New tool brings standards to epigenetic studies

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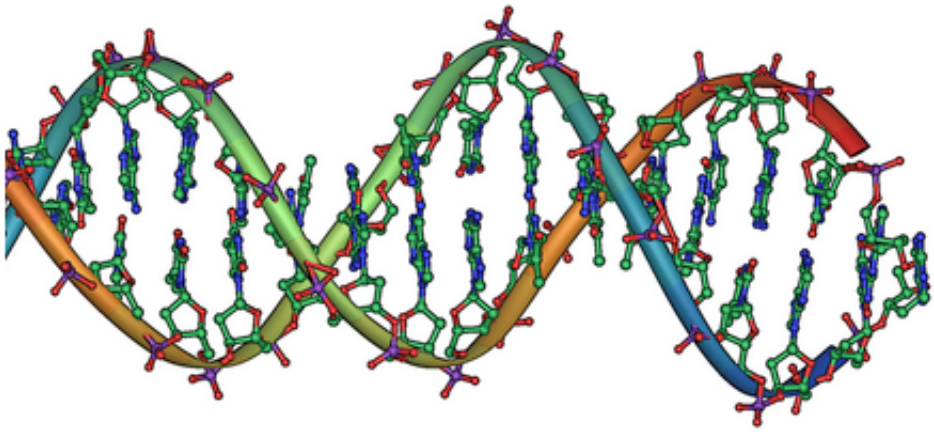

DNA double helix. Credit: public domain

One of the most widely used tools in epigenetics research - the study of how DNA packaging affects gene expression - is chromatin immunoprecipitation (ChIP), a technique that allows researchers to examine interactions between specific proteins and genomic regions. However, ChIP is a relative measurement, and has significant limitations that can lead to errors, poor reproducibility and an inability to be compared between experiments.

To address these issues, scientists from the University of Chicago have developed a new technique that calibrates ChIP experiments with an internal standard. The method, called Internal Standard Calibrated ChIP (ICeChIP), gives researchers an objective scale to assess ChIP measurements, enabling greater accuracy and reproducibility, better quality control and unbiased comparisons between experiments - which dramatically improves the accuracy of epigenetic studies and the development of therapeutics against diseases linked to epigenetic changes. ICeChIP is described in a paper published in *Molecular Cell* on June 4.

"Making accurate measurements has been impossible to do with ChIP before now," said senior author Alex Ruthenburg, PhD, assistant professor of molecular genetics and cell biology at the University of Chicago. "We see ICeChIP as an important step forward to measure real epigenetic phenomena in both experimental paradigms and patient samples."

For efficient cellular storage, DNA is typically spooled around histone proteins into complexes known as nucleosomes. Chemical modifications to histones - which are known to occur in response to cell signals or environmental conditions - can affect the expression of the underlying DNA. Although still poorly understood, these epigenetic changes are suspected to influence a variety of behavioral and health-related issues from cancer to cardiovascular disorders.

ChIP assays are the most commonly used tool to measure these changes. Central to the method are antibodies designed to bind to markers of interest, such as histone modifications. Antibodies allow researchers to extract specific histones and associated DNA from a general pool of genomic material gathered from cells or patient samples. These extracted complexes can then be sequenced and analyzed.

However, ChIP has several drawbacks. Commercial antibodies often have variable affinity, specificity and reproducibility when binding to markers of interest. Antibody quality cannot be measured within ChIP assays, which can lead to experimental errors. In addition, the data generated by ChIP are expressed on a relative scale. Coupled with variation in protocols, reagents, equipment and other factors, this makes comparisons between experiments challenging.

A new standard

Ruthenburg and his team tackled these issues by developing a method, dubbed ICeChIP, which adds an internal standard to ChIP assays, allowing results to be measured against an objective scale. They did so by designing semisynthetic nucleosomes - histones coupled to a variety of custom-synthesized DNA fragments. These fragments correspond to different DNA concentrations and act as barcodes that serve as a set of standards.

To use ICeChIP, semisynthetic nucleosomes are first engineered to represent the specific protein marker that a researcher is interested in. These nucleosomes are then added to the sample to be run. As antibodies extract the histone complexes and associated DNA of interest, ICeChIP nucleosomes are also extracted. During analysis, the synthetic DNA barcodes are identified and used to generate a calibration curve. This allows researchers to calculate the efficacy and specificity of the antibody used in the experiment, and to calculate the true amount of histones containing the marker of interest across the whole genome.

"What we've done is make the numbers produced by ChIP experiments correspond to something real," Ruthenburg said. "By putting in an internal standard in the experiment, we tether all our measurements to that standard. Not only can this be reproduced and compared, we're finally able to measure how much of a modification is present at a given

spot in the genome instead of relative amounts."

The researchers tested the efficacy of ICeChIP by measuring H3K4me3 modifications, a well-known epigenetic marker, in mouse and human stem cells. Samples were spiked with a small amount of synthetic nucleosomes and then run in ChIP assays. The team identified that roughly two percent of all histones expressed this marker, a number corroborated by mass spectrometry, radiolabeling experiments and other independent tests. Reproducibility was tested by simulating experimental handling disparities, and the results were found to be stable over multiple trials.

They also used ICeChIP to measure a variety of other common histone modifications, and made surprising findings. They discovered that roughly a third of all genes in stem cells are associated with both activating and deactivating histone modifications - a phenomenon known as bivalency. This was thought to be a regulatory mechanism for development, but unproven until now. Ruthenburg and his team found that bivalency is indeed a real characteristic, but its pervasiveness throughout the genome calls into question its role in developmental genetic regulation.

"Our work measures bivalency for first time at the genome scale, and its prevalence suggests that it is not as specialized as previously thought," Ruthenburg said.

The team also found that the efficacy of commonly-used antibodies for certain histone markers - specifically for H3K36me3 and H3K79me2 modifications - had poor specificity. As these antibodies have been used in hundreds of published studies and have led to the development of therapeutics undergoing clinical trials, additional studies to validate previous results could be required.

"We are deeply concerned that some of the measurements out there for these marks are far more noise than signal," Ruthenburg said.

The team are now working to extend ICeChIP toward other histone and protein markers, improve its suitability for commercial use and investigating ways to utilize it in the development of diagnostic assays for human diseases.

More information: Calibrating ChIP-Seq with Nucleosomal Internal Standards to Measure Histone Modification Density Genome Wide, *Molecular Cell*, 2015.

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