SYSTEMS BIOLOGY

Mapping RNA–RNA interactions

RIC-seq enables in situ mapping of intra- and intermolecular RNA–RNA interactions.

The functional versatility of RNA molecules is mediated by their structural diversity and interaction network. Current methods for mapping RNA–RNA interactions, which often involve a proximity ligation step, suffer from several limitations, such as high false-positive rates, low chimeric read percentage, and inefficiency in the in vitro ligation reaction, notes Yuanchao Xue of the Institute of Biophysics, Chinese Academy of Sciences. He and colleagues speculated that these limitations are mainly caused by the proximity ligation step performed in dilute solutions. To solve these problems, an in situ strategy could help.

Xue and colleagues developed the RIC-seq technology as a step in this direction. In RIC-seq, RNA proximity ligation is performed in situ to generate chimeric reads from interacting RNAs, followed by sequencing and bioinformatics analysis. One of the key steps uses pCp-biotin for chimeric junction marking and chimeric RNA enrichment. "This enhancement enables us to enrich chimeric RNAs selectively and to assign duplex positions correctly," says Xue.

Intramolecular RNA–RNA interactions identified by RIC-seq confirmed known features and suggested new details for ribosomal RNA and long non-coding RNA structures. After background correction, RIC-seq pinpoints reliable intermolecular interactions and facilitates interactome-level analysis. Xue and colleagues identified over 600 *trans*-interacting hub RNAs. "The most surprising result is the finding that pairwise interacting enhancer RNAs and promoter non-coding RNAs can be used to assign enhancer–promoter connectivity faithfully," says Xue, whose team further demonstrated that such interactions between enhancer RNA and promoter RNA can modulate long-range chromatin looping in an RNA-and-protein-dependent manner.

Xue plans to explore the application of RIC-seq to a small number of cells and even the single-cell level. He hopes the technology can also help others who study RNA viruses, non-coding RNA targets and non-coding mutations, which tend to be pervasively transcribed in the genome.

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