EpiCypher™
Bringing Epigenetics to Life

SNAP-ChIP™
For Histone Lysine Methylation

Sample **N**ormalization & **A**ntibody **P**rofiling for **ChIP** using EpiCypher's K-MetStat™ panel

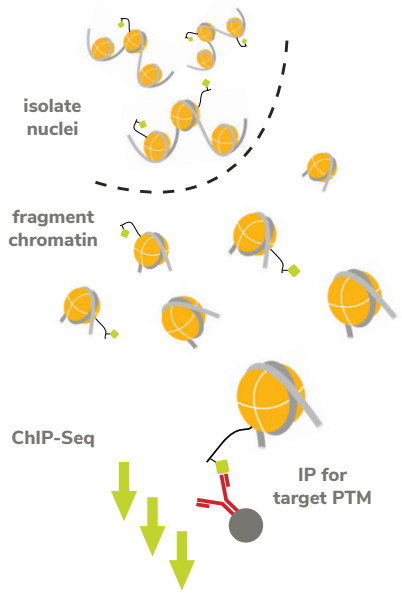
SNAP-ChIP uses DNA-barcoded recombinant designer nucleosomes (dNucs) as next-generation spike-ins for chromatin immunoprecipitation (ChIP). The first product in this family consists of a panel of dNucs carrying well-studied histone lysine methyl marks (K-MetStat panel).

EpiCypher's K-MetStat panel can easily be added to any ChIP workflow to standardize and normalize samples (across experiments) and rigorously validate antibody specificity. For the first time, users can monitor antibody specificity and evaluate technical variability within a ChIP experiment, setting SNAP-ChIP apart from any other spike-in controls currently available on the market.

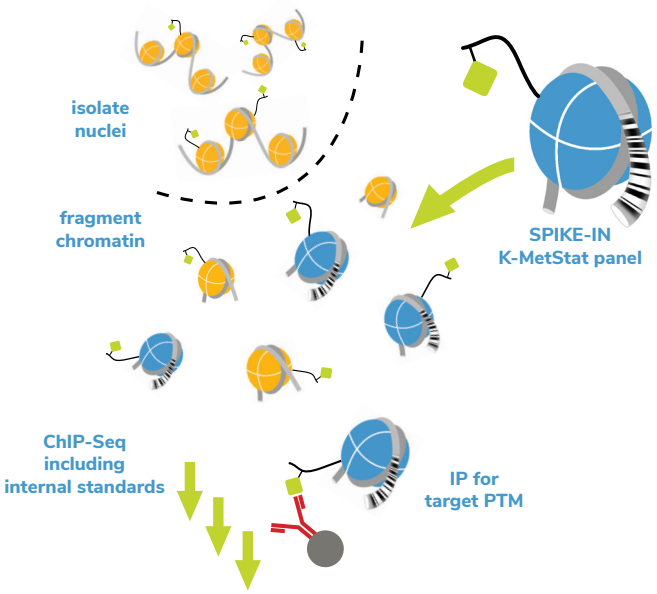
FIGURE 1

Overview of the SNAP-ChIP approach (adapted from ICe-ChIP technology: Grzybowski et al. Mol Cell, Vol. 58 , Issue 5 , 886 - 899, 2015). A pool of recombinant dNucs with defined post-translational modifications (PTMs) identified by unique DNA barcodes is added to sample chromatin prior to immunoprecipitation (IP). Capture of the barcoded nucleosomes (on / off target) allows the user to assess antibody specificity, monitor technical variability, and normalize experiments. Quantitative recovery of barcoded dNucs (via qPCR) provides a useful STOP / GO capability prior to advancing to next-generation sequencing.

Standard ChIP Protocol



SNAP-ChIP



Limitations

- No way to assess antibody specificity or efficiency
- Minimally reproducible
- Current methods to normalize experiments (e.g. exogenous chromatin spike-ins) are heterogeneous, poorly defined, and subject to lot-to-lot variability

SNAP-ChIP Advantages

- Determine antibody specificity and target pulldown efficiency
- Monitor technical variability within experiments
- Quantitative recovery of DNA barcodes (via qPCR) provides useful STOP / GO capability before advancing to NGS
- Homogenous, defined dNucs are subjected to rigorous quality control for lot-to-lot consistency

Sample **N**ormalization & **A**ntibody **P**rofiling for **ChIP** using EpiCypher's K-MetStat panel

K-MetStat panel (lysine-methylation status panel) is a single pool of 16 uniquely modified DNA-barcoded dNucs carrying disease-relevant lysine methylation modifications on histones H3 and H4.

Each modification site in the panel (H3K4, H3K9, H3K27, H3K36, H4K20) is represented by all lysine methylation states (*i.e.* me0, me1, me2, and me3). This allows for maximum user flexibility and provides the ability to gather detailed antibody cross-reactivity data. Additional modification-specific dNuc panels are currently in development (e.g. arginine methylation, lysine acetylation, etc.) and slated for release in 2018.

K-MetStat Panel

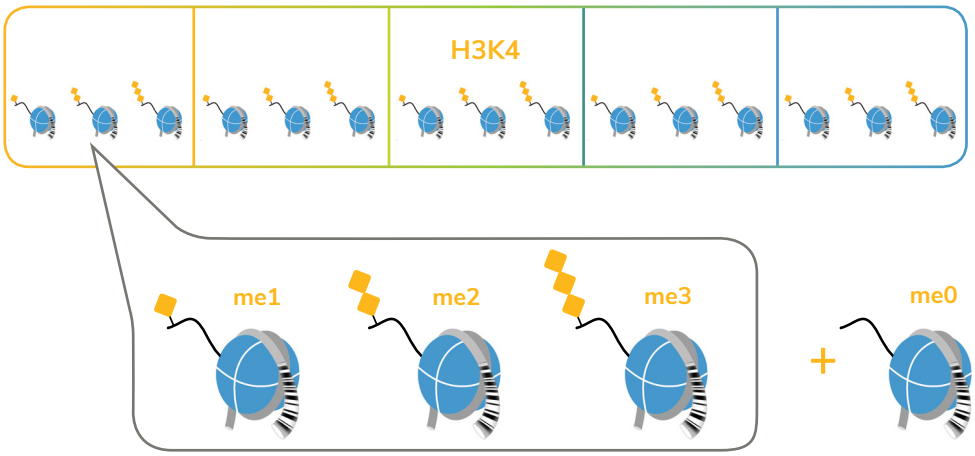


FIGURE 2

Schematic depicting the 16 dNucs included in the K-MetStat Panel, each uniquely DNA barcoded.

Why do I need to assess antibody specificity in ChIP?

- Antibody cross-reactivity can lead to gross misinterpretation of biological findings
- Methylation states (me0/1/2/3) are challenging targets for antibodies due to high degree of structural similarities
- SNAP-ChIP addresses these limitations by enabling quantification of antibody specificity within every ChIP experiment:

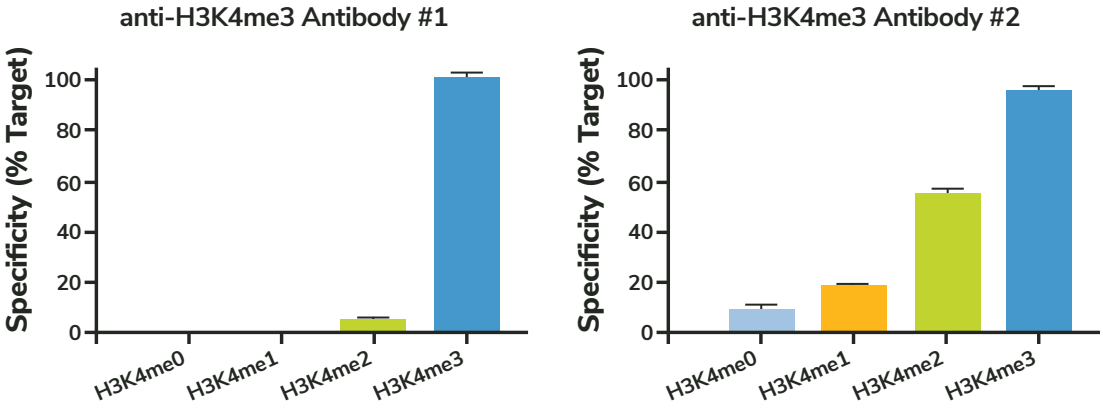


FIGURE 3

A SNAP-ChIP experiment (n = 3) using two H3K4me3 antibodies shows that Antibody #1 exhibits <3% cross-reactivity with alternate H3K4 methyl states; Antibody #2 shows ~60% cross-reactivity with H3K4me2, substantially compromising ChIP-studies.

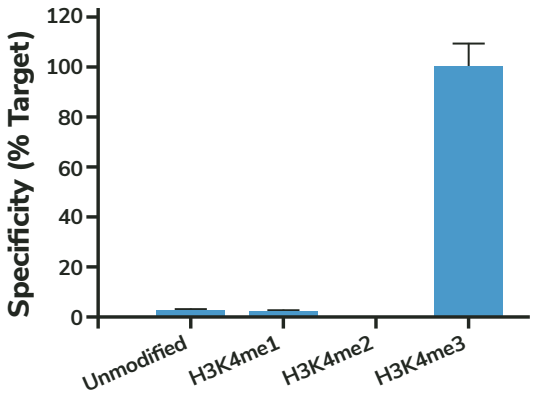
DO YOU REALLY KNOW WHAT YOU ARE PULLING DOWN IN YOUR CHIP?
DON'T LET FAULTY ANTIBODIES COMPROMISE YOUR RESEARCH

SNAP-ChIP Normalization Workflow

1

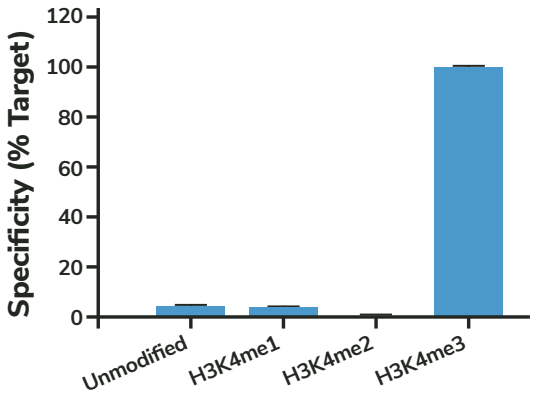
Check antibody specificity using SNAP-ChIP Spike-ins:

Experiment #1



Experiment #2 (50% bead loss)

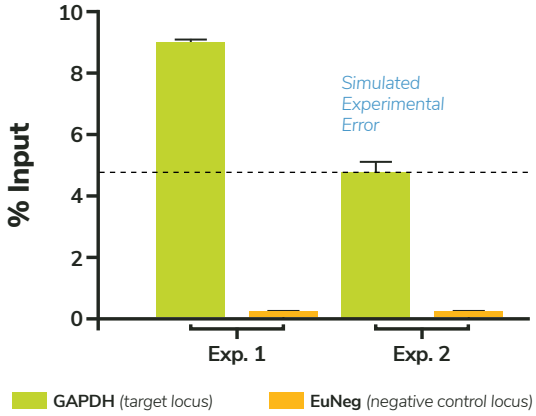
Simulated Experimental Error



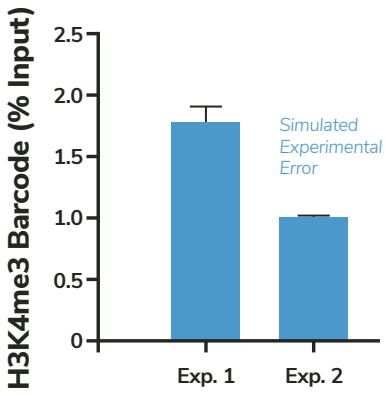
2

Calculate % input of gene loci and SNAP-ChIP Spike-ins:

Gene Loci



SNAP-ChIP Spike-in

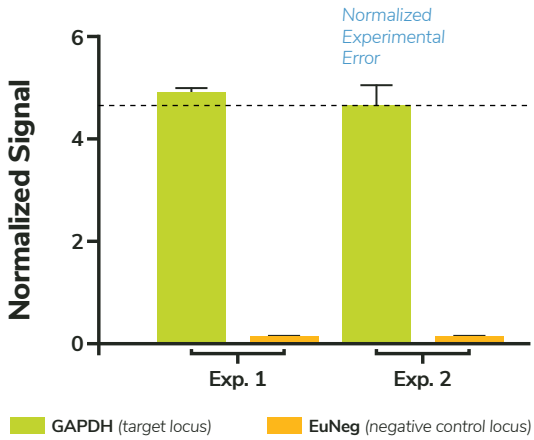


3

Normalize gene loci using a simple equation:

$$\text{NORMALIZED SIGNAL} = \frac{\text{\% Input of Gene Locus}}{\text{\% Input of SNAP-ChIP}}$$

Normalized Data



SNAP-ChIP for reliable sample normalization

ChIP is a proven technique that has been in use for over three decades, but surprisingly, few technical improvements have been incorporated in that time. The use of exogenous chromatin (e.g. Drosophila) as spike-in controls has been recently developed for ChIP sample normalization. However, these reagents are poorly defined and highly variable from batch-to-batch, limiting their use for consistent normalization.

SNAP-ChIP is the ideal tool for determining the consistency of ChIP/ChIP-Seq experiments, as fully defined recombinant nucleosomes enable for the first time — quantitative sample normalization.

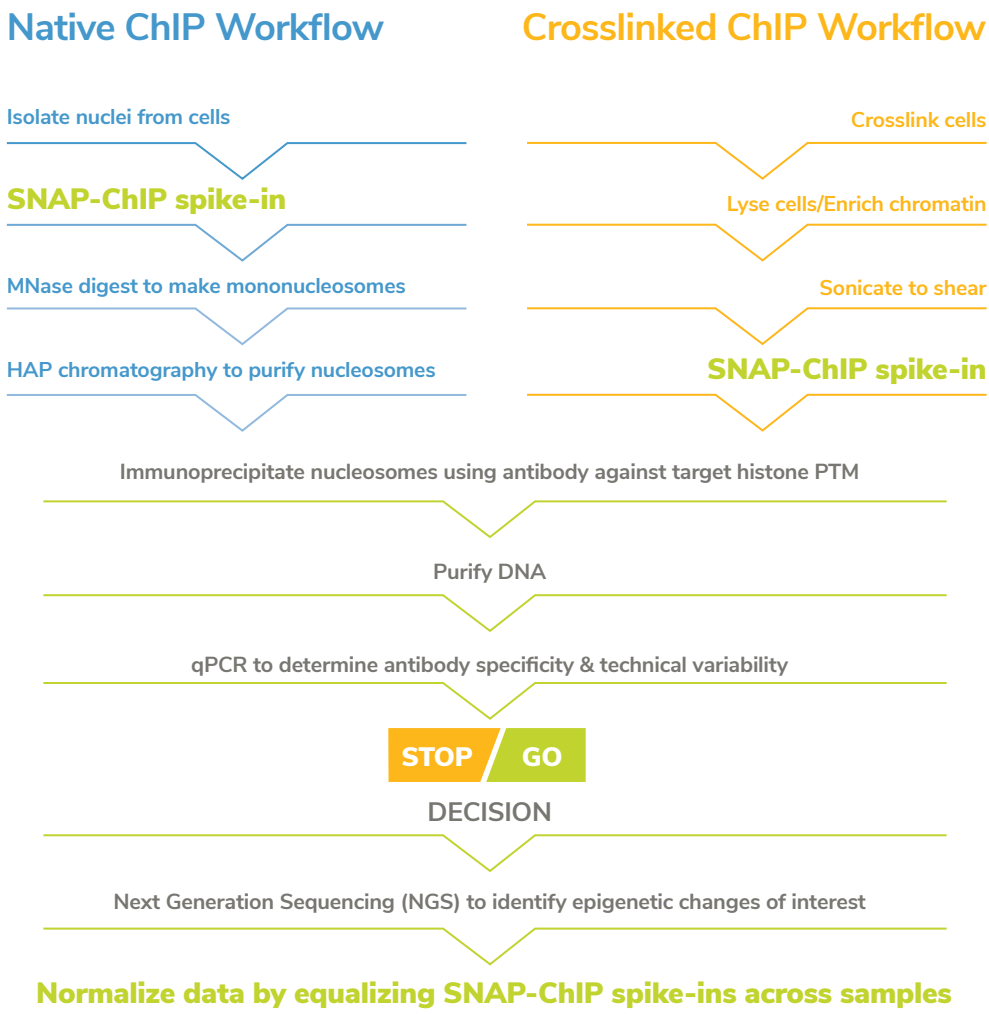
Spike-in controls for ChIP normalization

SNAP-ChIP	vs.	*Drosophila*
Fully defined PTMs • Proprietary semi-synthetic DNA-barcoded dNucs eliminate lot-to-lot variability		**Poorly defined PTMs** • Biologically-derived — resulting in significant lot-to-lot variability (sample preparation, etc.)
Biologically-relevant normalization • Simple to normalize across multiple experiments / users / labs • Quantitative normalization by adding in a defined DNA-barcoded dNuc standard		**Unreliable normalization** • Difficult to normalize across multiple experiments / users / labs • Values are arbitrary due to undefined spike-in quantity
MetStat panels allow antibody specificity / cross-reactivity analysis • First in class specificity testing in ChIP context		**No antibody specificity or cross-reactivity analysis** • *Drosophila*-specific spike-in antibodies do not provide valuable information regarding experimental antibody performance
Compatible with your sequencing depth • SNAP-ChIP spike-ins comprise <<1% of total nucleosomes in the sample		**Increased sequencing costs** • Additional reads are needed to include *Drosophila* genome — resulting in additional sequencing costs
Provide useful STOP/GO control before moving to NGS		**No STOP/GO control prior to investing in NGS**

MINIMIZE EXPERIMENTAL VARIABILITY TO GET RESULTS YOU CAN TRUST

Sample **N**ormalization & **A**ntibody **P**rofiling for **ChIP** using EpiCypher's K-MetStat panel

SNAP-ChIP seamlessly integrates into existing ChIP workflows. Just add SNAP-ChIP to your protocol and use it, it's that simple!



ORDERING INFO

SNAP-ChIP K-MetStat

Catalog No. 19-1001

Introductory Price: $349 / *10 ChIP equivalents*

Website: EpiCypher.com/SNAP-ChIP

Related Products *(Coming Soon)*

Lysine acetylation status panel (K-AcStat)

Aginine methylation status panel (R-MetStat)

Oncogenic mutation status panel (OncoStat)

Related Nucleosome Products

EpiDyne™ Nucleosome Remodeling Substrates

Recombinant Nucleosomes (rNucs)

Designer Nucleosomes (dNucs)

Variant Nucleosomes (vNucs)

Oncogenic Nucleosomes (OncoNucs)

Purified Nucleosomes (HeLa, Chicken)

EpiCypher™

Bringing Epigenetics to Life

EpiCypher.com

855.374.2461

info@epicypher.com