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Dr. Stephanie R. Weldon
Science Editor
JoVE

February 26, 2019

Dear Dr. Weldon,

This letter accompanies the manuscript titled "*Identification of footprints of RNA:protein complexes via RNA Immunoprecipitation in Tandem followed by sequencing (RIPiT-Seq)*" by Lauren Woodward, Pooja Gangras, and Guramrit Singh for publication alongside an instructional video in *JoVE*.

Within eukaryote cells, RNA is bound directly or indirectly by a myriad of RNA-binding proteins (RBPs) to form a dynamic ribonucleoprotein (RNP) network. Post-transcriptional gene regulation through RBPs is an ever-expanding frontier across many biological disciplines. Investigators often seek to identify endogenous targets of an RBP in mammalian cell culture, for which various options exist—the most famous being CLIP-Seq (UV-**C**ross**l**inking followed by **I**mmuno**p**recipitation). However, the identification of RNA targets of an RBP that does not bind the RNA directly or does not UV-crosslink well is a major limitation in the field. Further, RBPs generally have multiple roles and interaction partners. As such, it is challenging to deduce which RNA binding events represent a biologically active RBP binding network.

Our method, RIPiT, bypasses the need for UV-crosslinking and is designed to enrich compositionally distinct RBP complexes from a pool of RNPs with overlapping protein composition. This tool has been used successfully to characterize the biological role of compositionally distinct varieties of the exon junction complex (EJC). The EJC is a protein complex that interacts with many RBPs and participates in multiple compositionally distinct RNP structures.

In this manuscript, we describe in detail the steps, reagents, and controls an investigator will need to successfully perform RIPiT-Seq on their protein complex of interest. Schematic figures have been included to illustrate the major steps in the RIPiT workflow. RIPiT yields a low amount of RNA, so in addition to the RIPiT protocol, this manuscript includes our method for library preparation for deep sequencing. While our sample data focuses on EJC RIPiTs, the procedure is adaptable to virtually any other protein complex, and the *JoVE* video will provide a useful tool for someone new to the procedure.

We are excited about the opportunity to make this approach more widely accessible through *JoVE*, and look forward to hearing from you.

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