# Are your ChIP antibodies skewing your data?

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Video Abstract

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# Abstract

It’s a feared moment for every scientist: the discovery that years of painstaking research has led to results that can't be repeated. Many think that poorly characterized antibodies have contributed to this reproducibility crisis more than any other laboratory tool. A new study published in Molecular Cell supports this hypothesis, at least in the context of chromatin immunoprecipitation. Although accurate ChIP interpretation depends on near-perfect antibody specificity, the report shows that many of these reagents are far less capable than their advertising suggests, which calls into question several widely accepted paradigms on genomic regulation. The study focused on histone post-translational modifications; specifically all three methylation states of lysine 4 on histone H3. Through ChIP experiments, H3K4 methylation has been strongly linked to transcriptional control. But these conclusions largely assume that ChIP can correctly discriminate between H3K4 methylation states – a belief that hasn’t been systematically tested. To address this deficiency, four research teams collaborated to assess the capabilities of 52 commercial “ChIP grade” antibodies using two techniques: histone peptide microarrays, where antibody binding to slide-immobilized peptides is detected through fluorescence; and internally calibrated ChIP, where DNA-barcoded semisynthetic nucleosome standards are spiked into a chromatin sample to measure antibody specificity directly within the ChIP experiment. They identified some highly specific antibodies in both approaches, but noted that many reagents in common use don’t perform as expected. After comparing a subset of antibodies of varying proficiency in ChIP on K562 cells the team further found that high- and low-specificity reagents produce very different genome-wide profiles. In practical terms, these findings suggest that many conclusions drawn from prior ChIP reports – especially those using low-specificity antibodies – might not be as robust as previously believed. To prove this point, the researchers used internally calibrated ChIP to re-evaluate several currently accepted theories on the role of H3K4 methylation in genomic regulation. In several experiments, their findings obtained using highly specific antibodies substantially diverged from prior reports using low-specificity reagents. Overall, the study highlights the key role that antibody specificity plays in experimental interpretation. The results show both the danger of using unvalidated antibodies in ChIP and – more promisingly – the power of pairing highly specific antibodies with spike-in standards to drive new biological discovery.