miR-eCLIP™

Direct, transcriptome-wide identification of microRNA targets

miR-eCLIP Standard

Identify direct miRNA target sites for all expressed miRNAs transcriptome wide

miR-eCLIP +miR

Enrich miRNA target sites for miRNA(s) of interest transcriptome wide

miR-eCLIP +Gene

Enrich to identify miRNA target sites for all expressed miRNAs binding a gene of interest

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.

Specifications

| Input Range	5 M cells
Starting Material	UV Crosslinked cells or tissue
Sequencing Depth Suggestion	Standard miR-eCLIP: 50M reads miR-eCLIP +miR: 25M reads miR-eCLIP +Gene: 25M reads
PE/SE	Paired End

Direct miRNA target site detection

miR-eCLIP identifies unambiguous direct miRNA target sites by sequencing miRNA-mRNA chimeras.
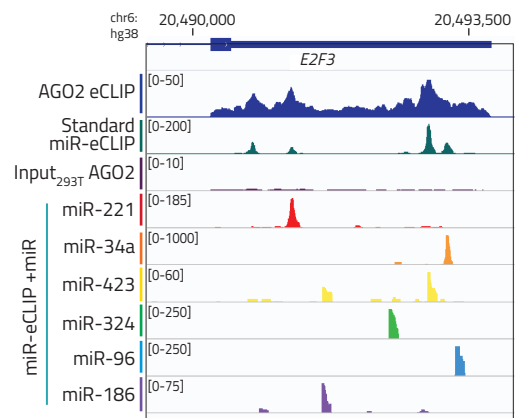
Figure 1. miR-eCLIP and AGO2-eCLIP read densities on the E2F3 gene 3'UTR illustrating several miRNA binding events. Bottom read densities indicate miR-eCLIP +miR enrichments for miR-221 (red), miR-34a (orange), miR-423 (yellow), miR-324 (green), miR-96 (blue), and miR-186 (purple).

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.
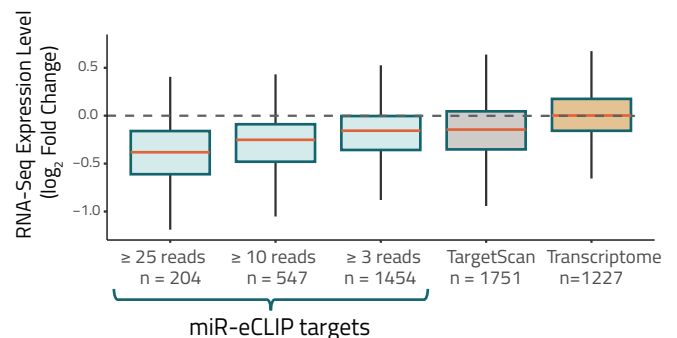

Figure 2. miR-eCLIP and RNA-Seq libraries were generated from HEK293xT cells transfected with miR-124 mimics. Expression levels of miR-eCLIP target genes show more downregulation with more miRNA-mRNA chimeric read coverage while only limited repression is observed for miR-124 TargetScan predicted target genes.

miR-eCLIP +Gene: In-depth profiling of miRNA binding on 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.
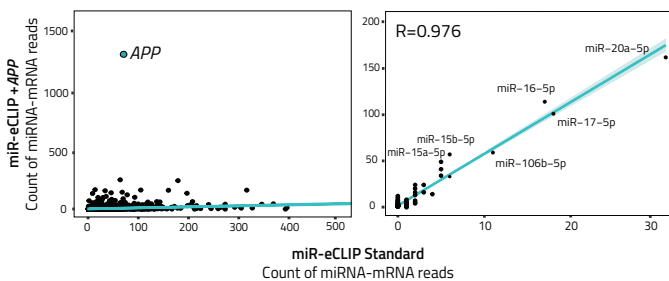
Figure 3. APP gene enriched miR-eCLIP libraries increase miRNA-mRNA reads on the gene of interest (left) and per targeting miRNA (right) with high correlation to miR-eCLIP Standard libraries (R=0.97).
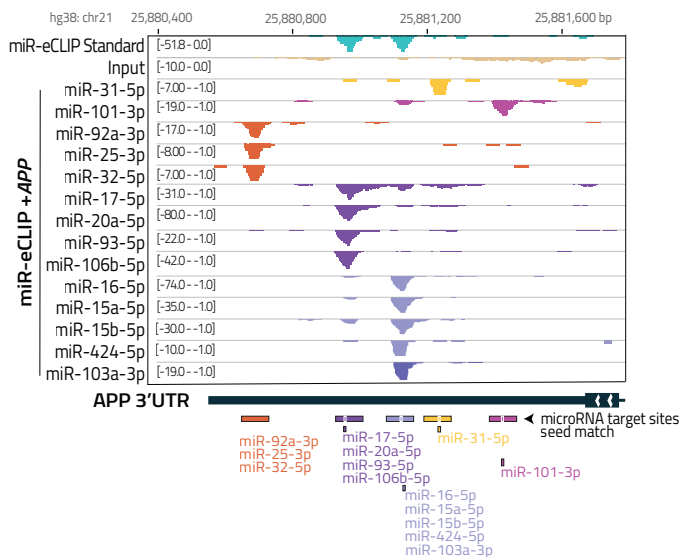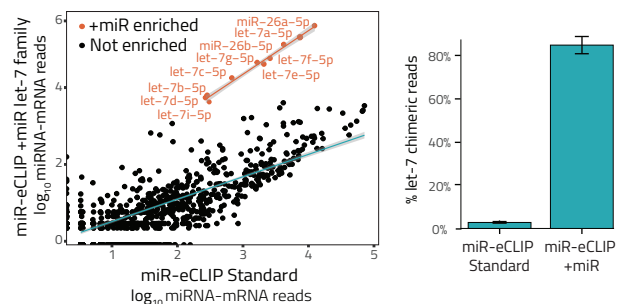

Figure 4. miR-eCLIP miRNA-mRNA chimeric read densities for +*APP* gene enriched and Standard unenriched libraries. miR-eCLIP +*APP* tracks display densities for individual miRNA binding events grouping those with similar seed sequence by color.

Ordering Information

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

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 5. 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 Workflow
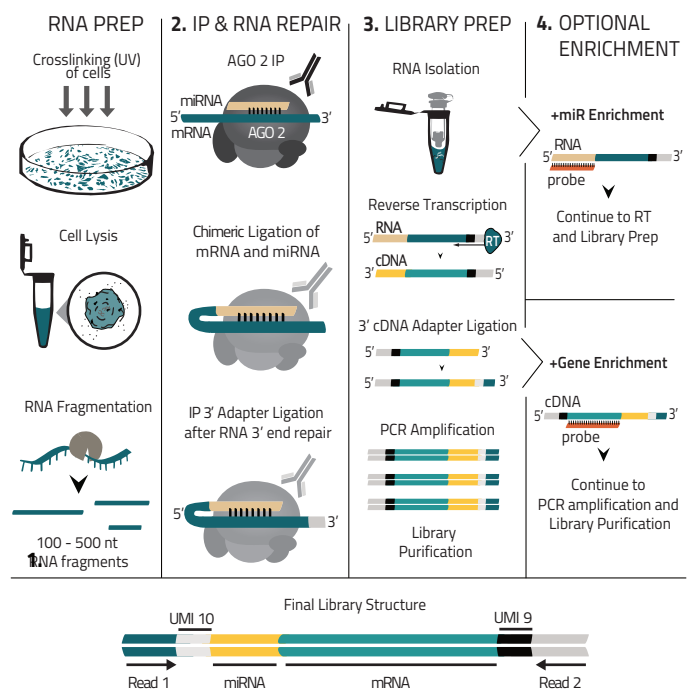
Figure 6. miRNA-mRNA molecules in the AGO2/RISC complex are immunoprecipitated using an Eclipse Bioinnovations AGO2 antibody. The miRNA and mRNA are then ligated to each other to form chimeric RNA molecules.