PROTEOMICS

Reverse ChIP

The combination of a DNA probe and mass spectrometric analysis allows the unbiased identification of chromatin-associated proteins.

Patience does pay off, as Robert Kingston and his postdoctoral fellow Jérôme Déjardin from Harvard Medical School can attest. Kingston has a longstanding interest in protein-mediated chromatin modification and decided five years ago to take another stab at an often-contemplated but never realized approach for the unbiased analysis of chromatin-binding proteins.

Traditionally, the interplay between DNA and its interacting proteins has been queried by chromatin immunoprecipitation (ChIP). The big limitation of ChIP is that it will only provide information on the DNA interaction of the protein chosen for precipitation. Kingston wanted to reverse the process and use a DNA probe to pull down all proteins associated with a targeted DNA locus *in vivo*.

He is quick to point out that this idea is not new: "People have been talking about it since the early '80s, but nobody has been able to realize it." But when Kingston received a letter from Déjardin five years ago proposing to give the approach another try, he thought it was timely, "because of the advances in mass spectrometry and [DNA] technologies."

With this decision, PICh (proteomics of isolated chromatin segments) was born. The principle is to use a DNA probe to hybridize to a specific chromatin locus and isolate it together with all associated proteins, which are then identified by mass spectrometry.

The main problem Déjardin and Kingston initially ran into was a low signal-to-noise ratio. They saw only very little, if any, enrichment of their specific probe over a scrambled control probe. It took three years of optimizing every aspect of the probe for them to see PICh succeed.

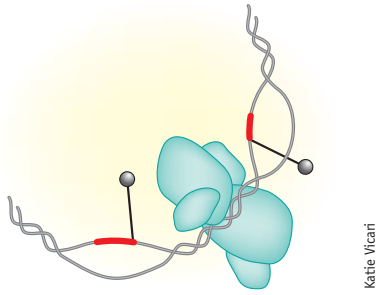
To further ensure enrichment, the researchers designed the DNA probe against human telomeres, which, with around 100 copies, are abundant in cells. PICh revealed nearly 200 proteins associated with telomeric sequences (Déjardin & Kingston, 2009). Some of these proteins had been previously identified as telomere binders; for several new ones, the scientists performed successful validation experiments.

Now that PICh has proved its mettle, Kingston wants to make it more sensitive, so that less abundant chromatin loci can be analyzed. To increase PICh's sensitivity, both the probe design and the mass spectrometric analysis of the proteins will need to be refined. Kingston is considering setting up collaborations with experts working on the cutting edge of mass spectrometry.

One such expert is Matthias Mann at the Max Planck Institute in Martinsried. Coincidentally, Mann and his team are also working on developing an unbiased approach to identify chromatin-protein associations. Some of their problems, such as high background, were similar to Kingston's. Their solution differed.

To eliminate the background, Mann used his SILAC (stable isotope labeling with amino acids in cell culture) approach. His team grew cells in the presence of either unlabeled or deuterium-labeled lysine and purified chromatin from the deuterium-labeled nuclear extracts with a specific DNA bait or from the unlabeled sample with a control bait. They separated the combined pulldowns by SDS-PAGE and analyzed them by mass spectrometry. Any protein that showed a 1:1 ratio between the labeled and unlabeled form was eliminated as background, as that indicated that it had also been pulled down with the nonspecific probe.

But Mann was not completely satisfied even when the method was up and running; he wanted to take the approach to a high-throughput level, which meant that the SDS-PAGE step needed to be eliminated. He describes the challenge as follows: "You have to elute directly from the bait, and then you have hundreds of proteins that bind nonspecifically, and you need to analyze them all."



Katie Vicari

DNA probes pull down proteins associated with a chromatin locus.

An essential element to this approach is a powerful mass spectrometer. Mann used the LTQ-Orbitrap from Thermo Fisher Scientific, a high-precision instrument that can sequence proteins very quickly to ensure that even the less abundant proteins are sequenced. In a proof-of-principle application, the researchers used a bait against the promoter region of a single gene; they confirmed two known interactors and found eight more (Mittler *et al.*, 2009).

Mann sees an important application of this approach in the extension of genome-wide association studies. In a GWAS scientists find SNPs that confer susceptibility to a certain disease, but they do not know how a given SNP causes a particular phenotype. Mann suggests synthesizing a probe with a disease-specific SNP, and a control with the wild-type SNP, and doing the SILAC experiment to find the specific transcription factor or repressor that may contribute to the disease.

With the right probe and a high-powered mass spectrometer, the protein occupancy at any genomic locus will now be open for investigation.

Nicole Rusk

RESEARCH PAPERS

Déjardin, J. & Kingston, R.E. Purification of proteins associated with specific genomic loci. *Cell* **136**, 175–186 (2009).

Mittler, G. *et al.* A SILAC-based DNA protein interaction screen that identifies candidate binding proteins to functional DNA elements. *Genome Res.* **19**, 284–293 (2009).