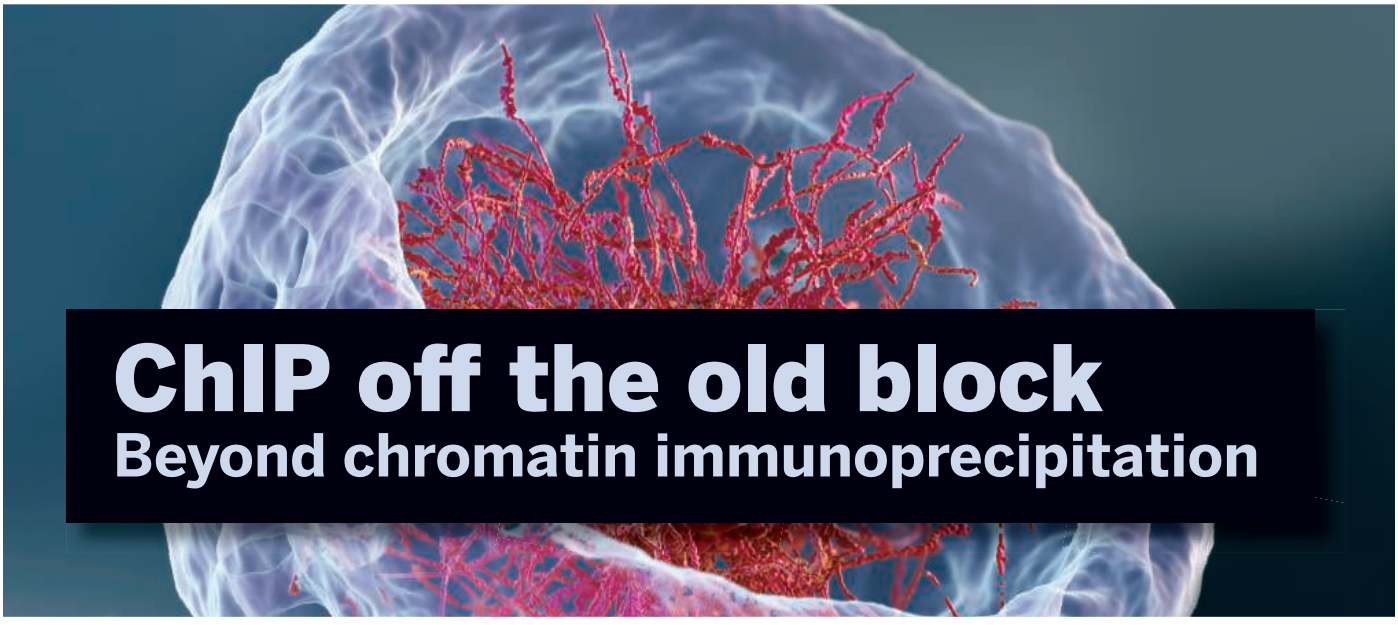

ChIP off the old block
Beyond chromatin immunoprecipitation

A host of techniques are building from a classic method—chromatin immunoprecipitation (ChIP)—to assess what binds to DNA and where. Emerging techniques whittle down the size of samples, interrogate DNA-bound protein complexes, or more closely assess the nucleotides involved. All of these approaches aim to overcome long-standing limitations of ChIP, and broaden the questions scientists can ask about gene regulation, development, and disease. **By Charlotte Schubert**

Chromatin immunoprecipitation, one of the most widely used techniques in molecular biology, was invented over 30 years ago—and some things about it have changed, while others have stayed the same.

The basic protocol is still similar to one developed in the 1980s, involving crosslinking proteins to DNA with formaldehyde and then fragmenting the DNA. A DNA-interacting protein is immunoprecipitated using an antibody, the crosslinks reversed with heat, and the associated DNA analyzed. Researchers later linked the technique to deep sequencing, developing the ChIP-seq technique to probe protein–DNA interactions at the genomic scale.

ChIP has been harnessed to address how transcription factors operate, how histones modulate gene expression, and other basic questions with implications for biological development and disease. In September, C. David Allis and Michael Grunstein won the prestigious Lasker award for their work on histones—research that relied on ChIP. And ChIP-seq is now a cornerstone of the ENCODE (ENCyclopedia Of DNA Elements) project, an effort to map regulatory regions of the genome in various cell types.

Chromatin biologists are also developing an array of spin-off or parallel technologies to go beyond what ChIP and ChIP-seq offer—to examine complexes of proteins, to more accurately assess the exact nucleotides a factor binds to, to look at small pools of cells, and to begin, tentatively, to assess protein–DNA interactions at the single-cell level.

All of these techniques aim to do things that ChIP-seq alone cannot, or does only sluggishly. And all of them have the same basic goal: to find out what molecules are associated with DNA and where.

"We really don't understand the fundamental principles by which regulatory functional sequences in our genome determine where and when genes come on," says Bradley Bernstein, director of the **Broad Institute's Epigenomics Program** in Cambridge, Massachusetts. He adds that ChIP is "limited in many ways. And so there are these efforts to try and innovate new approaches or adapt the technology in new ways."

Making it work

"Calling [ChIP-seq] a dark art is too much," says Nir Friedman, a professor of computer science and biology at **The Hebrew University of Jerusalem,** Israel. But despite it being a commonly used technique, "very few people are patient enough to calibrate their experiments," he says.

ChIP-seq experiments generate a lot of noise, notes Friedman. Formaldehyde can crosslink uninvolved molecules, antibodies can pull down nontarget proteins, and sonication—the most common way to break up DNA—tends to break up DNA that is in an open conformation. Says Friedman, "It can be that more than half of what you end up sequencing or looking at is nonspecific binding."

ENCODE publishes guidelines for assessing the quality of antibodies and screening out meaningless data, Friedman notes. The project also provides access to recent computational tools that are used, for instance, to normalize data to controls and to identify "peaks" or regions of possible DNA binding.

Choosing the right antibody, in particular, can be challenging, notes Michael-Christopher Keogh, chief scientific officer at **EpiCypher,** an epigenomics company in Research Triangle Park, Durham, North Carolina. Keogh was involved in a recent study showing that many antibodies popular for histone research perform poorly in ChIP, for instance, binding to off-target epitopes. The study also proposes validation steps beyond the ENCODE guidelines.

Some researchers bypass the antibody problem by engineering an epitope tag onto their target, as with CETCh-seq (CRISPR epitope tagging ChIP-seq). A long-standing technique, DamID (DNA adenine methyltransferase identification), involves engineering factors to tag neighboring DNA with molecular marks.

Upcoming features

Next-Gen Sequencing: RNA Seq—February 22 ■ Microscopy: Multiphoton Microscopy—March 22 ■ Molecular Biology: Epitranscriptomics—May 17