Novel nucleosome-based methods for rapid screening and identification of best-in-class antibodies: a community resource to improve genomic mapping approaches

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Background

Genomic mapping studies are critically reliant on the ability of antibodies to distinguish related histone post-translational modifications (PTMs). However inadequate validation of these reagents is widely recognized as contributing to the reproducibility crisis in biomedical research. To address this, we created DNA-barcoded semi-synthetic / fully-defined nucleosomes (Nucs; with histone PTMs or variants) as spike-in controls for **Sample Normalization and Antibody Profiling in Chromatin ImmunoPrecipitation (SNAP-ChIP®)**. These Nucs were assembled into rational panel sets for widely studied target classes (*e.g.* lysine methylation), and spiked into a ChIP experiment prior to immunoprecipitation. This provides a defined *in situ* metric of antibody capability and target enrichment. In parallel, we developed a high-throughput, multiplexed triage approach that assembles biotinylated Nucs from similar PTM / variant panels onto optically-barcoded Luminex beads to "decipher" antibody binding preference in a physiological context. In this **dCypher™ Luminex** approach these panels can be washed after antibody incubation under conditions representing a particular genomic approach (*e.g.* high stringency ChIP buffers). Of note we now have sufficient Nuc diversity to perform detailed 'motif walking' on a histone tail. As an example an antibody targeted to A31 that distinguishes histone H3.1/ 2 from H3.3 (S31) is unable to bind its epitope in the context of K27-me1-me2-me3 or S28phos (together found on >80% of *in vivo* nucleosomes$_4$). Such characterization will be essential to interpret results with these reagents.

Improved screening methods are needed to predict antibody behavior in ChIP

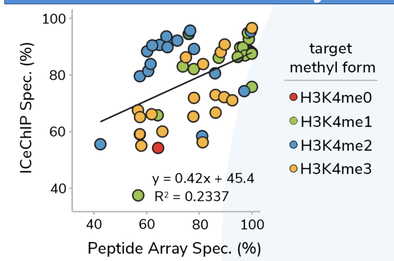
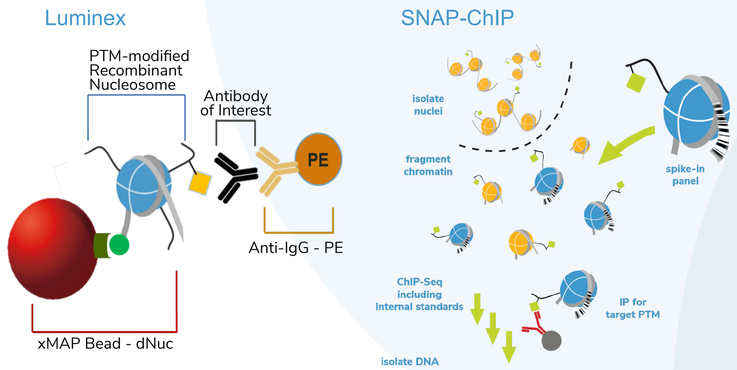


Figure 1. A comprehensive study$_2$ of 54 commercially available ChIP grade antibodies to H3K4 methyl states identified reagents with varying degrees of capability in peptide arrays and ChIP. Of note, antibody cross-reactivity in peptide array and ChIP were not correlated (R^2 = 0.2337; N=54). Percent specificity was calculated for each antibody as enrichment of target H3K4 methylation state normalized to the sum of enrichments of all H3K4 methylation states in ICe-ChIP (y-axis) and peptide arrays (x-axis), ICe-ChIP$_1$ is directly analogous to SNAP-ChIP.

Development of a novel nucleosome-based approach to rapidly screen for ChIP capable antibodies



- Multiplex assay
- High throughput wash platform
- High stringent ChIP washes
- Enables assessment of antibody specificity

- Spike-in controls enable assessment of antibody specificity
- Monitor technical variability and normalize experiments
- STOP/GO capability before advancing to NGS
- Homogenous, defined controls for lot-to-lot consistency

Figure 2. Graphical representation of EpiCypher Luminex (left) and SNAP-ChIP (right) approaches. Luminex is used to screen for antibodies with potential for use in ChIP.

Optimization of dCypher Luminex antibody screening approach

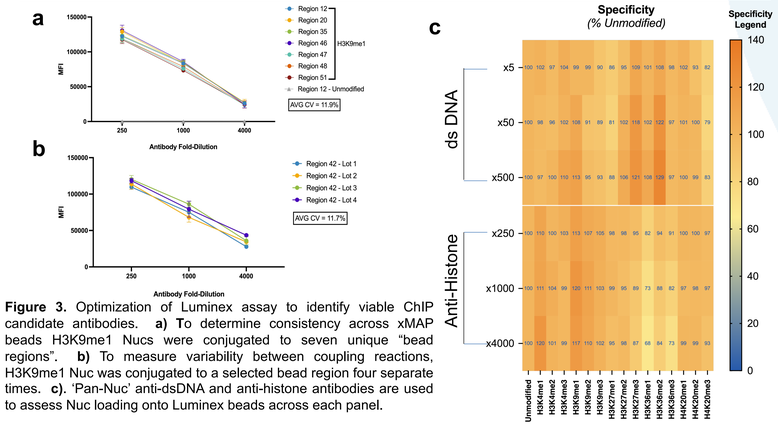


Figure 3. Optimization of Luminex assay to identify viable ChIP candidate antibodies. **a)** To determine consistency across xMAP beads H3K9me1 Nucs were conjugated to seven unique "bead regions". **b)** To measure variability between coupling reactions, H3K9me1 Nuc was conjugated to a selected bead region four separate times. **c).** 'Pan-Nuc' anti-dsDNA and anti-histone antibodies are used to assess Nuc loading onto Luminex beads across each panel.

Comparison between EpiCypher dCypher Luminex and SNAP-ChIP antibody screening

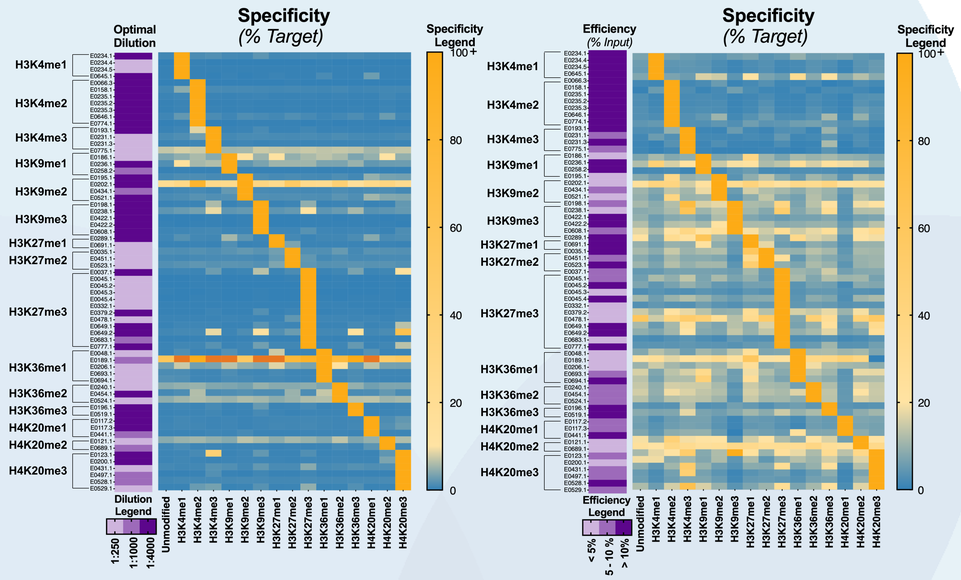


Figure 4. Nucleosome-based multiplexed Luminex analyses (Left) have high predictive validity for ChIP (Right). Dataset is 65 commercial reagents to lysine methyl states tested to K-MetStat panel [me0-1-2-3 for H3K4, H3K9, H3K27, H3K36 and H4K20]).

dCypher-Luminex prediction accuracy of ChIP-specificity is related to antibody efficiency

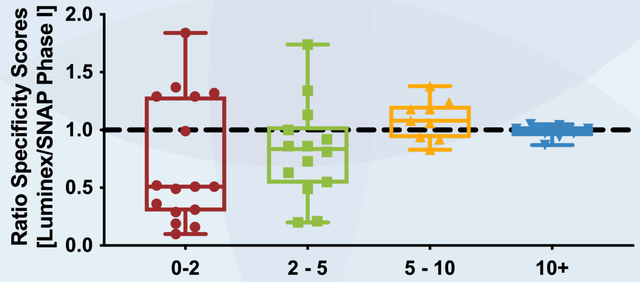


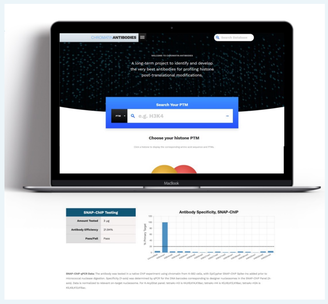
Figure 5. Luminex is more accurate at predicting ChIP specificity as the ChIP efficiency score (% spike-in recovery) increases. Data are a ratio of Specificity Scores: Luminex Full Panel *vs.* SNAP-ChIP Phase I (all methyl states at a target residue). Abs are binned by SNAP-ChIP Efficiency (**NOTE:** EpiCypher Pass/ Fail metric for ChIP is <20% cross reactivity / **>5% efficiency**).

dCypher Luminex / SNAP-ChIP screening have identified Fit-for-Purpose ChIP antibodies to widely studied Histone PTMs

a

SNAP-ChIP Panel	Lysine Methylation	Lysine Acylation	Total
Total Tested	263	129	392
Failure Rate	74.5%	64.5%	71.2%
$ Spent	$105,200	$51,600	$156,800
$ Wasted	$78,374	$33,282	$111,656
Top Cited: Citations w/"ChIP"	3,720 (N=18)	1,031 (N=6)	4,751 (N=24)
Top Cited: Failure Rate	74.5%	64.5%	79.2%

b



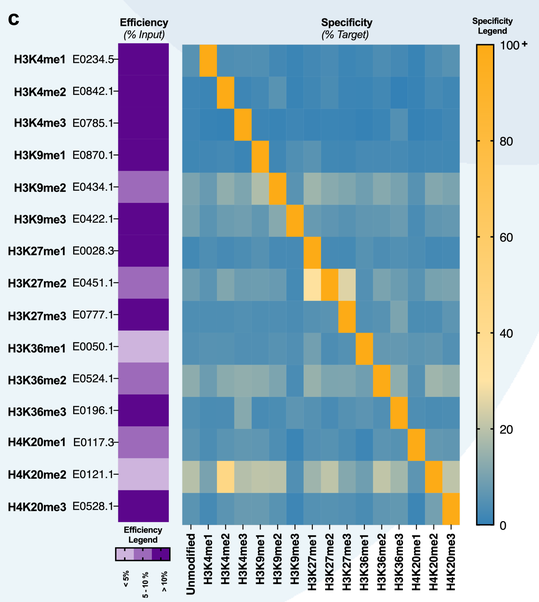
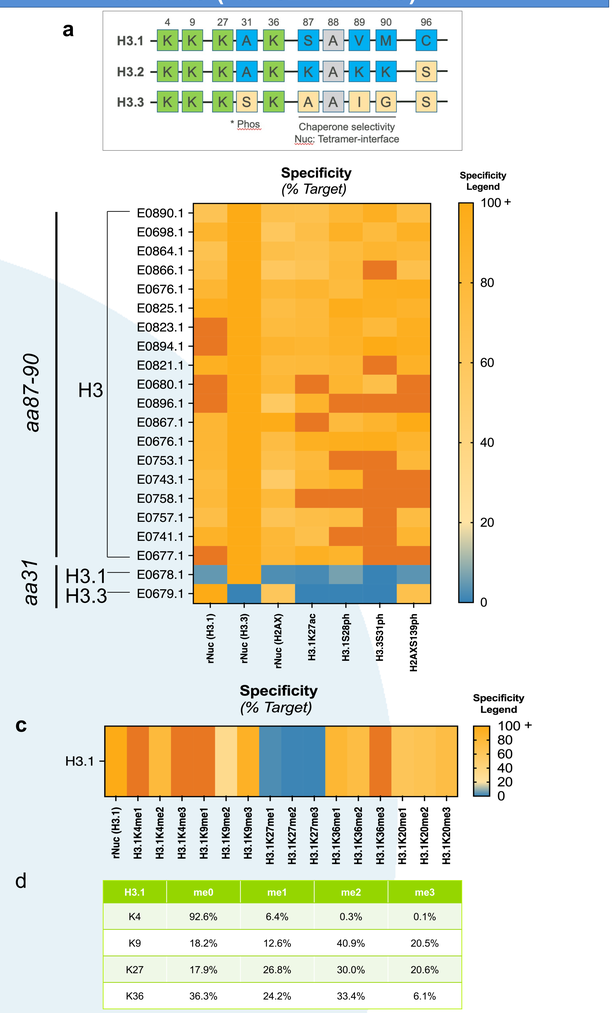


Figure 6. a) Testing of 400+ antibodies shows that >70% of commercial reagents are not fit for purpose (>20% cross-reactivity / <5% recovery). **b)** All data in this **(ongoing)** study can be found at chromatinantibodies.com. **c)** Specific antibodies do exist; heat map shows 15 best-in-class commercial reagents to the various PTM states in K-MetStat (Kme0-1-2-3 at H3K4, H3K9, H3K27, H3K36 and H4K20).

dCypher Luminex screening antibodies to histone variants (H3.1/2 *vs.* H3.3)



d

H3.1	me0	me1	me2	me3
K4	92.6%	6.4%	0.3%	0.1%
K9	18.2%	12.6%	40.9%	20.5%
K27	17.9%	26.8%	30.0%	20.6%
K36	36.3%	24.2%	33.4%	6.1%

Figure 7. a) H3.1 and H3.3 differ at only four amino acids, with three of these at the chaperone binding / H3-dimer interface. Most commercial reagents to distinguish the histones are to this location (with only one mAb pair focused on A31S). **b)** In Luminex testing to Nucs most Abs lack the capability to distinguish H3.1 and H3.3 (except mAb pair to the aa31 region). **c)** mAb to H3.1_A31 is unable to bind if H3.1 is methylated at K27me (1-2-3) (or S28ph) indicating that epitope spans H3 aa27 to aa31. **d)** Abundance of H3K27 methyl states *in vivo* (PTM-MS data).

Conclusions

- Peptide arrays do not predict antibody behavior in ChIP, leading to an abundance of widely used reagents that have generated compromised datasets
- dCypher Luminex is a groundbreaking, high-throughput approach to predict antibody capability for ChIP, especially with high efficiency antibodies
- dCypher Luminex antibody screening assay has undergone rigorous quality control experiments
- In testing of >400 commercially available antibodies (to Kme and Kacyl) >70% are unfit for purpose (>20% cross-reactivity / <5% efficiency)
- Our SNAP-ChIP antibody screening has identified capable antibodies for many widely studied PTM states: chromatinantibodies.com
- More screening panels are in development (including histone variants and phosphorylation)
- Our Nuc diversity now supports 'motif walking' to determine the *in vivo* combinatorial landscape that might compromise an antibody

References

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