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eCLIPsing CLIP: RNA-Protein Interactions Revealed

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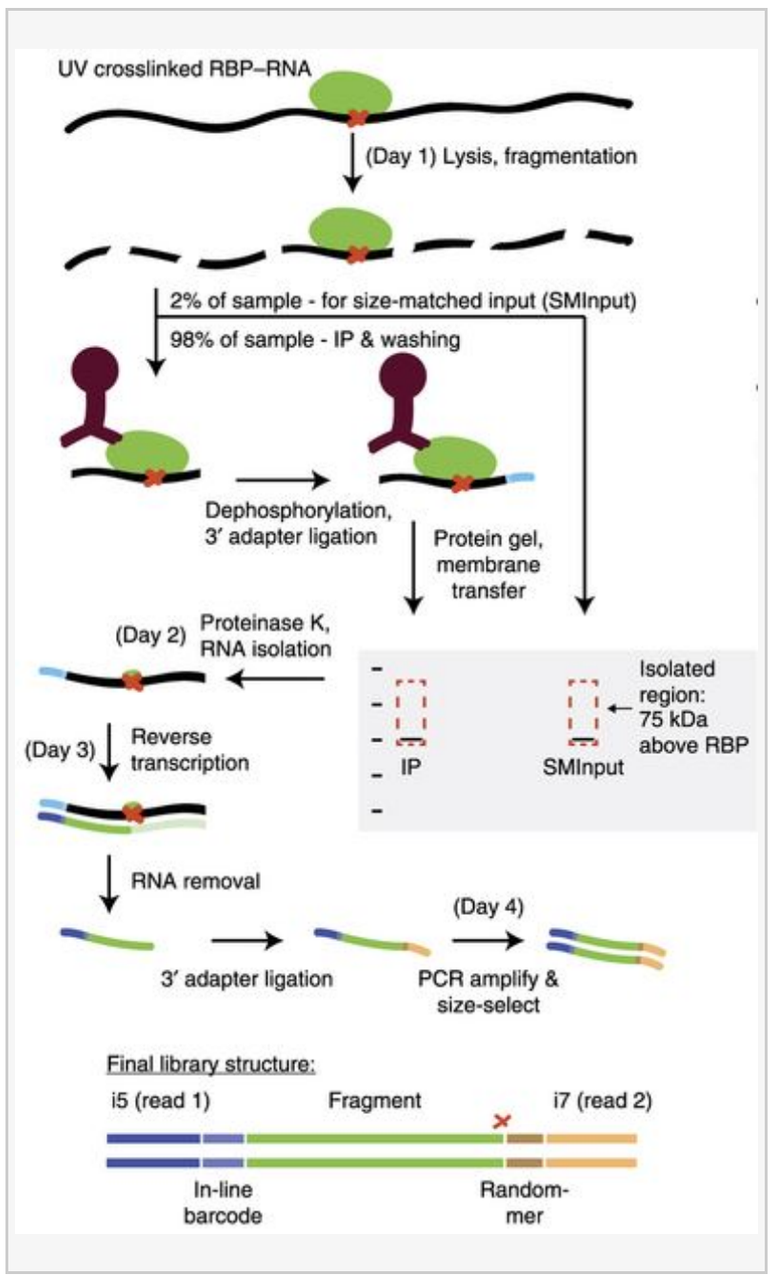
Hundreds of proteins can bind RNA, but finding out which ones bind where and what they do once they bind has been a challenge. That job just got a lot more manageable. Learn more...

There is more to genetic regulation than just transcriptional control. As researchers become increasingly aware of the diverse roles played by RNA, the search for interactions between these nucleic acids and RNA-binding proteins (RBPs) takes on even greater importance. Hundreds of RBPs are known–many of which have been implicated in a variety of diseases such as neurodegeneration, autoimmune defects, and cancer–but most of their targets remain to be discovered. Finding sequences where RBPs bind can give some insight into the mechanisms by which they manage the trafficking, processing, and sometimes translation of RNAs both big and small.

State-of-the-art methods to discover where RBPs bind rely on crosslinking and immunoprecipitation (CLIP) of the ribonucleoprotein complex, followed by sequencing. Such protocols are technically challenging, require radioactivity, have a high experimental failure rate, and generate a high percentage of duplicate reads. "When performing large-scale CLIP experiments as part of the ENCODE consortium, we determined that the current methods were not robust enough to profile hundreds of factors," related Eric Van Nostrand and Gene Yeo from the University of California, San Diego by email. They and their colleagues created enhanced CLIP (eCLIP) to solve these problems.



eCLIP borrows its 2-step multiplex library preparation technique from ChIP-seq. First, the researchers ligate an indexed 3´ adapter (which allows for subsequent pooling) to the RNA fragments attached to immunoprecipitation beads. They then separate the complexes by electrophoresis and digest away the protein. After reverse transcribing the RNA, they ligate a second 3' adapter containing an inline random 5- or 10-mer to the now single-stranded DNA before PCR amplification.

The fact that reverse transcription often terminates at the site of cross-linking allows the researchers to obtain nucleotide resolution. In the case of identical sequencing reads, the random single-stranded DNA adapter sequence will distinguish unique fragments from PCR duplicates, allowing the latter to be discarded, thus making the number of mapped reads more quantitative and minimizing wasted sequencing. In addition, eCLIP adds a size-matched pre-immunoprecipitation control for non-specific background and inherent biases.

These modifications mean that eCLIP requires about 1000-fold less amplification than its predecessors to achieve a higher percentage of useable reads and generate libraries with enough

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complexity to identify sequences used by more promiscuous RBPs. Now, it's time to use these sequences to figure out how RBPs actually do their jobs.

Reference

Van Nostrand EL, Pratt GA, Shishkin AA, Gelboin-Burkhart C, Fang MY, Sundararaman B, Blue SM, Nguyen TB, Surka C, Elkins K, Stanton R, Rigo F, Guttman M, Yeo GW. Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). Nat Methods 2016 Mar 28.

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