EpiCypher®

Bringing Epigenetics to Life

SNAP-ChIP®
For Histone
Lysine Methylation

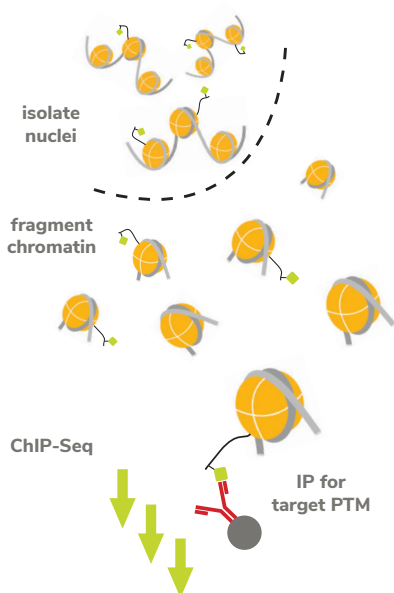Sample Normalization & Antibody Profiling for ChIP using EpiCypher's K-MetStat™ panel

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.
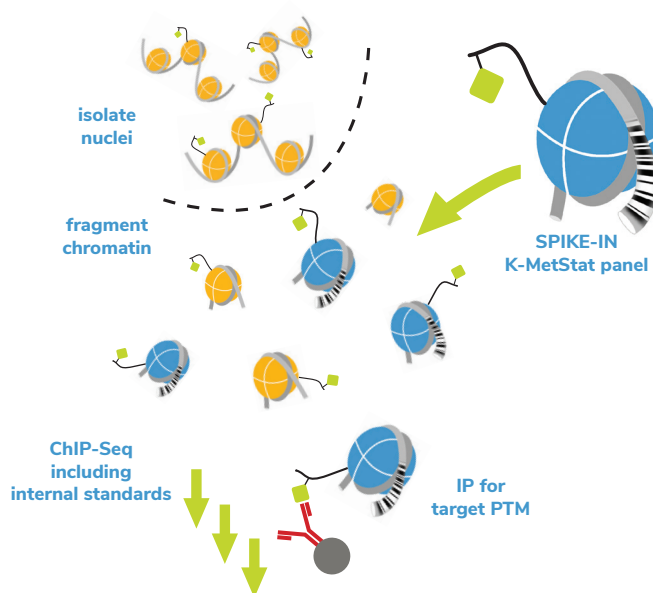
FIGURE 1

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

isolate nuclei

fragment chromatin

ChIP-Seq

IP for target PTM

SNAP-ChIP®

isolate nuclei

fragment chromatin

SPIKE-IN K-MetStat panel

ChIP-Seq including internal standards

IP for target PTM

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 Normalization & Antibody Profiling 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.
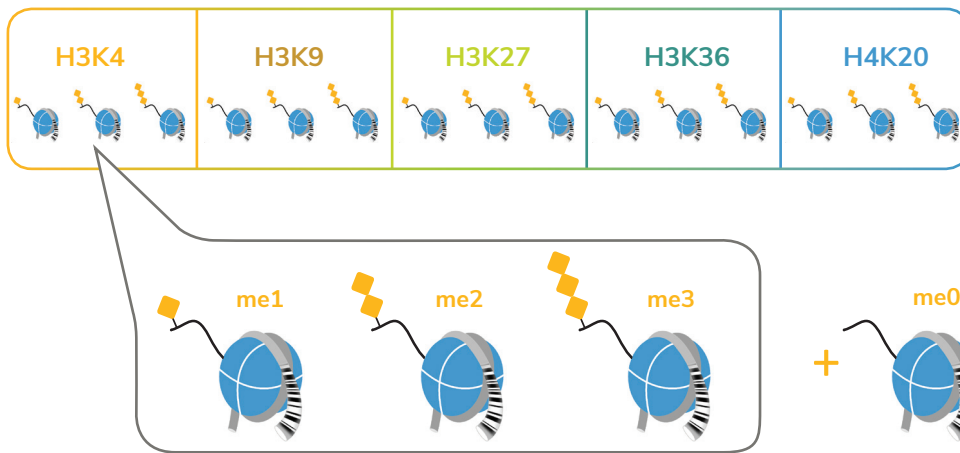
K-MetStat Panel

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:
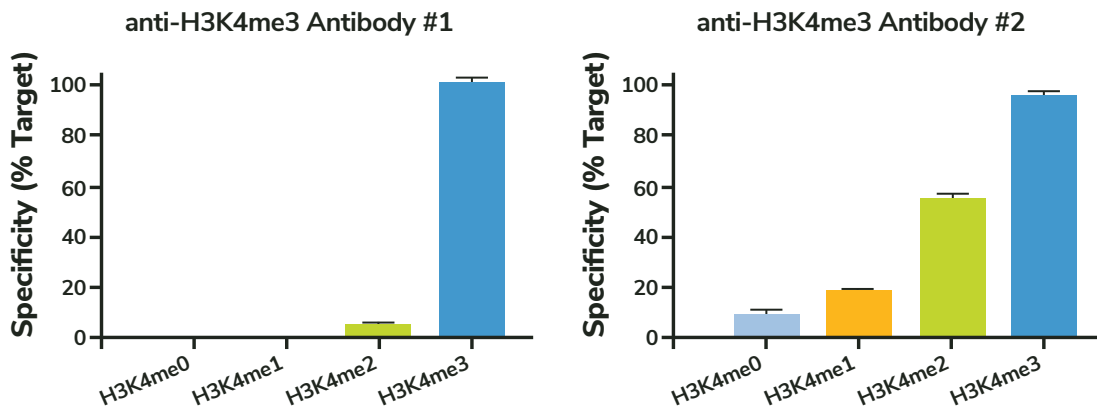

anti-H3K4me3 Antibody #1

anti-H3K4me3 Antibody #2

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.
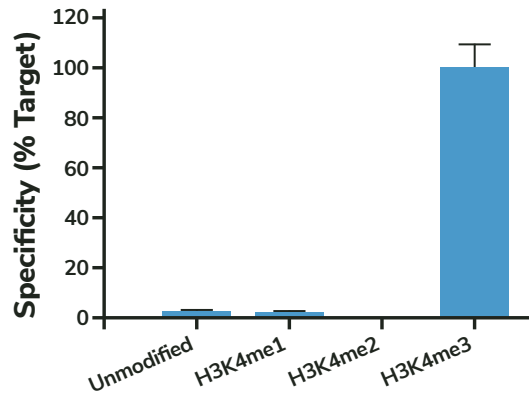
**DO YOU REALLY KNOW WHAT YOU ARE PULLING DOWN IN YOUR CHIP?
DON'T LET FAULTY ANTIBODIES COMPROMISE YOUR RESEARCH**
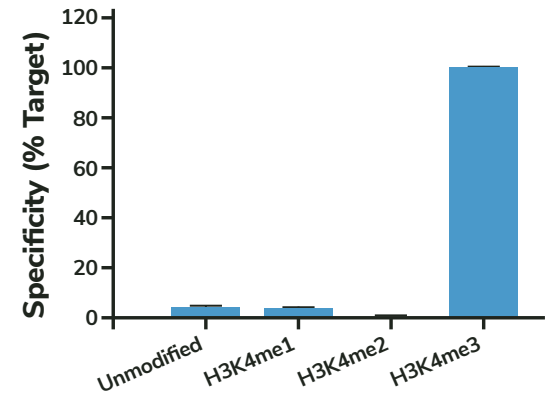
SNAP-ChIP® Normalization Workflow

1

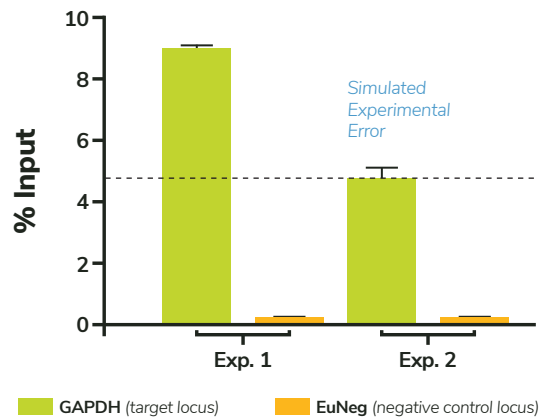**Check antibody specificity using SNAP-ChIP® Spike-ins:**

Experiment #1

Specificity (% Target) vs. Unmodified, H3K4me1, H3K4me2, H3K4me3

Experiment #2 (50% bead loss)

Simulated Experimental Error

Specificity (% Target) vs. Unmodified, H3K4me1, H3K4me2, H3K4me3

2

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

Gene Loci

% Input, Exp. 1 and Exp. 2

Simulated Experimental Error

GAPDH *(target locus)*   **EuNeg** *(negative control locus)*

SNAP-ChIP® Spike-in

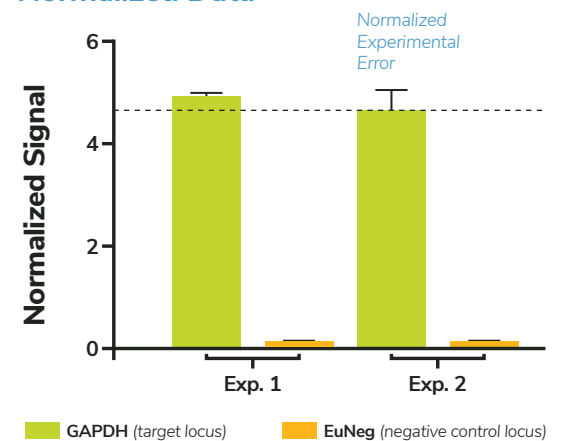H3K4me3 Barcode (% Input), Exp. 1 and Exp. 2

Simulated Experimental Error
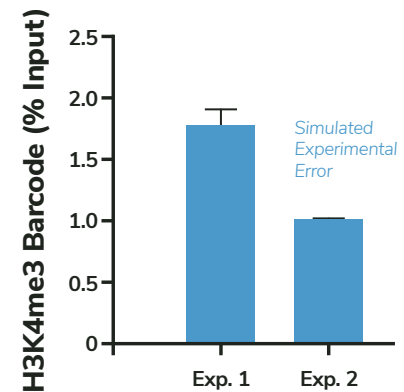
3

Normalize gene loci using a simple equation:

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

Normalized Data

Normalized Signal, Exp. 1 and Exp. 2

Normalized Experimental Error
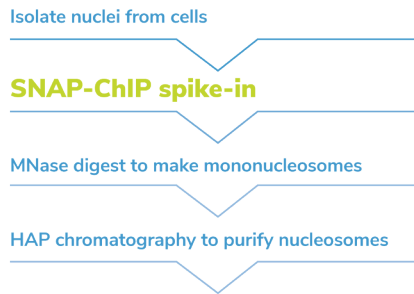
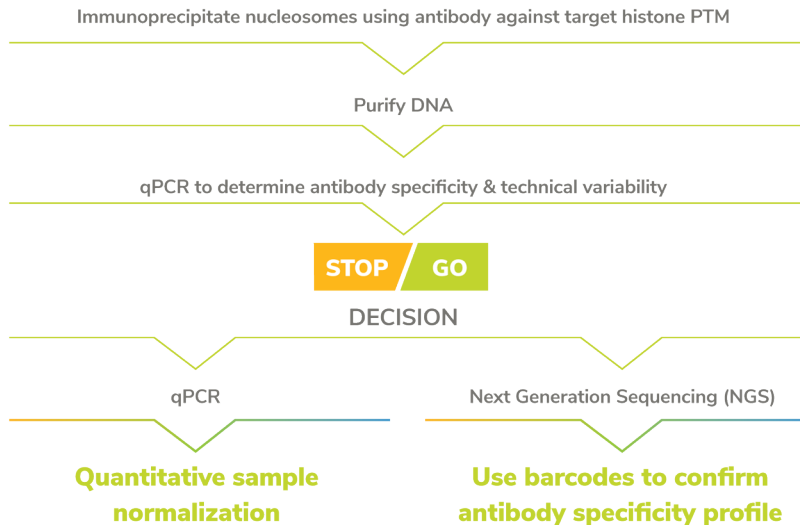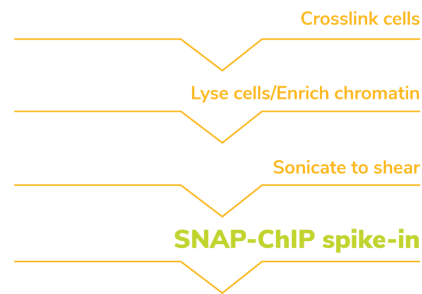**GAPDH** *(target locus)*   **EuNeg** *(negative control locus)*

Sample Normalization & Antibody Profiling 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!

Native ChIP Workflow

Isolate nuclei from cells

SNAP-ChIP spike-in

MNase digest to make mononucleosomes

HAP chromatography to purify nucleosomes

Crosslinked ChIP Workflow

Crosslink cells

Lyse cells/Enrich chromatin

Sonicate to shear

SNAP-ChIP spike-in

Immunoprecipitate nucleosomes using antibody against target histone PTM

Purify DNA

qPCR to determine antibody specificity & technical variability

STOP / GO

DECISION

qPCR

Next Generation Sequencing (NGS)

Quantitative sample normalization

Use barcodes to confirm antibody specificity profile

ORDERING INFO

SNAP-ChIP® K-MetStat
Catalog No. 19-1001
Introductory Price: $349 / *10 ChIP equivalents*

Website: EpiCypher.com/SNAP-ChIP

EpiCypher.
Bringing Epigenetics to Life

Related Products
SNAP-ChIP® Certified Antibodies
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)

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