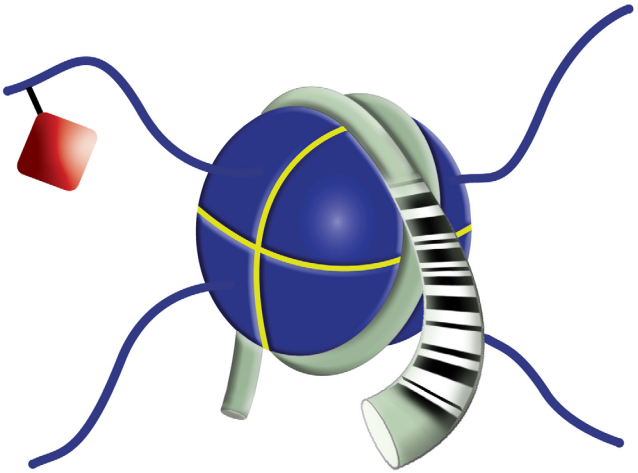
# SNAP-ChIP™

## K-MetStat Panel

Version 1.0



# SNAP-ChIP K-MetStat Panel

Version 1.0
Catalog No. 19-1001
Store at -20°C Upon Receipt
10 ChIP Reactions

SNAP-ChIP User's Manual ver 1.0

**EpiCypher, Inc.**
801 Capitola Dr
Suite 9
Research Triangle Park, NC 27713
www.epicypher.com
Phone: 855-374-2461
Fax: 855-420-6111
Email: info@epicypher.com
Tech Support: techsupport@epicypher.com

EpiCypher™

EpiCypher™

# Table of Contents

## Description

**SNAP-ChIP** (**S**ample **N**ormalization and **A**ntibody **P**rofiling **Ch**romatin **I**mmuno-**P**recipitation) uses DNA barcoded recombinant designer nucleosomes (dNucs) as next-generation spike-ins for ChIP. The first product in this family (our K-MetStat, or lysine-methylation status panel) consists of a pool of dNucs carrying fifteen well-studied histone lysine methyl marks and an unmodified control. EpiCypher's K-MetStat panel can easily be added to any ChIP workflow without altering the protocol. However, users can monitor antibody specificity and evaluate technical variability within a ChIP experiment for the first time, setting SNAP-ChIP apart from any other spike-in controls currently available.

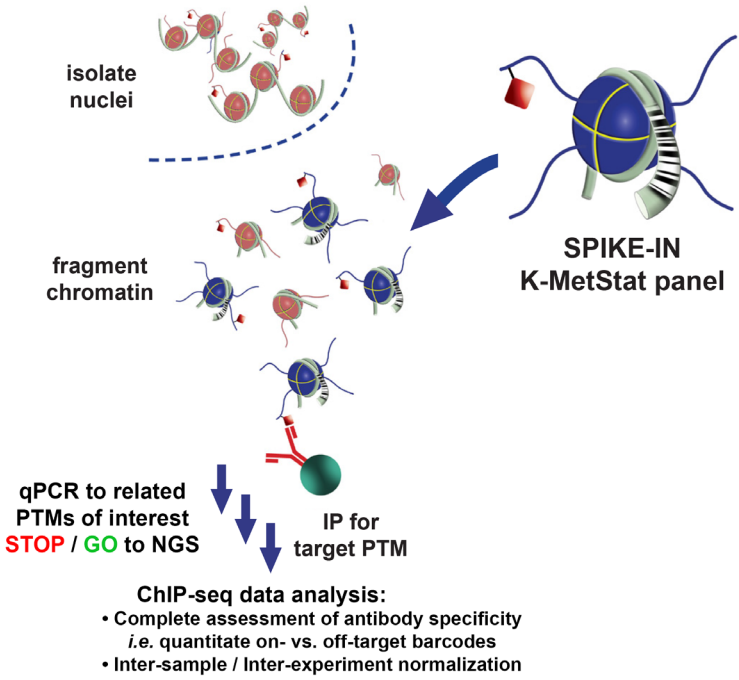



**Figure 1:** Overview of the SNAP-ChIP approach (adapted from ICe-ChIP technology: Grzybowski et al. 2015. *Mol Cell* 58: 886 - 899). A pool of recombinant dNucs with defined post-translational modifications (PTMs) identified by unique DNA barcodes is added to sample chromatin prior to immunoprecipitation (IP). Capture of the barcoded nucleosomes (on- and 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 next-generation sequencing.

EpiCypher's K-MetStat panel is comprised of sixteen uniquely modified dNucs (15 with PTMs and an unmodified control) carrying disease-relevant lysine methylation modifications on histones H3 and H4. All of the modification sites in the panel (H3K4, H3K9, H3K27, H3K36, H4K20) are represented by a nucleosome containing a distinct lysine methylation state: 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 acylation, etc) and slated for release in early 2018.

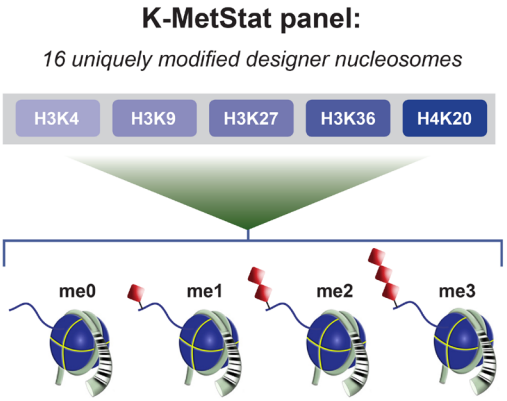



**Figure 2:** Schematic depicting the 16 dNucs included in the K-MetStat Panel, each uniquely DNA barcoded.

## SNAP-ChIP Advantages

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

## Materials Required but not Supplied

SNAP-ChIP K-MetStat panel contains enough reagent to perform 10 ChIP experiments. Some additional materials will need to be obtained in order to fully perform the SNAP-ChIP protocol.

**Note:** TaqMan™ qPCR recommended for greater sensitivity; however, you can also try SBYR green qPCR.

**Note:** Bio-Rad qPCR reagents referenced; however the user can adapt the protocol to alternate platforms.

- Standard ChIP Reagents (user specific)
- TaqMan qPCR Master Mix: e.g. Bio-Rad iTaq™ Universal Probes Supermix
- Universal SNAP-ChIP Forward Primer:
  5'- CGT ATC GCG CGC ATA ATA -3'
- Universal SNAP-ChIP TaqMan Probe (IDT Technologies):
  5'-/56-FAM/TCT AGC ACC GCT TAA ACG CAC GTA/3IABkFQ/-3'
- qPCR Plates: *e.g.* Bio-Rad HSP9601
- qPCR Machine: *e.g*. Bio-Rad CFX Connect™
- SNAP-ChIP Reverse Primers: The reverse primers are unique to each of the 16 Barcoded dNucs in the K-MetStat Panel (see **Appendix 1**). Each user has flexibility to determine which primers are most appropriate to order based on their own particular needs. At minimum, it is recommended to assess IP enrichment for the antibody target of interest as well as off-target PTMs most likely to cross react with the antibody.

## Experimental Overview

Three simple steps to incorporate SNAP-ChIP into your ChIP experiments:

1. Spike-in the SNAP-ChIP K-MetStats to your samples at the earliest appropriate step in your ChIP protocol (see **Experimental Protocol, Step 1**, on page 6).
2. After immunoprecipitation and DNA isolation, run a quantitative PCR (qPCR) reaction to assess whether your ChIP successfully enriched the target PTM. Use this information to decide whether to invest in NGS (STOP/GO capability). For example, does your anti-H3K4me3 antibody cross-react with related methyl species (H3K4me0/1/2).
3. NGS data will give further information regarding antibody specificity for all K-MetStats (H3K4/9/27/36 and H4K20), identifying additional potential cross-reactivity. If all is as expected, NGS data can be corrected for technical variability by using the SNAP-ChIP K-MetStats as a normalization standard.

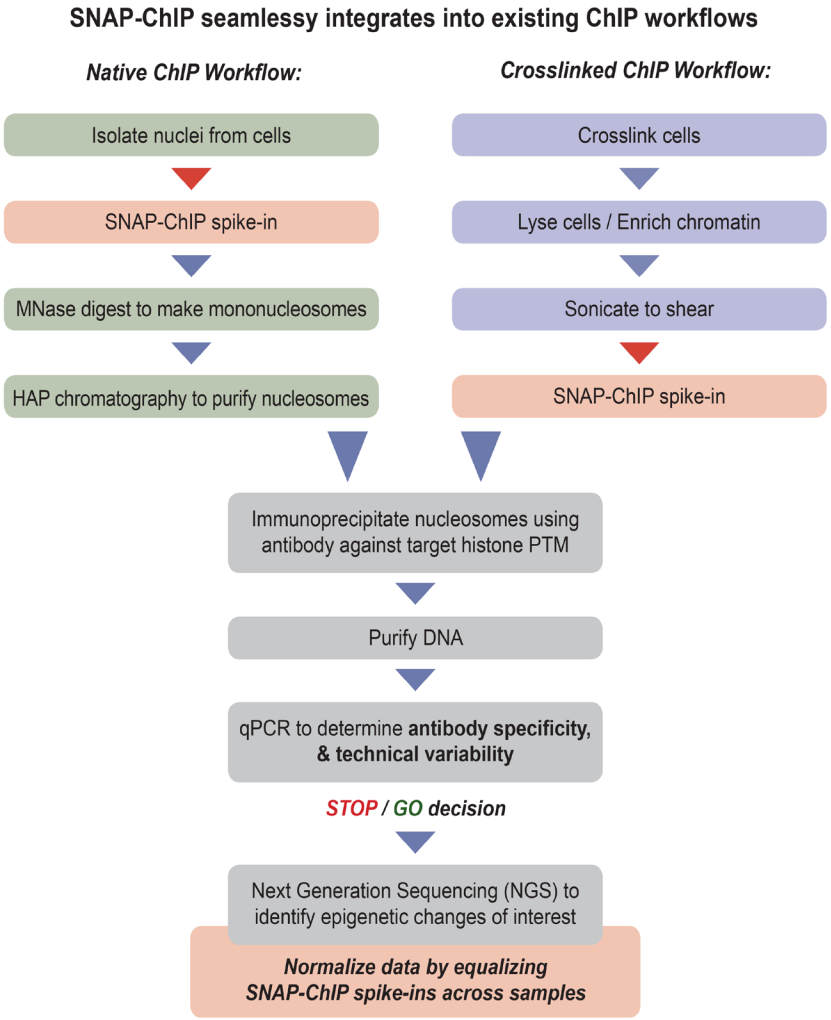



**Figure 3.** SNAP-ChIP sample ChIP workflow. SNAP-ChIP K-MetStats are compatible with both native and crosslinked ChIP protocols, where a simple spike-in of the panel enables the assessment of antibody specificity and technical variability prior to investment in NGS (STOP/GO decision). SNAP-ChIP K-MetStats can also be used to normalize experimental data, improving quantitative comparisons in qPCR or sequencing studies.

## Experimental Protocol

1. Prepare experimental samples using desired protocol, either crosslinked or native.

   **Note:** If using a crosslinked protocol, spike-in SNAP-ChIP K-MetStats post-sonication. If using a native protocol, spike in SNAP-ChIP K-MetStats prior to micrococcal nuclease digestion.

2. Spike-in 20 µL SNAP-ChIP K-MetStats (0.6 nM stock) per 100 µg sample chromatin

   **NOTE:** in a native ChIP protocol, 100 µg chromatin is sufficient for approximately 10 ChIP samples

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 amount of antibody:chromatin can be varied, but a good starting place for determining specificity is a 1:1 ratio (check the lot-specific capacity of the beads to determine saturation).

4. Purify immunoprecipitated DNA (*e.g.* Serapure, Qiagen PCR Purification Kit).
5. Run a qPCR experiment to determine antibody specificity, efficiency, and to assess technical variability:

   **qPCR Reaction mix (per 10 µL PCR reaction)**

   3.5 µL 1:10 diluted ChIP DNA or Input DNA
   5.0 µL 2X TaqMan qPCR Master Mix
   0.5 µL 20X Universal SNAP-ChIP Forward Primer (250 nM final)
   0.5 µL 20X Barcode-specific SNAP-ChIP Reverse Primer (250 nM final)
   0.5 µL 20X SNAP-ChIP TaqMan Probe (250 nM final)

6. Run qPCR using reaction conditions specified for the Master mix (*e.g.* BioRad iTaq Universal Probes Supermix = polymerase activation/DNA denaturation @ 95°C for 3 min followed by 40 cycles of denaturation @ 95°C for 5 sec, annealing/extension @ 60°C for 30 sec).
7. Analyze data using standard ΔΔCt Calculations with Input DNA as a control.

## Data Analysis: Determining Antibody Specificity

**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 their high degree of structural similarity. Refer to **Figure 4** on page 8.
- SNAP-ChIP addresses these limitations by enabling quantification of antibody specificity within every ChIP experiment:

**Due to structural similarities between methylation states at a single lysine (Kme0, Kme1, Kme2 and Kme3), an antibody could possess undesirable off-target recognition of one or more of these PTMs. For example, to test the specificity of an H3K4me3 antibody, it is recommended to run qPCR for the barcodes corresponding to H3K4me0, H3K4me1, H3K4me2, and H3K4me3 K-MetStats. If an antibody looks specific at this stage, the user can decide whether to actively check the remaining barcodes (H3K9, H3K27, H3K36, and H4K20) by qPCR, or proceed to NGS where post-IP recovery of all the barcodes is passively captured in the sequencing data.**

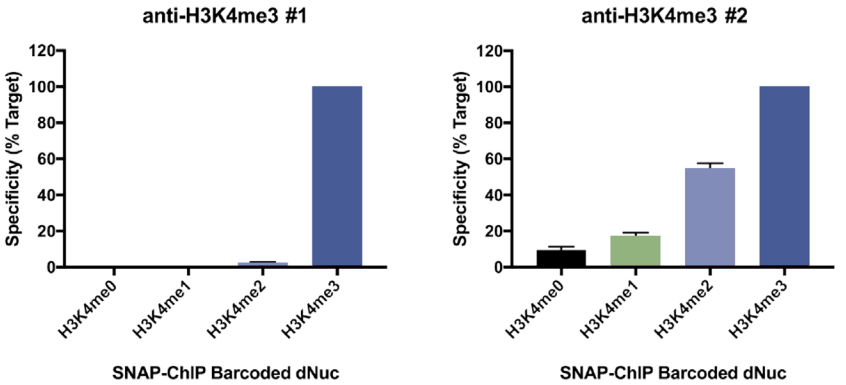



**Figure 4.** A SNAP-ChIP experiment (n = 3) using two commercially available H3K4me3 antibodies shows that Antibody #1 exhibits less than 3% cross-reactivity with alternate H3K4 methylation states. In contrast, Antibody #2 shows roughly 60% cross-reactivity with H3K4me2, substantially compromising ChIP studies.

| Ct Mean | ΔCt | ΔΔCt (relative to antibody target) | Relative Quantification (RQ) | Specificity (% Target) |
|---|---|---|---|---|
| Average of 3 replicates | = $Ct^{ChIP\ Mean}$ - $Ct^{Input\ Mean}$ | =ΔCt - ΔCt of Ab Target | $=2^{-\Delta\Delta Ct}$ | =RQ*100 |

**Table 1:** Method for determining antibody specificity. Ct = Cycle Threshold.

# Appendix 1 - qPCR Primer Sequences

| Internal Barcode ID | dNuc | TaqMan Reverse Primer |
|---|---|---|
| B058 | Unmod. (me0) | CGCGTAACGACGTACC |
| B017 | H3K4me1 | CGTTAACGCGTTTCGT |
| B079 | H3K4me2 | CGTACGTCGTGTCGAA |
| B038 | H3K4me3 | GTTCGCGACACCGTTC |
| B056 | H3K9me1 | TCGCGACGGACGTA |
| B092 | H3K9me2 | CGCATATCGCGTCGT |
| B005 | H3K9me3 | GAACGATTCGACGATCGT |
| B093 | H3K27me1 | TACGTGTCGCGCGTA |
| B032 | H3K27me2 | GAACGTTCGTCGACGAT |
| B008 | H3K27me3 | GAATCGTCGACGCGTATA |
| B022 | H3K36me1 | CGAAATTCGTATACGCGTCG |
| B034 | H3K36me2 | GTGATATCGCGTTAACGTCG |
| B077 | H3K36me3 | CGTAATGCGCGACGTTA |
| B033 | H4K20me1 | CGCGAACTATCGTCGATTC |
| B087 | H4K20me2 | CGATACGCCGATCGATC |
| B090 | H4K20me3 | CGATTCGACGGTCGC |

**Table 2.** qPCR primer sequences corresponding to the unique DNA barcodes used in the SNAP-ChIP K-MetStat Panel. These unique reverse primers for each barcode are combined with a Universal SNAP-ChIP Forward Primer and a Universal SNAP-ChIP TaqMan Probe for qPCR experiments (see **Materials Required but not Supplied** on page 3). Primer sequences are available for download at http://www.epicypher.com/k-metstat, where the Excel sheet can be edited and uploaded online for simple ordering from primer companies that support this feature (*e.g.* IDT).

## Appendix 2 - Barcode Sequences

| Internal Barcode ID | dNuc | Barcode sequence |
|---|---|---|
| B058 | Unmodified-A | TTCGCGCGTAACGACGTACCGT |
| B097 | Unmodified-B | CGCGATACGACCGCGTTACGCG |
| B017 | H3K4me1-A | CGACGTTAACGCGTTTCGTACG |
| B096 | H3K4me1-B | CGCGACTATCGCGCGTAACGCG |
| B079 | H3K4me2-A | CCGTACGTCGTGTCGAACGACG |
| B019 | H3K4me2-B | CGATACGCGTTGGTACGCGTAA |
| B038 | H3K4me3-A | TAGTTCGCGACACCGTTCGTCG |
| B041 | H3K4me3-B | TCGACGCGTAAACGGTACGTCG |
| B056 | H3K9me1-A | TTATCGCGTCGCGACGGACGTA |
| B021 | H3K9me1-B | CGATCGTACGATAGCGTACCGA |
| B092 | H3K9me2-A | CGCATATCGCGTCGTACGACCG |
| B067 | H3K9me2-B | ACGTTCGACCGCGGTCGTACGA |
| B005 | H3K9me3-A | ACGATTCGACGATCGTCGACGA |
| B088 | H3K9me3-B | CGATAGTCGCGTCGCACGATCG |
| B093 | H3K27me1-A | CGCCGATTACGTGTCGCGCGTA |
| B070 | H3K27me1-B | ATCGTACCGCGCGTATCGGTCG |
| B032 | H3K27me2-A | CGTTCGAACGTTCGTCGACGAT |
| B044 | H3K27me2-B | TCGCGATTACGATGTCGCGCGA |
| B008 | H3K27me3-A | ACGCGAATCGTCGACGCGTATA |
| B025 | H3K27me3-B | CGCGATATCACTCGACGCGATA |
| B022 | H3K36me1-A | CGCGAAATTCGTATACGCGTCG |
| B100 | H3K36me1-B | CGCGATCGGTATCGGTACGCGC |
| B034 | H3K36me2-A | GTGATATCGCGTTAACGTCGCG |
| B039 | H3K36me2-B | TATCGCGCGAAACGACCGTTCG |
| B077 | H3K36me3-A | CCGCGCGTAATGCGCGACGTTA |
| B075 | H3K36me3-B | CCGCGATACGACTCGTTCGTCG |
| B033 | H4K20me1-A | GTCGCGAACTATCGTCGATTCG |
| B078 | H4K20me1-B | CCGCGCGTATAGTCCGAGCGTA |
| B087 | H4K20me2-A | CGATACGCCGATCGATCGTCGG |
| B076 | H4K20me2-B | CCGCGCGATAAGACGCGTAACG |
| B090 | H4K20me3-A | CGATTCGACGGTCGCGACCGTA |
| B061 | H4K20me3-B | TTTCGACGCGTCGATTCGGCGA |

**Table 3** (facing page). DNA sequences corresponding to the barcodes used in the SNAP-ChIP K-MetStat Panel. Each PTM is wrapped with two distinct barcoded DNAs, sequence A and sequence B. Each SNAP-ChIP spike-in can be used to generate internal technical replicate data sets for each PTM, such as to assess antibody performance and provide experimental normalization. Barcode sequences are available for download at http://www.epicypher.com/k-metstat.

## Appendix 3 - FAQ

- **Will the barcodes overlap with genomic DNA sequences from my samples?**

SNAP-ChIP barcodes are designed to be compatible with multiple species (*e.g.* human, mouse, fly, and yeast) such that their genomic DNA can be readily distinguished without issue.

- **Why would I use a native ChIP-protocol with micrococcal nuclease (MNase) digestion vs. crosslinking/sonication?**

SNAP-ChIP is directly compatible with both approaches though native ChIP is recommended. Crosslinking can impact antibody specificity and efficiency because crosslinked chromatin becomes more sticky and susceptible to epitope masking. Signal-to-noise ratios are often decreased in crosslinked samples compared to native ChIP.

In contrast, a native nuclei preparation that is micrococcal nuclease digested to yield >95% pure mononucleosomes will yield samples that look nearly identical to the SNAP-ChIP spike-ins (*i.e.* recombinant mononucleosomes). As a result, data obtained from the SNAP-ChIP controls will be most representative of the experimental samples.

- **Are there any guidelines for how to run the sequencing?**

Paired end sequencing is recommended so you can eliminate any dinucleosome data in your sample. Dinucleosomes are immunoprecipitated at least 7 times more efficiently than mononucleosomes (Grzybowski et al, 2015 Mol Cell 58:886), so they will be overrepresented in the sequencing data. This bias can be mitigated by using paired end sequencing and excluding fragments greater than 220 bp in size from analysis.

- **Will the spike-ins affect the required sequencing depth?**

The K-MetStat set represents much less than 1% of the total nucleosomes in the sample so sequencing depth is unaffected.

- **Can you recommend specific histone PTM antibodies?**

  EpiCypher and our collaborators have performed extensive antibody specificity studies for H3K4 methylation, with additional PTM studies ongoing. For more information, inquire at info@epicypher.com.