Concentration = OD_{260} ***x Dilution factor x 50 ng/µL***

Crosslinked ChIP

1. After chromatin sonication, follow steps in the user-specific protocol to digest proteins and reverse crosslinking. In general:
   1. Add elution buffer.
   2. Incubate samples at 65°C while shaking 4 hours – overnight.
   3. Add Proteinase K.
   4. Incubate samples at 60°C while shaking for 1 hour.
2. Purify DNA (e.g. PCR Purification Kit, phenol:chloroform extractionor equivalent method).
3. Remove a small aliquot of purified DNA. Dilute 1:100 using TE buffer.
4. Use a spectrophotometer to determine nucleic acid content (OD_{260}).
5. Use molar extinction coefficient for DNA to calculate concentration:

   DNA Concentration = OD_{260} ***x Dilution factor x 50 ng/µL***

References

1. Grzybowski, A. T., Chen, Z. & Ruthenburg, A. J. Calibrating ChIP-Seq with Nucleosomal Internal Standards to Measure Histone Modification Density Genome Wide. *Molecular cell* **58**, 886-899, doi:10.1016/j.molcel.2015.04.022 (2015).
2. Shah, R. N. *et al.* Examining the Roles of H3K4 Methylation States with Systematically Characterized Antibodies. *Molecular cell*, doi:10.1016/j.molcel.2018.08.015 (2018).
3. Lowary, P. T. & Widom, J. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *Journal of molecular biology* **276**, 19-42, doi:10.1006/jmbi.1997.1494 (1998).
4. Brand, M., Rampalli, S., Chaturvedi, C. P. & Dilworth, F. J. Analysis of epigenetic modifications of chromatin at specific gene loci by native chromatin immunoprecipitation of nucleosomes isolated using hydroxyapatite chromatography. *Nature protocols* **3**, 398-409, doi:10.1038/nprot.2008.8 (2008).

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