RBP-eCLIP

Robust transcriptome-wide identification of RNA binding protein targets

HIGHLIGHTS

Highly efficient library prep

Low PCR duplication rate

Transcriptome-wide targets

RBP binding sites discovered in all gene regions

Precise RBP binding motifs

RNA binding sites resolved at single nucleotide resolution

Introduction

RNA binding proteins (RBPs) bind to RNAs through recognition of sequence and structural motifs to regulate RNA function in a cell-type, condition-specific, or temporal manner. Recent studies have estimated that there are over 1500 RBPs in the human genome and these RBPs play an integral role modulating RNA stability and function throughout the RNA life cycle. Mutations in RBPs have been linked to cancer, Amyotrophic Lateral Sclerosis (ALS), and numerous other diseases.

Enhanced crosslinking and immuno-precipitation followed by high-throughput sequencing (eCLIP) was developed to provide a robust and reproducible framework to map RBP binding sites on RNAs transcriptome-wide. Eclipse Bio has optimized the eCLIP technology developed at UCSD and described in the 2016 Nature Methods paper to improve the efficiency of converting immunoprecipitated RNA into high-throughput sequencing libraries.

References

Van Nostrand EL, et. al. Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). Nat Methods. 2016 Jun;13(6):508-14

RBP-eCLIP Workflow

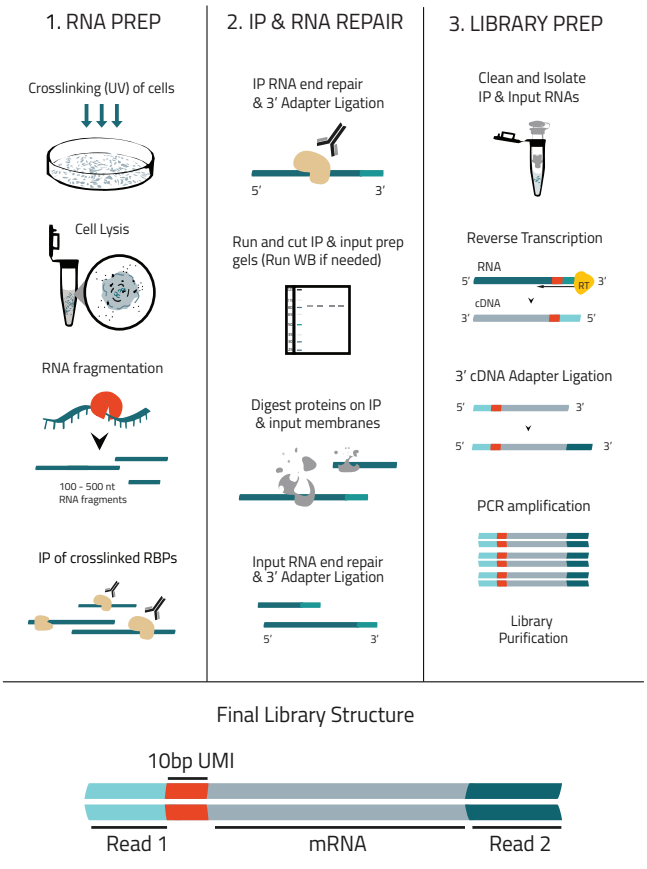

Figure 1. RBP-RNA interactions are UV crosslinked. RNA is fragmented, and an RBP of interest is immunoprecipitated. After ligation of a 3' RNA adapter, IP material is run on denaturing protein gels and reverse transcribed to ssDNA (when a second adapter is ligated). PCR amplification is then used to obtain sufficient material for high-throughput sequencing.

Specifications

Sample Input Requirement	UV Crosslinked Cells	20M cells
Sequencing Recommendations	Instrument Sample Depth Run Parameters	Illumina 20M reads SE100

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High efficiency library preparation with decreased PCR duplication

Optimization of the enzymatic steps of RBP-eCLIP improve library preparation by 1000-fold compared to other CLIP methods, thereby increasing experimental success rates and decreasing wasted sequencing due to PCR duplication.

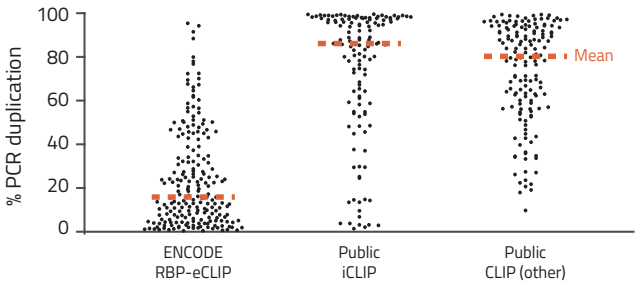

Figure 2. PCR duplication rates comparing many samples across methodologies, eCLIP, iCLIP, and other CLIP methodologies. RBP-eCLIP yields significantly lower PCR duplications rates on average.

Transcriptome-wide identification of RNA targets

RBP-eCLIP identifies RBP target binding sites across all genic regions: exons, introns and untranslated regions (UTRs), and in both coding and non-coding RNAs, including lincRNAs, microRNAs, and retrotransposons.

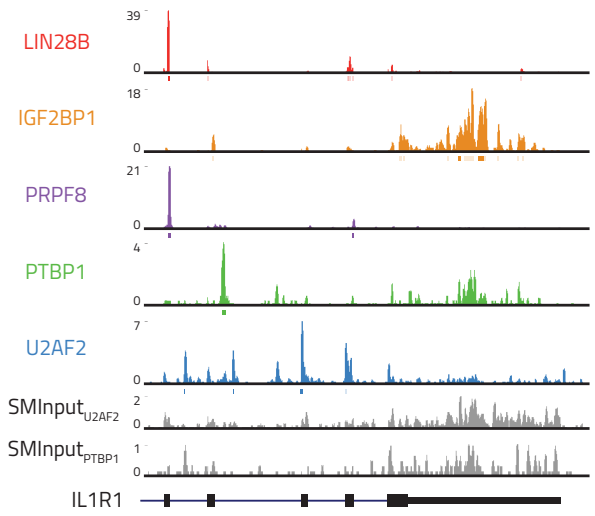

Figure 3. Read coverage showing peaks of reads representing binding sites for RBPs LIN28, IGF2BP1, PRPF8, PTBP1, and U2AF2 on the IL1R1 gene transcript.

Single nucleotide resolution binding sites

Crosslink sites resolve in RBP-eCLIP data to reveal precise RBP binding motifs and enable identification of RBP binding sites with single nucleotide resolution.

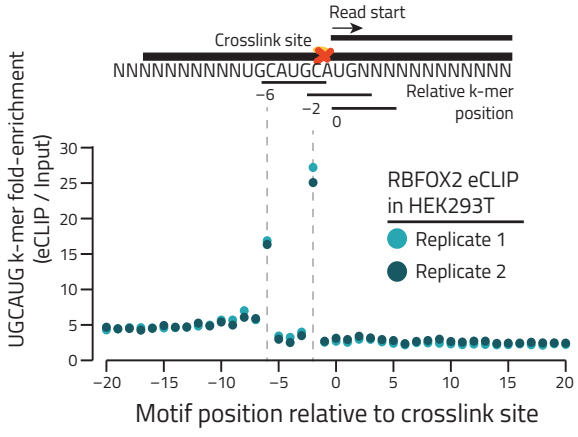

Figure 4. eCLIP reads begin at crosslink sites allowing for simple single base resolution of RBP motifs. RBFOX2 motif UGCAUG is detected at crosslink sites in eCLIP from HEK293T cells.

RBP-eCLIP Kit

Ordering information

More information about RBP-eCLIP kit and services online at www.eclipsebio.com or contact us at info@eclipsebio.com.