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Technology Watch

HITS-CLIP HITS TARGET

Many efforts to determine microRNA (miRNA) targets rely on computational approaches, which are hampered by high false-positive rates. This problem has been overcome by a recent study, which combined HITS-CLIP (high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation) with bioinformatics to produce a map of functionally relevant miRNA-binding sites. HITS-CLIP was first developed by the Darnell laboratory in 2008 and works by covalently crosslinking RNA–protein complexes. A partial RNA digest followed by high-throughput RNA sequencing allows the protein-bound RNA molecules to be identified. Darnell and colleagues used HITS-CLIP in mouse brain tissue to crosslink the ternary complex that is formed when Argonaute (Ago) binds to a miRNA and its mRNA target. A bioinformatics approach was used to identify nucleotide motifs of 6–8 base pairs — the length of the seed sequence miRNAs use to find targets — that were enriched in the Ago–mRNA data. The most significant seed match was for miR-124, consistent with previously published data. The approach was extended to produce a map of predicted sites for nearly 90% of the miRNAs expressed in the mouse brain. A clear advantage of Ago HITS-CLIP is that it identifies direct Ago targets and specific interaction sites, an improvement over correlative predictions of target mRNAs identified by previous approaches.

ORIGINAL RESEARCH PAPER Chi, S. W. *et al.* Argonaute HITS-CLIP decodes microRNA–mRNA interaction maps. *Nature* 17 Jun 2009 (doi:10.1038/nature08170)

RNA–PROTEIN LIAISONS

Ray *et al.* developed RNAcompete, a method for the rapid and systematic characterization of RNA-binding specificities. They created a pool of various short RNA sequences and structures, which was used in a single pull-down reaction to find RNAs bound to a tagged RNA-binding protein (RBP) of interest. The enrichment of each RNA in the bound fraction relative to the total pool signal was determined in a microarray — representing a measure of the binding affinity and therefore sequence preference. Analysis of nine RBPs, which encompass four classes of RNA-binding domains, resulted in expected and previously unknown binding preferences. Interestingly, the authors found that preferences for individual sequences provided a more accurate representation of RNA-binding preferences than position weight matrix models.

Butter *et al.* developed a quantitative proteomics approach to capture RBPs from cell extracts after stable isotope labelling of amino acids in cell culture, using aptamer-tagged RNA as bait. This unbiased approach enables specific binders to the RNA sequence to be distinguished from background binders by their isotope ratios between bait and control. In all experiments, known RBPs were unambiguously identified in a single step.

ORIGINAL RESEARCH PAPERS Ray, D. *et al.* Rapid and systematic analysis of the RNA recognition specificities of RNA-binding proteins. *Nature Biotech.* 28 Jun 2009 (doi:10.1038/nbt.1550) | Butter, F. *et al.* Unbiased RNA–protein interaction screen by quantitative proteomics. *Proc. Natl Acad. Sci. USA* 16 Jun 2009 (doi:10.1073/pnas.0812099106)

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