EPIGENOMICS

QC for ChIP

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Methods for mapping histone post-translational modifications on a genome-wide scale have been central to generating and testing hypotheses on epigenetic gene regulation. Chromatin immunoprecipitation (ChIP) remains a foundational technology in which antibodies against specific histone modifications are used to produce an affinity-purified pool of nucleosomal fragments, whose associated DNA is then analyzed by techniques such as qPCR (ChIP-qPCR) or next-generation DNA sequencing (ChIP-Seq) to reveal the genomic localization of the histone modification. Despite their broad usage, ChIP-based profiling methods are limited by factors such as the specificity of antibody reagents and variability in sample handling and amplification. More broadly, data collection and normalization protocols complicate the comparison of similar experiments. To address these challenges, Grzybowski *et al.* now integrate analytical chemistry principles and chemical biology tools in a method they call internal standard calibrated ChIP (ICeChIP). Using native chemical ligation, the authors prepared semisynthetic histones containing a unique modification (for example, H3K4me3). To create internal standards, these modified histones were incorporated into a set of reconstituted nucleosomes, each of which contained a DNA barcode to report on the modification's concentration in the analyte solution. These internal standards, when added to experimental samples and subjected to the same protocols, produced a standard calibration curve from which quantitative histone modification density values (HMDs) were calculated genome wide. In addition to reporting extensive validation of the precision and accuracy of the method in mouse and human cell lines, the authors showed that the approach can monitor the specificity of the IP step, information that may be used to better calibrate data sets. Further, their method provided the necessary resolution to better characterize the concept of 'bivalency', whereby activating and repressive marks colocalize in the genome. ICeChIP, and future extensions to other histone marks (or combinations thereof), offers potential for more quantitative and reproducible ChIP data that can be used to answer precise questions in chromatin biology. *TLS*

PEPTIDES

A noncanonical conotoxin

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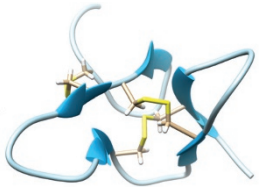
ASWANI KUMAR AND SIDDHARTHA SARMA



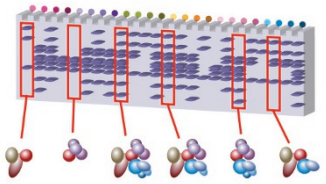
Conotoxins are short peptides that are multiply crosslinked with disulfide bonds. These extremely stable protein scaffolds are potent ion-channel inhibitors, used by venomous animals to disable their prey. In addition to their use in ion-channel research, these peptides are increasingly employed as modifiable scaffolds, necessitating the elucidation of their three-dimensional structures. More than 2,000 conotoxins and 26 disulfide-linked frameworks are known, but structural data is available for only 10 of these frameworks, raising questions about what other conformations might exist. Kancherla *et al.* now characterize Mo3964, a recently identified conotoxin from *Conus monile*. The authors first confirmed that the purified peptide contains oxidized cysteines and exists as a monomer that is stable to denaturation in the absence of a reducing agent. Functional assays using expressed sodium channels and DRG neurons suggested that Mo3964 acts on voltage-gated sodium channels, decreasing ion selectivity—thus increasing permeability to calcium ions—as well as increasing voltage sensitivity, and on voltage-gated potassium channels. NMR analysis identified five short β-strand-like segments with two structured and two unstructured loops. Solvent-exchange experiments confirmed the stability of the scaffold, as the majority of residues did not show any exchange over ~200 hours. Analysis of disulfide connectivity established an unusual pattern in which the first and third, the second and fifth, and the fourth and sixth cysteines in the sequence are linked; when these data were combined with further NMR analysis to generate a tertiary structure, the authors were able to determine that the latter two bonds form a Gordian knot. The tertiary structure is an unusual β-sandwich, and explains circular dichroism data that suggested the existence of a sterically constrained aromatic amino acid, as the aromatic ring of Trp25 is tightly packed by the side chains of six other residues. Mo3964 has no structural relative, suggesting it will find particular utility in further basic and applied research. *CG*

INTERACTOMICS

Connecting the dots

Nat. Methods **12**, 553-560 (2015)

NATURE METHODS



Although it is now relatively straightforward to sequence the genome or characterize the proteome of an organism, it is still quite challenging to identify protein-protein interaction networks and study their individual components. This is due in part to the need to optimize the conditions required to carry out the affinity-capture protocol—which is used to separate the target protein complex from the other constituents of the cell—so as to ensure that none of the components of that complex are lost during the purification step. Hakhverdyan *et al.* now report a new high-throughput method to rapidly and comprehensively screen different parameters that affect the stability of protein complexes. Cells that express a tagged protein of interest are cryo-milled and dispensed into the wells of a 96-well plate; the protein complexes are then extracted with different combinations of salts, buffers and detergents. The extracts are filtered, and magnetic beads are used to facilitate the affinity capture of the tagged protein and any proteins with which it associates. The resulting protein–protein complexes can be separated into their components using SDS-PAGE and then characterized by mass spectrometry (MS) or subjected to MS analysis directly. The authors first showed that their method could be used to identify the individual components of the ~50-MDa nuclear pore complex, generating data that recapitulated the macromolecular architecture reported in a previous paper. They then determined that the approach could be used to identify protein partners for a range of other proteins, including components of the exosome, the U1 small nuclear ribonucleoprotein complex and the integral endoplasmic reticulum membrane. Because this approach was shown to work for yeast, bacterial and human cells and is amenable to robotic automation, the authors believe that it will be possible to use it to efficiently obtain high-quality interactomics datasets for a range of cell types and pathophysiological conditions. *JMF*

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