EpiCypher®

Bringing Epigenetics to Life

SNAP-ChIP®
Spike-in Controls
for Quantitative ChIP

Sample **N**ormalization & **A**ntibody **P**rofiling for **ChIP**

SNAP-ChIP® is a multi-use spike-in control for chromatin immunoprecipitation (ChIP) that uses DNA-barcoded recombinant designer nucleosomes (dNucs) for assay quantification and antibody validation.
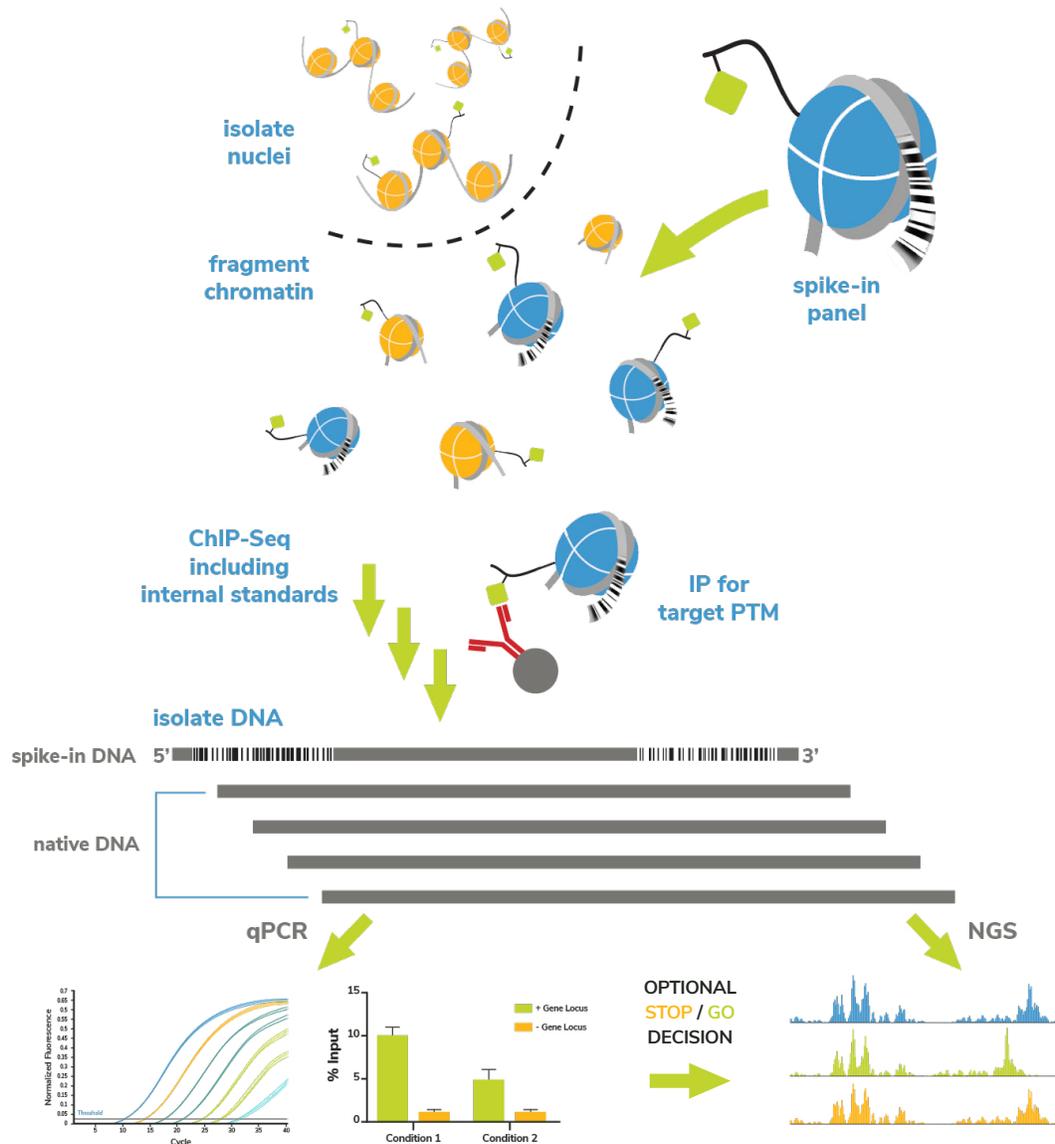
SNAP-ChIP can easily be added to any ChIP workflow.

Advantages

- Determine antibody specificity and target pulldown efficiency
- Monitor experimental variability
- Quantitative recovery of DNA barcodes (via qPCR) provides useful **STOP / GO** capability before advancing to **NGS**
- Sample normalization for reliable cross-sample comparisons
- Homogenous, fully defined dNucs are subjected to rigorous quality control for lot-to-lot consistency

FIGURE 1

Overview of the SNAP-ChIP® approach: 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 experimental variability, and normalize experiments. Quantitative recovery of barcoded dNucs (via qPCR) provides a useful STOP / GO capability prior to advancing to next-generation sequencing.

SNAP-ChIP spike-ins are adapted from ICeChIP technology (Shah et al. Mol Cell, Vol. 72, Issue 1, 162-177, 2018 and Grzybowski et al. Mol Cell, Vol.58 , Issue 5 , 886 - 899, 2015)

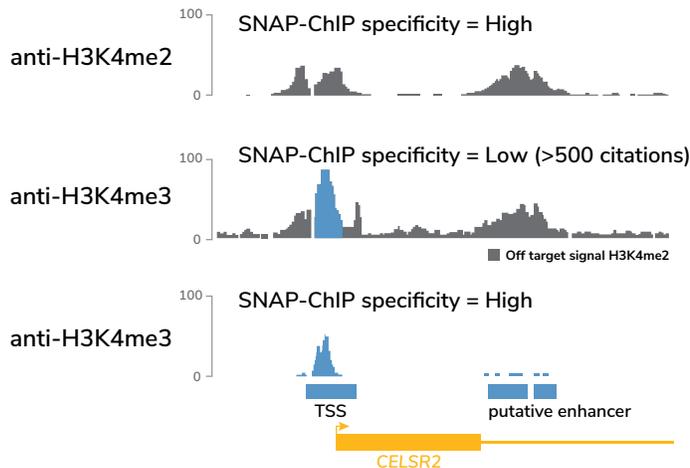Sample Normalization & Antibody Profiling for ChIP

Why should I worry about antibody specificity?

As published in Shah et *al., Mol Cell* 2018, we tested the performance of 54 "ChIP-grade" commercial antibodies to H3K4 methyl states using both peptide array and SNAP-ChIP®. This study establishes SNAP-ChIP as the new gold standard for ChIP antibody validation.

Here is what we found...

- Peptide arrays fail to predict antibody specificity in ChIP

- SNAP-ChIP specificity predicts ChIP-seq peak profiles

- Most commonly used H3K4me3 antibodies (including ENCODE recommended antibodies) are highly cross-reactive to H3K4me2 in SNAP-ChIP

- Use SNAP-ChIP to validate antibody specificity and monitor antibody performance when it matters ⟶ IN YOUR EXPERIMENT

Don't let non-specific antibodies compromise your research

anti-H3K4me2

SNAP-ChIP specificity = High

anti-H3K4me3

SNAP-ChIP specificity = Low (>500 citations)

■ Off target signal H3K4me2

anti-H3K4me3

SNAP-ChIP specificity = High

TSS   putative enhancer

CELSR2

- **Antibody specificity matters.** Figure compares ChIP tracks using H3K4me3 antibodies with low (center) or high (bottom) specificity. A highly specific H3K4me2 antibody is shown for reference (top).

- **When using a low specificity antibody,** genomic areas reported as containing H3K4me3 are actually a result of a contaminating H3K4me2 signal (gray).

- **Use SNAP-ChIP to validate your antibody and control your ChIP experiments**

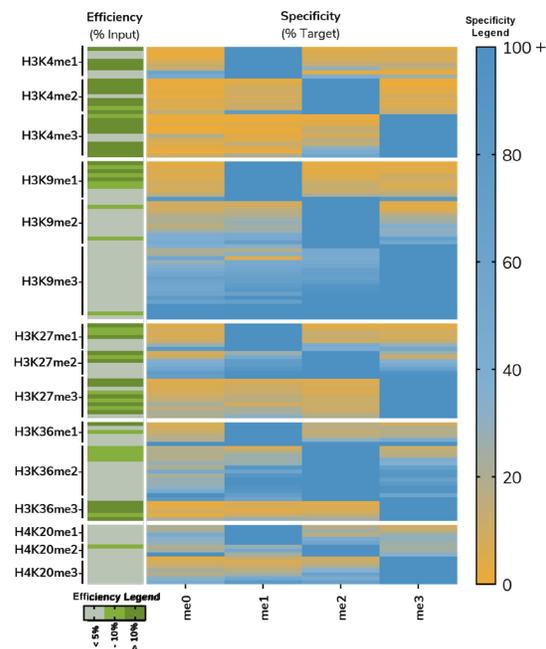# Do you really know what you are pulling down in your chip?

Efficiency (% Input)   Specificity (% Target)   Specificity Legend

H3K4me1
H3K4me2
H3K4me3
H3K9me1
H3K9me2
H3K9me3
H3K27me1
H3K27me2
H3K27me3
H3K36me1
H3K36me2
H3K36me3
H4K20me1
H4K20me2
H4K20me3

me0   me1   me2   me3

Efficiency Legend
<5%   5 - 10%   > 10%

FIGURE 2

Specificity survey of commercially available antibodies using EpiCypher's SNAP-ChIP K-MetStat panel (Cat. No. 19-1001).

- *While many reagents are not fit-for-purpose, highly specific and efficient antibodies exist and are for the first time identifiable as such using SNAP-ChIP*

- *Driven to deliver the best reagents to the field, EpiCypher has screened **hundreds of antibodies** to identify antibodies that are truly "ChIP-grade".*

- *With EpiCypher's SNAP-ChIP certified antibodies you no longer need to second guess the performance of your antibody.*

Sample Normalization & Antibody Profiling for ChIP

SNAP-ChIP® seamlessly integrates into existing ChIP workflows.
Just add SNAP-ChIP® to your protocol.

Native ChIP Workflow

Isolate nuclei from cells

SNAP-ChIP spike-in

MNase digest to make mononucleosomes

HAP chromatography to purify nucleosomes

Cross-linked 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

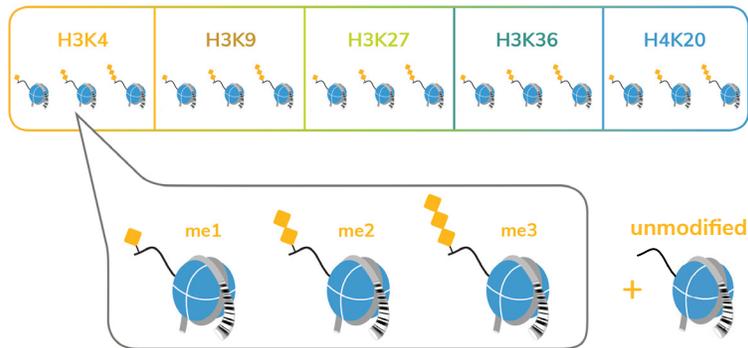Next Generation Sequencing (NGS)

Quantitative sample normalization

Use barcodes to confirm antibody specificity profile

SNAP ChIP® spike-in panels

SNAP-ChIP® spike-in panels are composed of a pool of uniquely modified DNA-barcoded dNucs carrying disease-relevant modifications.
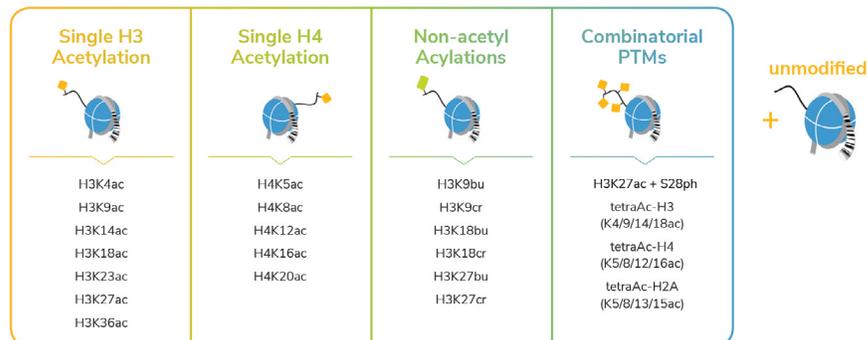
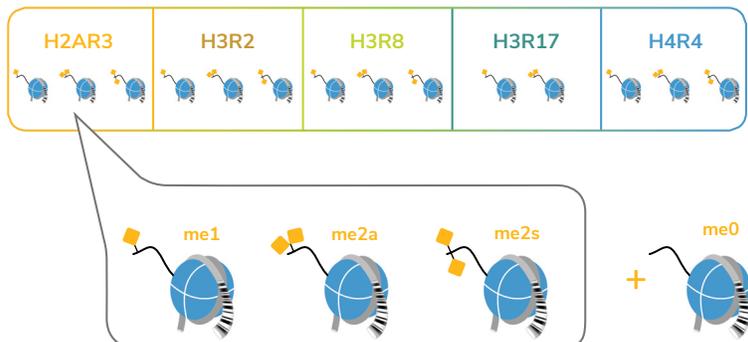## Pick your favorite panel

K-MetStat Panel *(Catalog No. 19-1001)*

H3K4	H3K9	H3K27	H3K36	H4K20

me1   me2   me3   unmodified

+

OncoStat Panel *(Catalog No. 19-2001)*

H3.3	H3.3K4M	H3.3K9M	H3.3K27M	H3.3G34R	H3.3G34V	H3.3G34W	H3.3K36M

K-AcylStat Panel *(Catalog No. 19-3001)*

Single H3 Acetylation	Single H4 Acetylation	Non-acetyl Acylations	Combinatorial PTMs	unmodified
H3K4ac	H4K5ac	H3K9bu	H3K27ac + S28ph	+
H3K9ac	H4K8ac	H3K9cr	tetraAc-H3 (K4/9/14/18ac)	
H3K14ac	H4K12ac	H3K18bu	tetraAc-H4 (K5/8/12/16ac)	
H3K18ac	H4K16ac	H3K18cr	tetraAc-H2A (K5/8/13/15ac)	
H3K23ac	H4K20ac	H3K27bu		
H3K27ac		H3K27cr		
H3K36ac				

R-MetStat Panel *(Catalog No. 19-4001)* Coming Soon

H2AR3	H3R2	H3R8	H3R17	H4R4

me1   me2a   me2s   me0

+

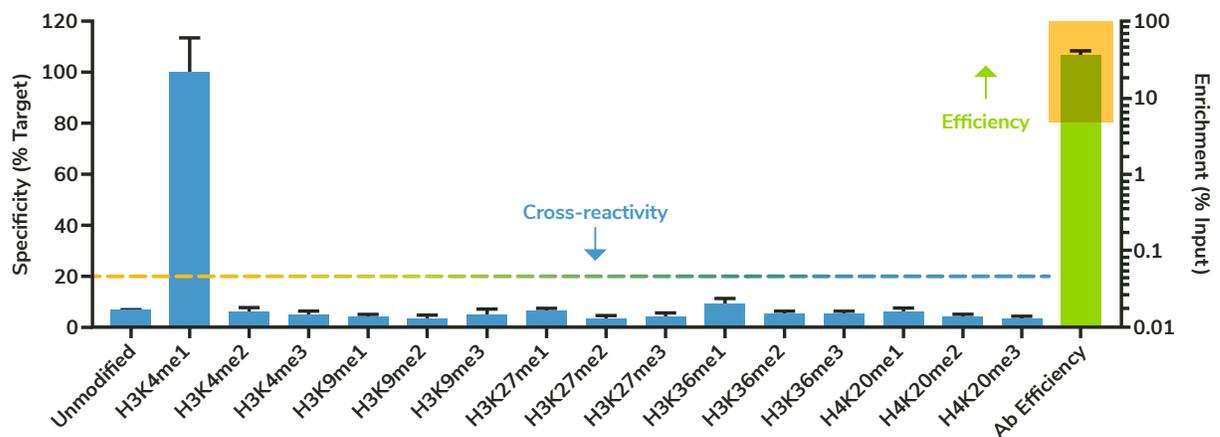**SNAP-ChIP® Certified Antibodies**

EpiCypher has embarked on a massive effort to identify the highest quality ChIP-certified antibodies using our proprietary SNAP-ChIP® technology.

SNAP-ChIP® certified antibodies set a new higher standard for antibody performance.

We have screened **hundreds of antibodies** using SNAP-ChIP so you don't have to. SNAP-ChIP certified antibodies are the highest quality available -- Don't let faulty antibodies compromise your research.

What is a SNAP-ChIP Certified Antibody?

ChIP Metric	Definition	Criteria	Significance
Specificity	Percentage of off-target immunoprecipitation relative the the on-target PTM	<20% cross-reactivity	Have confidence that ChIP signal is specific for your target
Efficiency	Percentage of PTM recovered after immunoprecipitation relative to input	>5% enrichment	High IP efficiency generates greater Signal-to-Noise
Relative PTM Abundance	SNAP-ChIP spike-in controls are corrected for any differences in loading, whereas PTMs in experimental samples vary relative to each other (e.g. see Peach et. al., Mol. Cell. Proteomics 2012).	Antibodies to low abundance PTMs tolerate less cross-reactivity compared to high abundance PTMs	Providing highest confidence in your ChIP data

SNAP-ChIP® Certified Antibodies

Name	Catalog No.	Size	Name	Catalog No.	Size
H3K4ac	13-0034	100 µg	**H3K36ac**	13-0035	100 µg
H3K4me1	13-0026	100 µg	**H3K36me3**	13-0031	100 µg
H3K4me2	13-0027	100 µg	**H4K8ac**	13-0036	100 µg
H3K4me3	13-0028	100 µg	**H4K12ac**	13-0037	100 µg
H3K9ac	13-0033	100 µg	**H4K20ac**	13-0039	100 µg
H3K9me1	13-0029	100 µg			

For more information, visit **www.epicypher.com/snap-chip-abs/**
Don't see your PTM of interest? **Contact us at info@epicypher.com**

Spike-in control to track ChIP experimental variability

# SNAP-ChIP® spike-in controls for reliable sample normalization

The use of exogenous chromatin (*e.g. Drosophila*) as spike-in controls has been adopted for ChIP sample normalization. However, these reagents are poorly defined (*i.e.* contain unknown PTM levels) and highly variable from batch-to-batch, limiting their use for consistent sample normalization.

SNAP-ChIP® spike-ins are homogeneous and fully defined, making them the ideal tool for generating reliable ChIP data. By including in your ChIP experiments, SNAP-ChIP can be used to monitor experimental variation and normalize samples for reliable cross-sample comparisons. **Get results you can trust with SNAP-ChIP.**
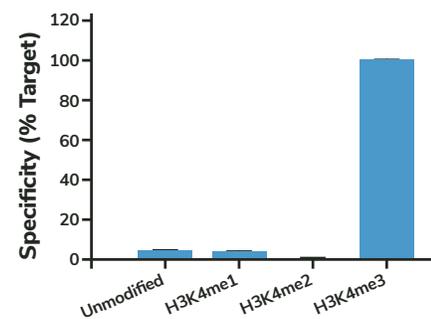
1

Check antibody specificity using SNAP-ChIP® Spike-ins:
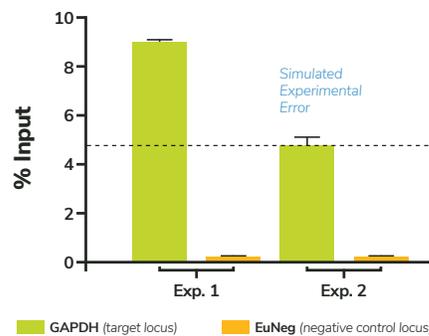
Experiment #1
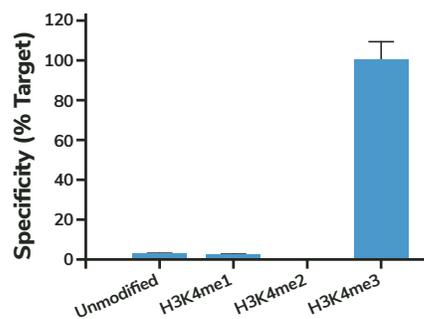
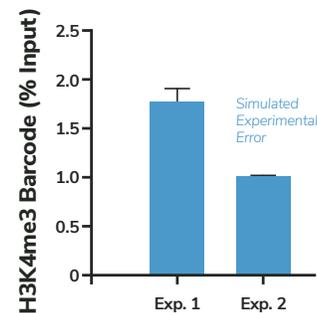**Experiment #2 (50% bead loss)**
Simulated Experimental Error

2

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

Gene Loci qPCR

■ **GAPDH** (*target locus*)   ■ **EuNeg** (*negative control locus*)
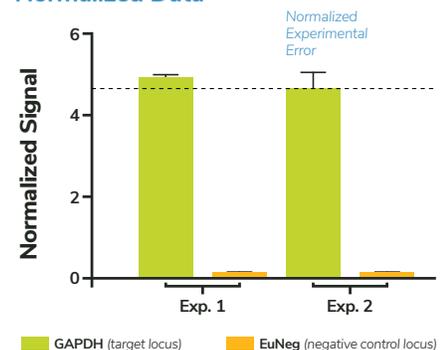
SNAP-ChIP® Spike-in qPCR

3

Normalize gene loci using a simple equation:

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

Normalized Data

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

Ordering Information

SNAP-ChIP Spike-in Panels

K-MetStat Panel
19-1001	10 ChIP reactions	$365
19-1100	100 ChIP reactions	$2799

OncoStat Panel
19-2001	10 ChIP reactions	$365
19-2100	100 ChIP reactions	$2799

K-AcylStat Panel
19-3001	10 ChIP reactions	$365
19-3100	100 ChIP reactions	$2799

Website: EpiCypher.com/SNAP-ChIP

SNAP-ChIP Primer Sets and Probe

SNAP-ChIP Dual Labeled Hydrolysis Probe
18-6001	100 reactions	$95.00
18-6005	500 reactions	$395.00

SNAP-ChIP K-MetStat Full Panel Primer Set
18-6101	100 reactions	$195.00

SNAP-ChIP K-MetStat Mini Panel Primer Set
H3K4	18-6102	100 reactions	$95.00
H3K9	18-6103	100 reactions	$95.00
H3K27	18-6104	100 reactions	$95.00
H3K36	18-6105	100 reactions	$95.00
H4K20	18-6106	100 reactions	$95.00

SNAP-ChIP OncoStat Full Panel Primer Set
18-6201	100 reactions	$195.00

SNAP-ChIP Certified Antibodies

H3K4ac
13-0034	100 µg	$405.00

H3K4me1
13-0026	100 µg	$405.00

H3K4me2
13-0027	100 µg	$405.00

H3K4me3
13-0028	100 µg	$405.00

H3K9ac
13-0033	100 µg	$405.00

H3K9me1
13-0029	100 µg	$405.00

H3K36ac
13-0035	100 µg	$405.00

H3K36me3
13-0031	100 µg	$405.00

H4K8ac
13-0036	100 µg	$405.00

H4K12ac
13-0037	100 µg	$405.00

H4K20ac
13-0039	100 µg	$405.00

Website: EpiCypher.com/snap-chip-abs/

EpiCypher®
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