Deep quantitative profiling of miRNA and siRNA targets using miR-eCLIP enrichment of sRNA:target chimeras

Sergei A Manakov[1], Alexander A Shishkin[2,3], Brian A Yee[2], Kylie A Shen[1], Diana C Cox[1], Samuel S Park[2], Heather M Foster[1], Karen B Chapman[1], Gene W Yeo[2,4], Eric L Van Nostrand[2,5,6]

1. Eclipse BioInnovations, San Diego, CA
2. Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA
3. (Present address) Eclipse BioInnovations, San Diego, CA
4. Institute for Genomic Medicine, University of California San Diego, La Jolla, CA
5. (Present address) Verna & Marrs McLean Department of Biochemistry & Molecular Biology, Baylor College of Medicine, Houston, TX.
6. (Present address) Therapeutic Innovation Center, Baylor College of Medicine, Houston, TX

Abstract

Experimental identification of interactions between small regulatory RNAs (sRNA) and their targets is critical for understanding RNA biology development RNAi therapeutics. We developed miR-eCLIP, an AGO2 eCLIP assay with chimeric ligation, where gel and nitrocellulose transfer steps can be omitted to simplify the technique, increase yield and enable probe capture enrichment of chimeric reads. We show that this assay can be applied to deeply profile targets of one or more microRNAs (miRNAs) of interest, precisely map binding sites of a plurality of miRNAs targeting a gene of interest, as well as to detect target and off-target interactions of small interfering RNAs (siRNAs). In recent work (Manakov et al. bioRxiv 2022) we validated this technology by applying it to identify targets of miR-1 and miR-124 in HEK293xT cells transfected with mimics of these miRNAs.

Building on the miR-eCLIP proof of concept to study direct targets of miRNAs, we are now exploring how this technology can be utilized to identify off-target interactions of siRNAs. We applied miR-eCLIP assay to HEK293xT cells transfected with siRNA duplexes designed to specifically silence APP expression and detected interactions of the siRNA not only with the APP target transcript but also with hundreds of other transcripts. RNA-seq analysis of siRNA transfected cells showed that expression of siRNA-interacting transcripts was reduced, while enrichment of k-mers complementing the siRNA seed region indicated that these transcripts were silenced via a mechanism resembling miRNA-mediated silencing. Thus, miR-eCLIP provides a snapshot of miRNA-target and siRNA off-target interactions that can both be useful for researchers studying the biology of these sRNAs as well as for robust development of RNAi-based therapeutics.

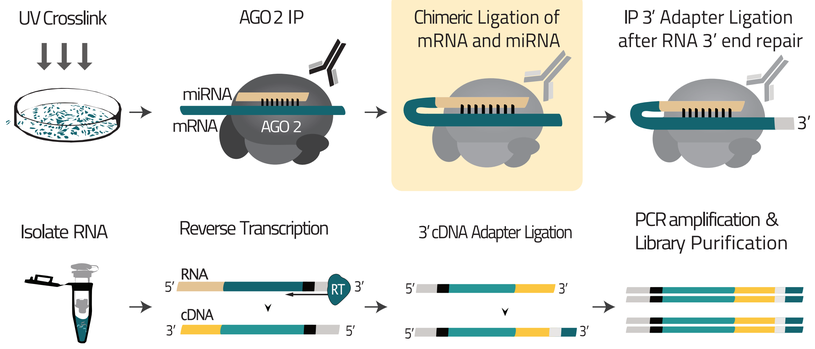
Assay work flow[1]

miR-eCLIP is a modified Enhanced Crosslinking and Immunoprecipitation (eCLIP) protocol

miRNAs are directly ligated to AGO2-bound target RNAs to generate chimeras

Sequencing of chimeras results in miRNA binding maps

Seed match is not required to detect targets



[1]Manakov et al., bioRxiv 2022.02.13.480296

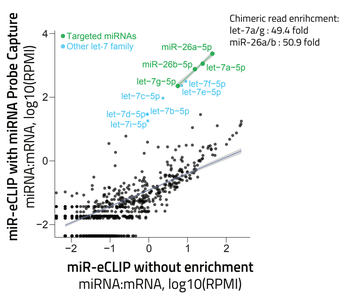
Probe capture enrichment of chimeric reads

Optional on bead probe capture enrichment step can be added to enrich chimeric reads for a target gene of interest or for one or more miRNAs of interest

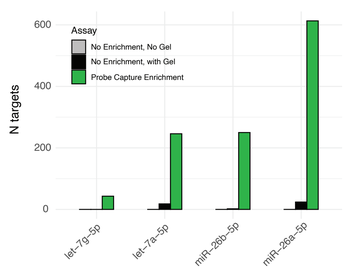
Capture with probes matching miRNAs resulted in 30 to 175 fold increase in recovery of chimeric reads for miRNAs of interest

Capture with probes for a gene of interest led to 75 fold increase in chimeric reads for miRNAs targeting the gene of interest

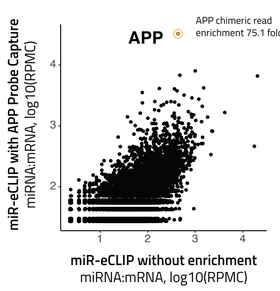
Enrichment of chimeric reads for miRNAs of interest



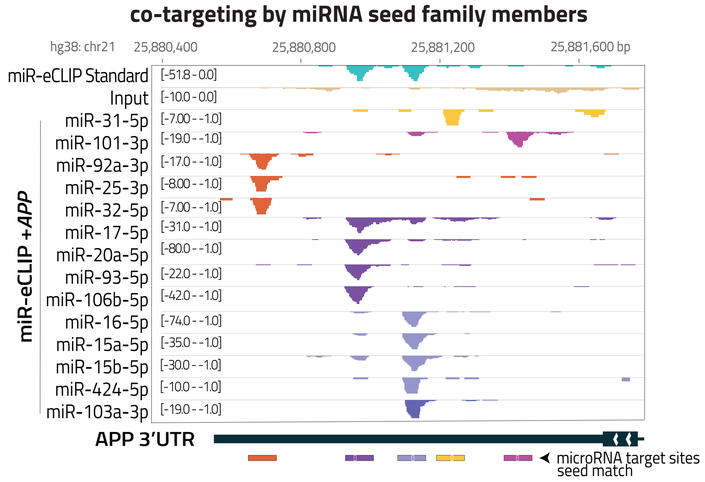
Increase in capacity to identify reproducible miRNA target sites



Enrichment for a gene of interest

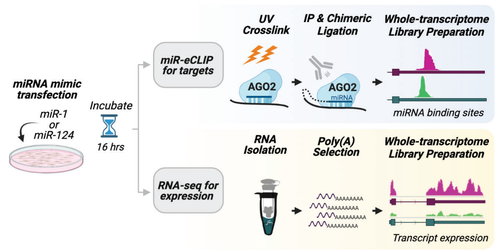


Enrichment with probes for a target gene of interest suggests co-targeting by miRNA seed family members



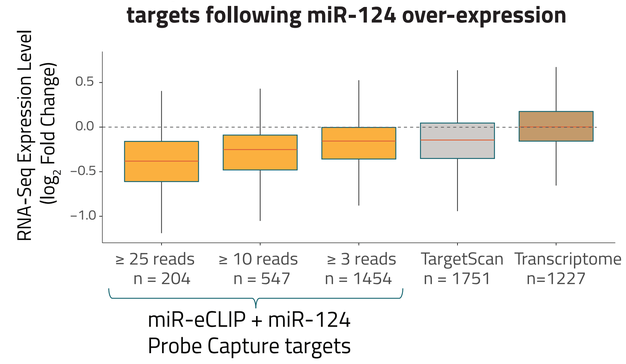
Validation

Expression of functional miRNA targets is reduced following miRNA over-expression



miRNA-overexpression paradigm is commonly used for genome-wide validation of miRNA targets. Elevated level of miRNAs results in destabilization of targeted transcripts (detectable with RNA-seq)

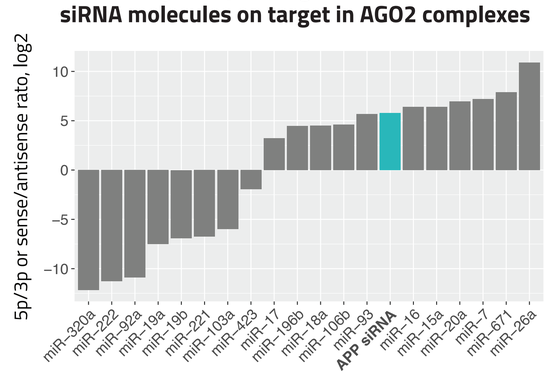
RNA-seq confirms reduction of miR-eCLIP miR-124 targets following miR-124 over-expression



Greater number of chimeric reads per target site predicts greater repression of the target

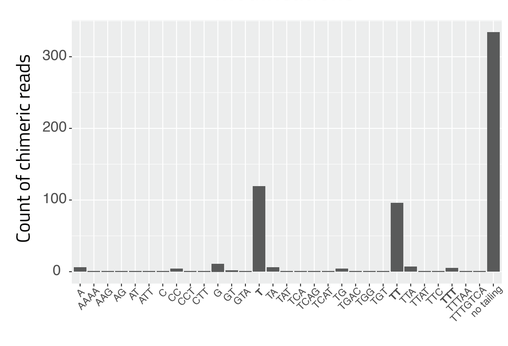
siRNA strand selection, tailing & off-target activity

Profile of "guide/passenger" ratio of miRNA and siRNA molecules on target in AGO2 complexes



Guide/passenger (miRNA) or sense/antisense (sRNA) strand ratio is readily discernible from sRNA sequences in chimeric reads, i.e. sRNA molecules in AGO2 complexes & engaged with targets

Profile of miRNA and siRNA 3′-end sequence modifications

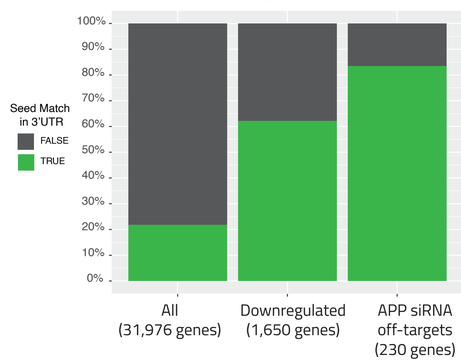


Analysis of non-templated "tailing" nucleotides at 3′-end of APP siRNA molecules engaged with targets in AGO2 complexes points at a widespread uridylation

APP siRNA miR-eCLIP off-targets show downward shift in expression following siRNA transfection

siRNAs off-target interactions reduce gene expression via a miRNA-like mechanism.

APP siRNA miR-eCLIP off-targets show enrichment in sites complementing the siRNA seed region



Off-targets were defined as genes with a 3′UTR peak of chimeric reads for APP siRNA. Seed match was not required to define the off-targets, yet the seed match enrichment was observed in 3′UTRs

Conflict of Interest Statement

GWY and ELVN are listed inventors on technology disclosures related to eCLIP and chimeric eCLIP to University of California San Diego that have been licensed by Eclipse BioInnovations. ELVN is co-founder, member of the Board of Directors, on the SAB, equity holder, and paid consultant for Eclipse BioInnovations. ELVN's interests in Eclipse BioInnovations and UCSD-owned intellectual property have been reviewed and this financial conflict of interest is managed by the Baylor College of Medicine in accordance with its financial conflicts of interest policies and procedures. GWY is co-founder, member of the Board of Directors, on the SAB, equity holder, and paid consultant for Eclipse BioInnovations. GWY's interests have been reviewed and approved by the University of California San Diego in accordance with its conflict-of-interest policies. SAM, KAS, and AAS are inventors on a patent filed by Eclipse BioInnovations on Methods and Kits for Enriching for Polynucleotides that covers probe capture methods.

Stay connected

miR-eCLIP on bioRxiv

Download data sheet