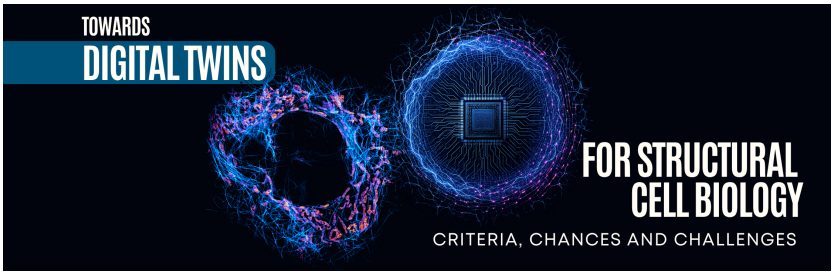

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iCLIP3: A streamlined, non-radioactive protocol for mapping protein-RNA interactions in cellular transcripts at single-nucleotide resolution

UV-C crosslinking and immunoprecipitation (CLIP)–based methods are the gold standard for identifying direct RNA binding protein (RBP) interaction sites on cellular RNA in vivo. Here, we describe individual nucleotide resolution CLIP version 3 (iCLIP3), an optimized protocol for generating transcriptome-wide maps of RBP-RNA interaction sites at single-nucleotide resolution from low-input material. iCLIP3 introduces several key improvements over previous iCLIP variants, including rapid and safe infrared-based visualization of RBP–RNA complexes, silica column–based RNA isolation, and the incorporation of TruSeq adapter sequences with unique dual indexing. These modifications streamline library preparation, facilitate multiplexing, and enable concurrent sequencing of iCLIP3 libraries alongside unrelated RNA-seq libraries. In addition, we provide a detailed bioinformatics workflow for identifying RBP crosslinking events and defining RBP binding sites.

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