DATASHEET

Direct, transcriptome-wide identification of microRNA targets

Introduction

MicroRNAs (miRNAs) have been shown to be involved in nearly every physiological system, and their misregulation is linked to many human diseases; therefore precise miRNA target identification is essential to understand post-transcriptional gene regulation. In contrast to standard techniques that provide indirect methods to identify miRNA targets, miR-eCLIP enables the identification of direct miRNA-mRNA interactions transcriptome wide utilizing AGO2 immunoprecipitation, RNA-RNA ligation, and high-throughput sequencing (similar to methods such as CLASH or CLEAR-CLIP). miR-eCLIP also has the option of enriching for specific miRNAs or genes of interest, enabling profiling of miRNA-target interactions at an unprecedented depth.

Highlights

miR-eCLIP Standard

Unbiased transcriptome-wide detection of direct miRNA binding sites

miR-eCLIP +miR

Transcriptome-wide enrichment for binding sites of miRNA(s) of interest

miR-eCLIP +Gene

Target-specific enrichment to identify miRNA binding sites for gene(s) of interest

Specifications

Input Sample	20M Cells or 80mg Tissue
Starting Material	UV Crosslinked Cells or Tissue
Sequencing Depth Suggestion	Standard miR-eCLIP: 50M reads miR-eCLIP +miR/+Gene: 20M reads
PE/SE	SE100

miR-eCLIP Workflow

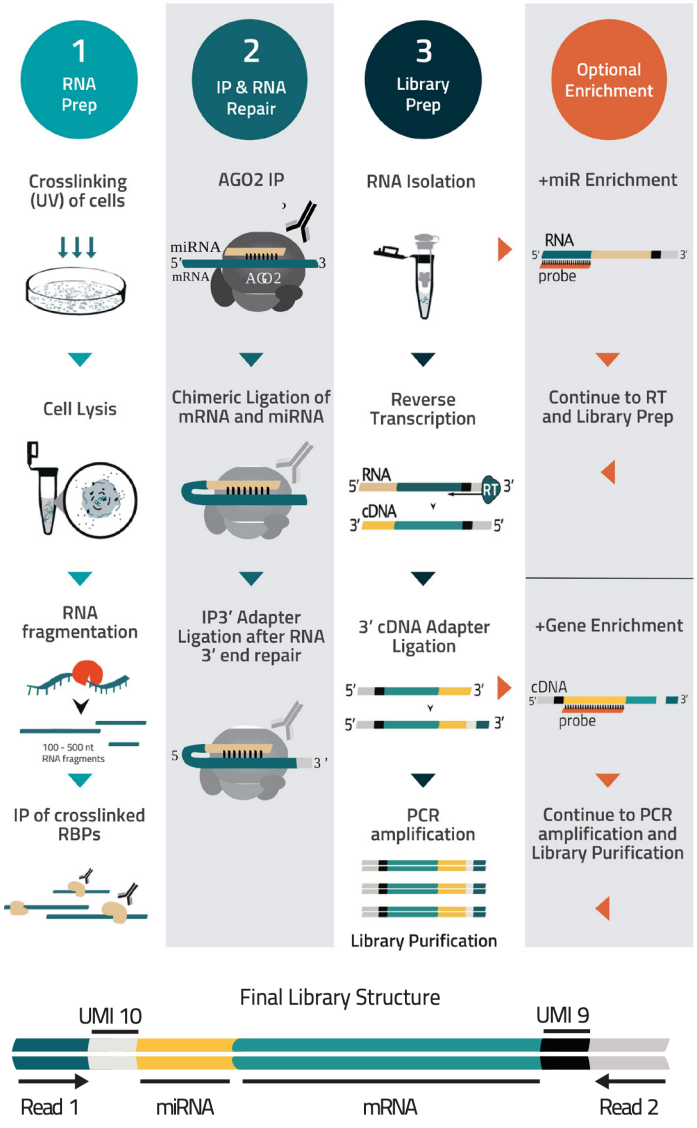

Figure 1. miRNA-mRNA molecules in the AGO2/RISC complex are immunoprecipitated using an Eclipsebio AGO2 antibody. The miRNA & mRNA are then ligated to each other to form chimeric RNA molecules.

Ordering Information

More information about miR-eCLIP services online at **eclipsebio.com** or contact us at **info@eclipsebio.com**.

Direct miRNA target site detection

miR-eCLIP identifies direct miRNA target sites by sequencing miRNA-mRNA chimeras.

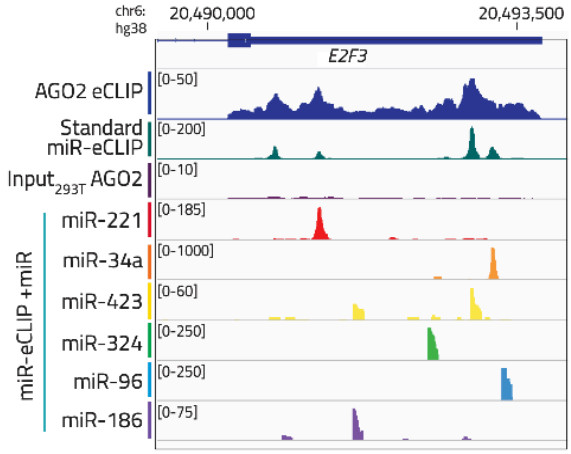

Figure 2. miR-eCLIP and AGO2-eCLIP read densities on the E2F3 gene 3'UTR illustrating several miRNA binding events. Bottom read densities indicate miR-eCLIP +miR enrichments for miR-221 (red), miR-34a (orange), miR-423 (yellow), miR-324 (green), miR-96 (blue), and miR-186 (purple).

Quantify functional miRNA targeting

miR-eCLIP quantitatively detects functional miRNA target genes where higher miRNA-mRNA chimeric read coverage indicates increased binding strength and increased repression in corresponding RNA-seq experiments, in contrast to computational predictions that produce many false positives.

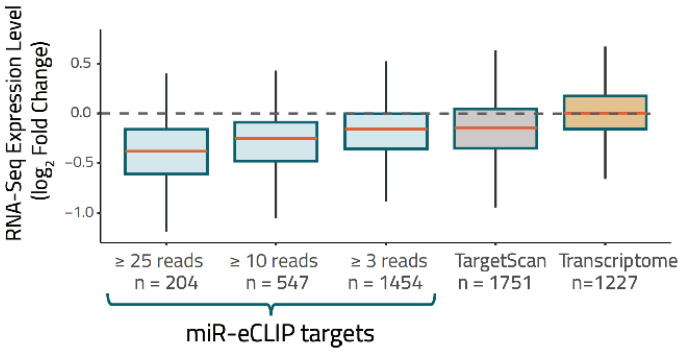

Figure 3. miR-eCLIP and RNA-Seq libraries were generated from HEK293xT cells transfected with miR-124 mimics. Expression levels of miR-eCLIP target genes show greater downregulation with more miRNA-mRNA chimeric read coverage while only limited repression is observed for miR-124 TargetScan predicted target genes

miR-eCLIP +miR: Distinct target profiling for miRNAs of interest

miR-eCLIP +miR enrichment increases miRNA-mRNA chimeric reads specific to miRNA(s) of interest to deeply profile the target repertoire for miRNAs of varying abundance.

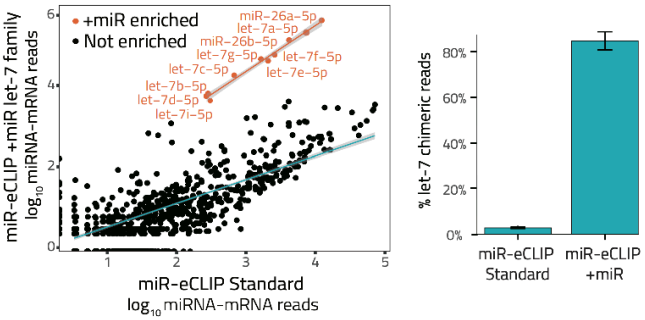

Figure 4. miR-eCLIP +miR was performed enriching for the let-7 family and miR-26a/b simultaneously (orange). miRNA-mRNA chimeric read counts relative to miR-eCLIP Standard (black) is shown, indicating ~25-fold enrichment for the miRNAs of interest. Bar plot indicates percentage of let-7 specific chimeric reads out of total miRNA-mRNA chimeric reads using miR-eCLIP Standard and miR-eCLIP +miR.

miR-eCLIP +Gene: In-depth profiling of miRNA binding a gene of interest

miR-eCLIP +Gene enrichment increases miRNA-mRNA chimeric reads on a gene of interest 50 to 300-fold. Added miRNA-mRNA read coverage in miR-eCLIP +Gene enrichment identifies additional targeting miRNAs and reveals sites co-targeted by several different miRNAs, many with the same seed matching sequence.

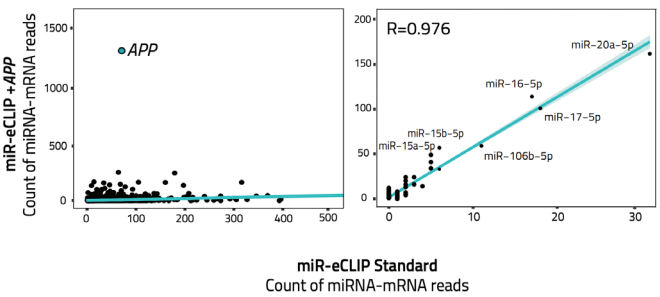

Figure 5. *APP* gene enriched miR-eCLIP libraries increase miRNA-mRNA reads on the gene of interest (left) and per targeting miRNA (right) with high correlation to miR-eCLIP Standard libraries (R=0.98).