invitrogen

SNAP-ChIP™: A novel platform for ChIP standardization and antibody development

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ABSTRACT

Histones are decorated by post translational modifications (PTMs) that serve as epigenetic signatures for gene expression. Chromatin immunoprecipitation (ChIP) is a common application to examine these PTMs at individual loci and globally to guide our understanding of many biological processes. Researchers have become increasingly concerned by the precise specificity of histone PTM antibodies, especially in ChIP applications. Peptide arrays have traditionally been the gold standard for determining the capability of these reagents, but screening by array is most analogous to a denaturing western blot. Increasing evidence suggests that the array does not recapitulate antibody performance in a ChIP experiment, due (in part) to the requirement for the antibody to recognize the modified histone in a nucleosome context. EpiCypher Inc. has developed a method (SNAP-ChIP™ : **S**ample **N**ormalization and **A**ntibody **P**rofiling) for normalizing ChIP experiments that can be further employed to rigorously test antibody specificity in this experimental approach. Spiking-in a panel of recombinant semi-synthetic modified nucleosomes allows one to determine if an antibody is enriching the target of interest compared to other histone PTMs (i.e. off-target) in the panel. A pilot SNAP-ChIP study (to H3K4) has identified a group of Invitrogen antibodies with incredible specificity for their target PTM in ChIP (Shah et al, Submitted). We are currently expanding this endeavor to all 15 lysine methylation states included in the current panel (SNAP-ChIP - K-MetStat : Figure 1). The goal is to develop a comprehensive antibody portfolio comprising at least one SNAP-ChIP validated antibody for each represented histone PTM. SNAP-ChIP provides a unique validation approach and represents the strongest evidence to date for the capability of histone PTM antibodies in ChIP.

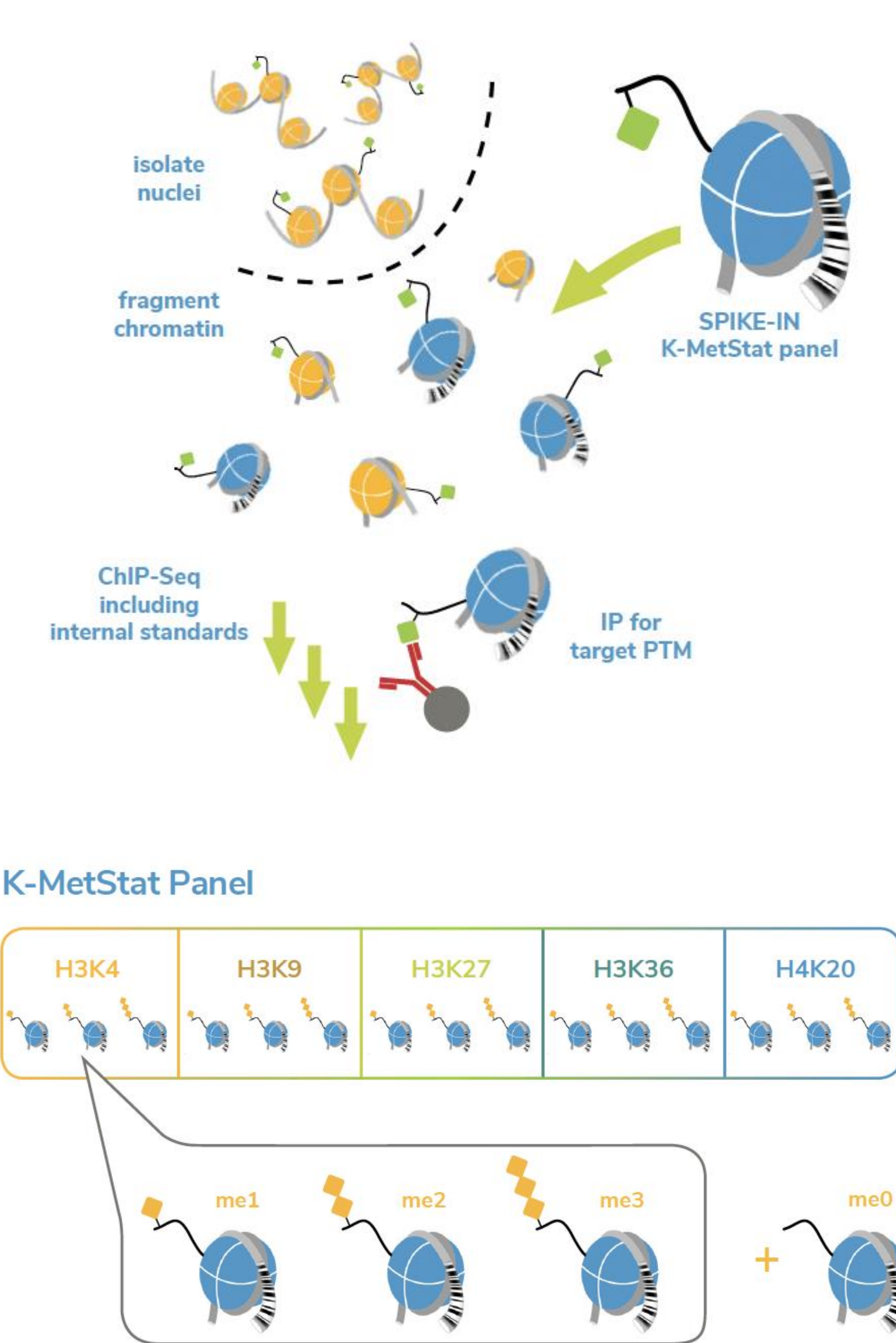
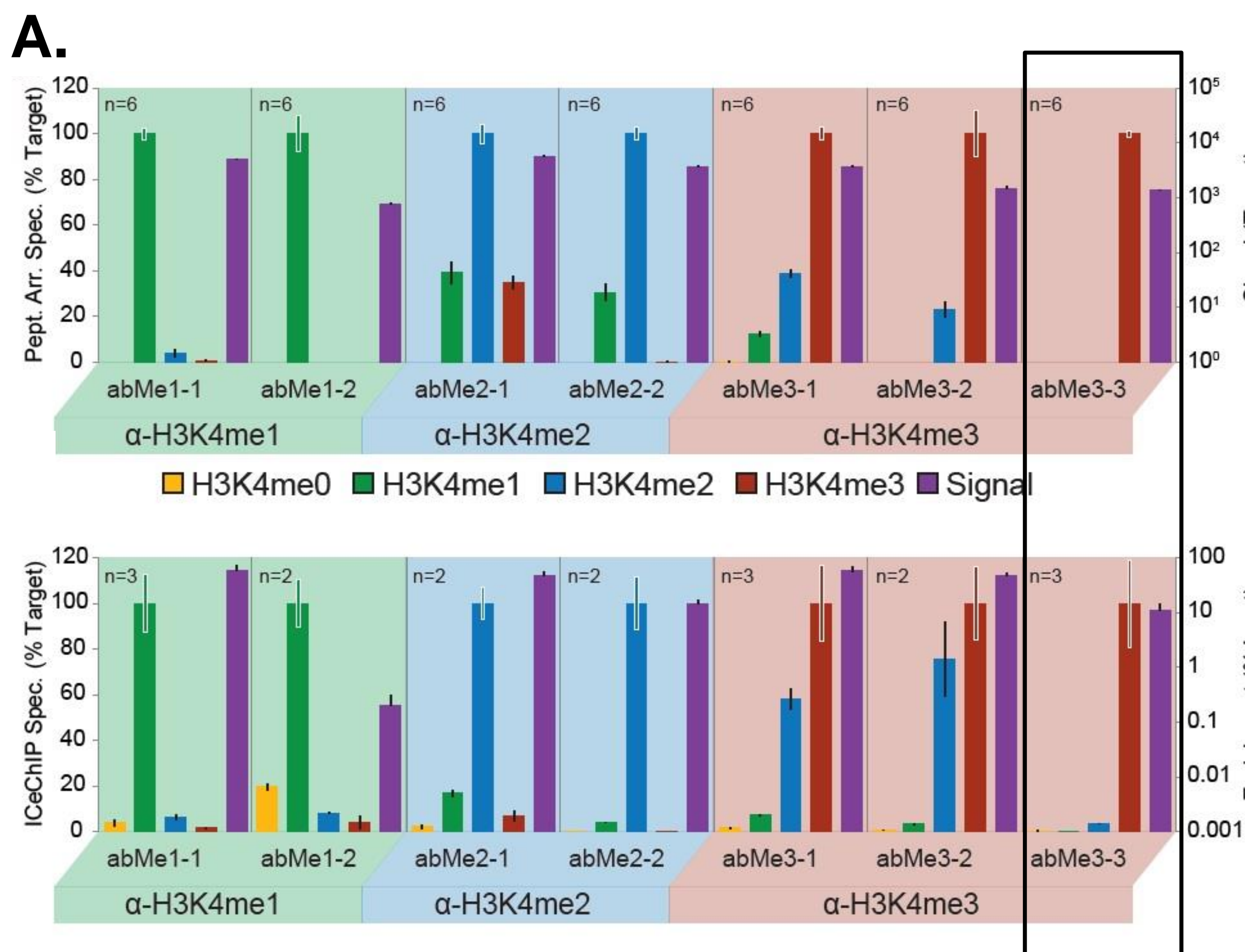


Figure 1. SNAP-ChIP™ K-MetStat™.
Approach spikes in panels of post-translationally modified semi-synthetic nucleosomes during the normal ChIP workflow. As these nucleosomes are individually barcoded, they can later be quantified to determine how much of each PTM is immunoprecipitated in a ChIP reaction. The full K-MetStat panel includes H3K4, H3K9, H3K27, H3K36 and H4K20 in unmethylated and [mono-, di- and tri-] methyl forms. ICe-ChIP (Grzybowski et al, 2015) is similar to SNAP-ChIP but includes multiple concentrations of each barcoded modification to calibrate ChIP experiments.

RESULTS



B.

y = 0.42x + 45.4
R^2 = 0.2337
ICeChIP Spec. (%)
Pept. Array Spec. (%)

Figure 2. Peptide array and ChIP specificity do not correlate. A. Peptide array (top) and ICe-ChIP (bottom) specificity / Ab efficiency for representative H3K4 antibodies. abMe3-3 is Invitrogen antibody PA5-40086 (highlighted in black box). **B.** Specificity of 52 H3K4 antibodies in peptide array and ICe-ChIP are not correlated. Figure adapted from Shah *et al.*

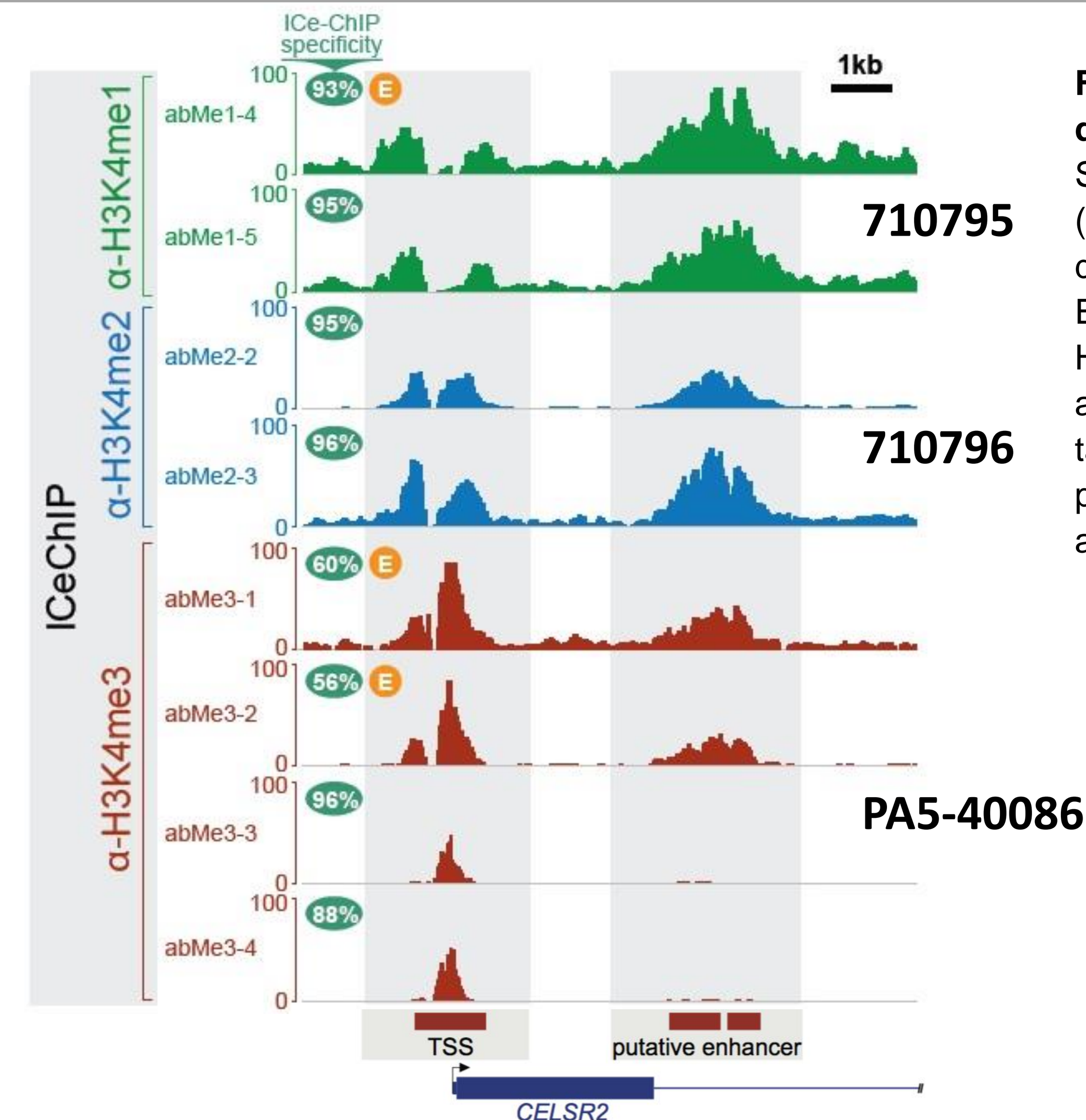
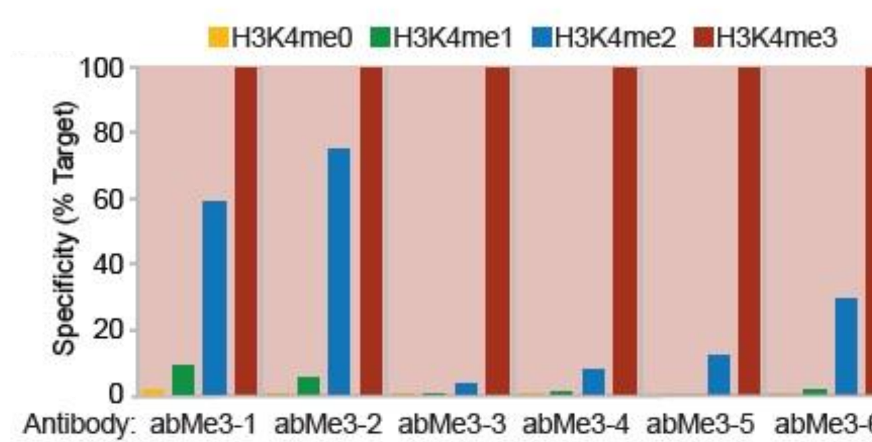


Figure 3. Antibodies with varied specificity in ICe-ChIP have different ChIP-seq profiles in K562 cells. Invitrogen antibody SKUs are highlighted on right. Chromosomal coordinate view (graphic on bottom) of a putative promoter-enhancer connection defined by Pol II ChIA-PET. E represents antibodies used by ENCODE Project Consortium. Detailed specificity for these H3K4me3 antibodies is directly below. Low-specificity abMe3-1 and abMe3-2 ChIP significant amounts of H3K4me2 in addition to target H3K4me3: this is demonstrated by additional ChIP-seq peaks consistent with H3K4me2 (compare with high-specificity abMe3-3 and abMe3-4). Figure adapted from Shah *et al.*



H3K4me1 #710795, Lot QL230605

H3K4me3 #PA5-27029, Lot SG2419844A

H3K4me2 #710796, Lot QL230606

Specificity (% Target)
Enrichment (% Input)
Unmodified, H3K4me1, H3K4me2, H3K4me3, H3K9me1, H3K9me2, H3K9me3, H3K27me1, H3K27me2, H3K27me3, H3K36me1, H3K36me2, H3K36me3, H4K20me1, H4K20me2, H4K20me3, Ab Efficiency

Figure 4. Complete K-MetStat SNAP-ChIP testing of H3K4 Invitrogen antibodies. 710795 (H3K4me1), 710796 (H3K4me2) and PA5-27029 (H3K4me3) exhibit specificity to their target PTM. Each antibody was tested in native ChIP with chromatin from HEK-293 cells. Specificity (left Y-axis; all blue bars mean ± SEM) was determined by qPCR for the duplicate DNA barcodes for each modified nucleosome in the K-MetStat panel (X-axis). Black bar is antibody efficiency (right Y-axis; log scale) and indicates % target nucleosome immunoprecipitated relative to Input.

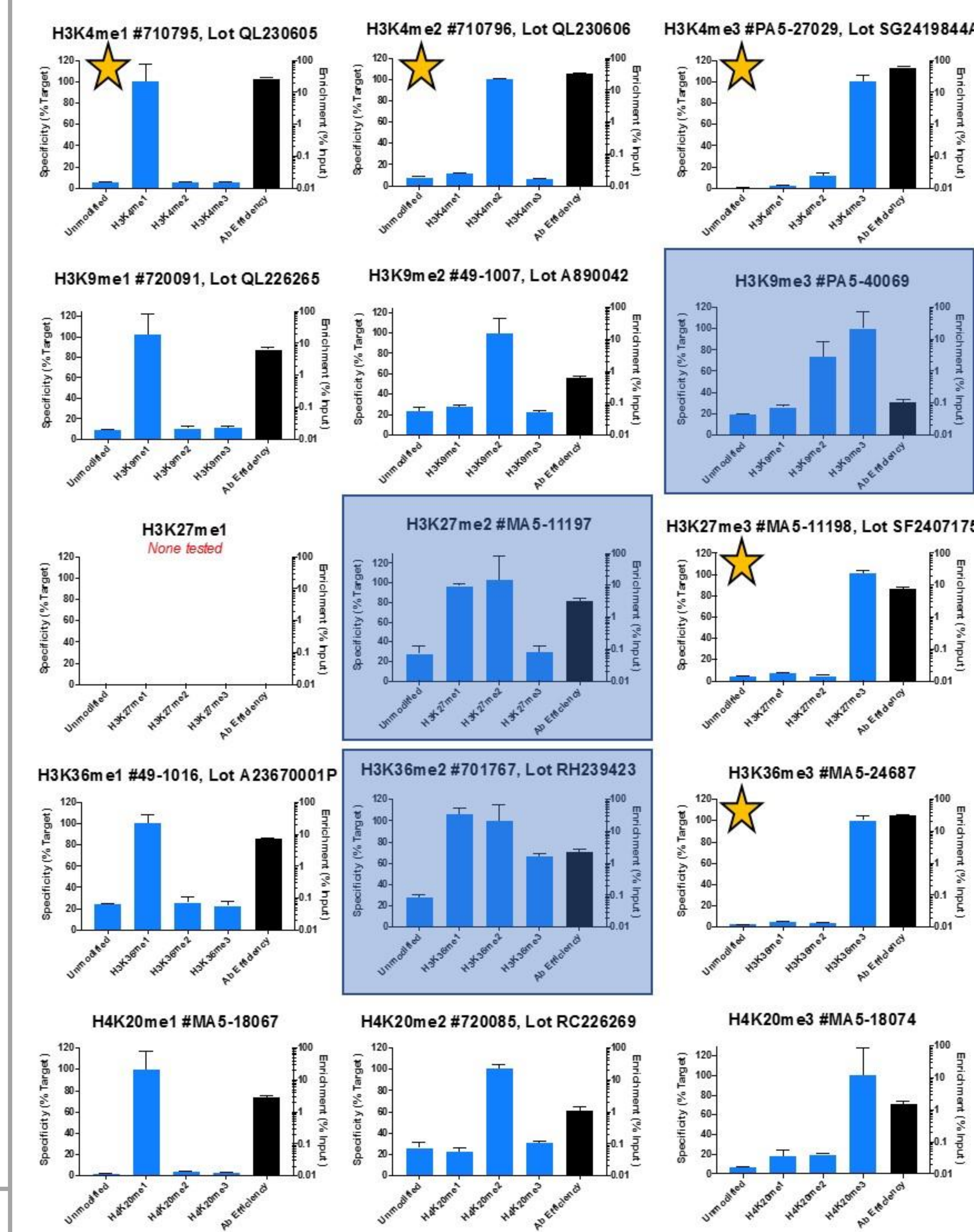


Figure 5. K-MetStat SNAP-ChIP testing of Invitrogen antibodies. Shown are best in class (tested to date) from Invitrogen antibody portfolio for specificity towards the target residue [i.e. Phase I testing]. Antibodies in blue boxes display unacceptable cross-reactivity and will not be further tested in the full K-MetStat panel [i.e. Phase II testing as in Figure 4]. Antibodies to H3K27me1 have not yet been tested. Starred antibodies have passed the full K-MetStat panel and are very specific to their target PTM in ChIP.

CONCLUSIONS

We have identified exquisitely specific ChIP grade antibodies for some of the most relevant histone modifications. Interestingly, the antibodies identified as most specific in SNAP-ChIP do not directly correlate with specificity testing using a peptide array demonstrating the importance of determining antibody suitability based upon desired application. Histone modification targets presented in the context of nucleosomes, like SNAP-ChIP, more closely resemble the local chromatin environment and represent an appropriate validation of ChIP antibody specificity. Indeed, this specificity is essential when using ChIP to identify gene specific histone modification associations. Thus, SNAP-ChIP provides an innovative and exciting platform for identifying ChIP-grade antibodies for histone modification targets.

REFERENCES

Grzbowski, Chen & Ruthenburg (2015) Calibrating ChIP-Seq with Nucleosomal Internal Standards to Measure Histone Modification Density Genome Wide. *Molecular Cell* 58:886.

Shah *et al.* Examining the roles of H3K4 methylation states with systematically characterized antibodies. *Manuscript under review.*

ACKNOWLEDGEMENTS

Thank you very much to the Bangalore Antibody and Immunoassays team at Thermo Fisher for all their helpful discussions and data sharing and to the Epigenetics Antibody working group (Afzal Husain, Remanika Edolia, Deepan Narayanan, Natalie Oxford, Dylan Malayter, Wayne Considine and Chandan Bhambhani).