AN RNA-BINDING AGREEMENT

Understanding the function of an RNA-binding protein (RBP) requires identifying its target RNA sequences. One approach for doing this is systematic evolution of ligands by exponential enrichment (SELEX), but this in vitro technique is not quantitative and favors the strongest binding sequences. Another approach is cross-linking immunoprecipitation (CLIP), but this in vivo method requires large numbers of cells and may select for highly abundant, low-affinity non-target sequences while missing low-abundance, high-affinity target sequences. In this issue of *BioTechniques*, researchers led by Kee K. Kim at Chungnam National University describe how they overcame the limitations of these two approaches by combining them. As with SELEX, their method uses RNA synthesized from a random oligonucleotide library, but the RNA contains the photo-activatable ribonucleoside analog 4-thio-uridine, used in CLIP. RNAs from the random sequence pool that bind the RBP are then UV crosslinked to the RBP and selected for by immunoprecipitation of the RBP. This isolates low- and high-affinity sequences without being affected by target RNA abundance in the library pool.

See “An in vitro technique to identify the RNA binding-site sequences for RNA-binding proteins” on page 28.

A DECLARATION OF TEMPLATE-DEPENDENCE

Multiple displacement amplification (MDA) using phi29 DNA polymerase is an important method for amplifying low quantities of genomic DNA, such as for single-cell genomics studies. Compared to PCR, this isothermal amplification method provides superior template coverage and sequence amplification fidelity. A major issue with MDA, however, is production of junk DNA by template-independent amplification (TIA), which occurs when using higher concentrations of random hexamers and longer incubations in order to amplify very low quantities of DNA. In this month's issue, researchers at the Saint Louis University School of Medicine led by Adrian Di Bisceglie and Xiaofeng Fan describe their template-dependent MDA (tdMDA) technique, which overcomes this problem. The team tested the ability of various types of primers to suppress TIA and found that random pentamers blocked at the 5' end with a C18 spacer resulted in the strongest and most consistent suppression of TIA. Using tdMDA, the team was able to carry out transcriptome profiling of circulating RNA in human serum samples.

See “Template-dependent multiple displacement amplification for profiling human circulating RNA” on page 21.

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CHANGING CELL VOLUME TO STUDY PROTEIN INTERACTIONS

Understanding where and when proteins interact in cells is critical to decoding cell-signaling networks and, ultimately, to understanding how cells function. There are a number of different techniques for studying protein interactions, but these approaches often encounter difficulty when the interactions are weak or transient. Sukenik et al. present a clever solution to quantifying such protein interactions by modulating the cell's volume to induce changes in the status of protein complexes. Sudden changes in cell volume can lead to the re-equilibration of protein monomers and complexes within the cell. The authors realized that by changing cell volume at specific times, the interactions between proteins tagged with fluorescent labels could be visualized, and binding properties and kinetics could be quantified, even in the case of weak or transient interactions. To validate the method, they studied the association between GAPDH and PGK, proteins thought to form a transient complex in cells.

Sukenik, S., Ren, P. and Gruebele, M. 2017. Weak protein-protein interactions in live cells are quantified by cell-volume modulation. *Proc Natl Acad Sci U S A*. pii: 201700818.

DNA SEQUENCING: IMPROVING SINGLE LIFE

When it comes to preparing DNA sequencing libraries from highly degraded ancient DNA samples, single-stranded library preparations, where each DNA strand is used as input, has proven to be more effective than the traditional double-stranded library preparation approach. Gansauge et al. now provide an optimized protocol for generating single-stranded sequence libraries from ancient DNA samples that builds on earlier efforts. The new method, which is called ssDNA 2.0, uses T4 DNA ligase and a splinter oligonucleotide possessing a stretch of random bases hybridized with a 3'-biotinylated donor oligonucleotide. Using these modifications, the authors show that higher concentrations of DNA can be ligated to the adaptors, larger amounts of starting DNA can be used for library preparation, and the method is less costly and more suitable for automation. They also provide intriguing data showing that ssDNA 2.0 increases library yields from tissues stored in formalin by several orders of magnitude.

Gansauge et al. 2017. Single-stranded DNA library preparation from highly degraded DNA using T4 DNA ligase. *Nucleic Acids Res*. 45:e79.

Selected and written by Nathan S. Blow, Ph.D.

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