ECLIPSEBIO
THE RNA GENOMICS COMPANY

Research Summary

Using miR-eCLIP to Identify microRNA Targets Involved in Skeletal Muscle Disease

Introduction

MicroRNAs (miRNAs) are short, non-coding RNA molecules that bind to mRNA targets and down regulate their expression[1,2]. Several hundred miRNAs have been identified in humans, and many more are predicted to exist. It is estimated that more than half of all human mRNAs are controlled by one or more miRNAs. The number of diseases known to be caused by miRNA dysregulation continues to increase, making miRNAs and their mRNA target sites of growing interest as potential targets for therapeutic intervention.

Comprehensive mapping of miRNAs to their targets is crucial to understanding gene regulation. Eclipsebio's miR-eCLIP™ (miRNA-enhanced cross-linking followed by immunoprecipitation and next-gen sequencing[3]) assay provides an accurate method to unambiguously map direct miRNA target sites transcriptome-wide (Figure 1). The AGO2 protein carrying a miRNA is first cross-linked to its mRNA target. The complex is immunoprecipitated using a highly specific AGO2 antibody. A ligation step covalently joins the miRNA to its target, forming a chimera. The chimeric molecules are then deep sequenced and mapped to the genome. An optional enrichment step, for the miRNA or target transcript, can also be performed to enhance sensitivity.

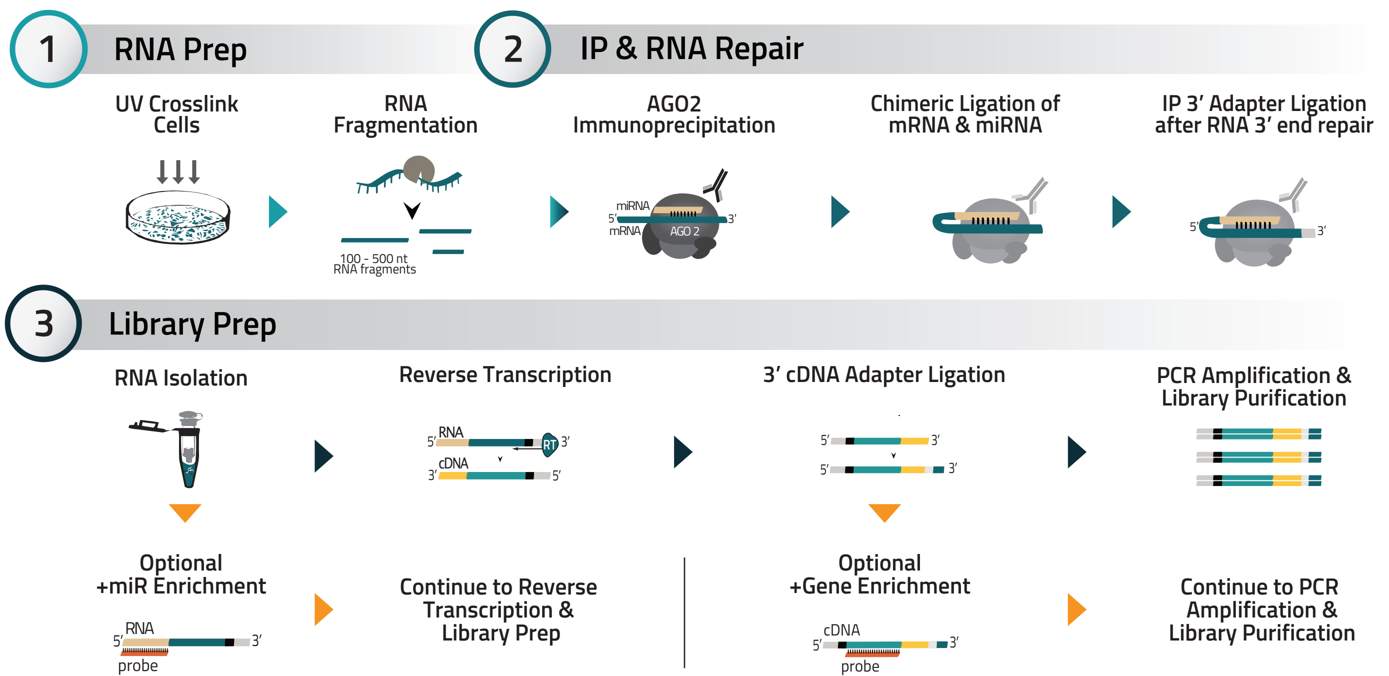

Figure 1. The Eclipsebio miR-eCLIP workflow. miRNA-mRNA molecules in the AGO2/RISC complex are immunoprecipitated using an Eclipsebio AGO2 antibody. The miRNA and mRNA are then ligated to each other to form chimeric RNA molecules.

Direct Identification of *in-vivo* miR-486 Gene Targets that Enable Dystrophic Disease

Samani *et al.*[6] have applied miR-eCLIP in the context of Duchenne muscular dystrophy (DMD). DMD is a debilitating muscular disease caused by mutation of the X-linked dystrophin gene, which produces a protein crucial for normal muscle development and function. Great strides have been made in the application of antisense oligonucleotides for DMD therapy, with several approved treatments available[5]. miRNAs are also potentially attractive therapeutic targets for DMD. Specifically, miR-486 is one of several muscle-enriched miRNAs known to be dysregulated in dystrophic muscle, and disease progression is associated with diminishing miR-486 levels. miR-486 is critical for normal muscle development and function and can compensate for a lack of dystrophin in mice.

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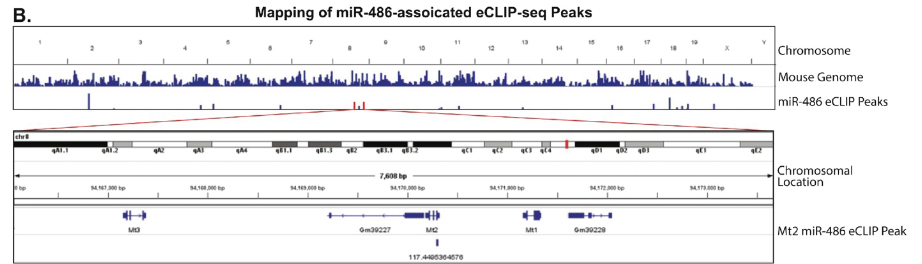
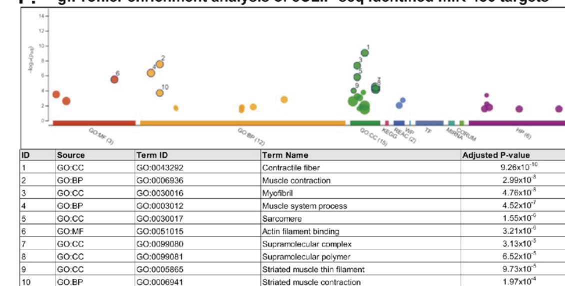
In this study the authors first characterize miR-486 knockouts in mice, showing pronounced skeletal and cardiac muscle phenotypes, in development as well as function, and perform RNA-Seq of the miR-486 knockouts revealing 286 genes with significant changes in expression. Next, the authors map miR-486 to its mRNA targets transcriptome-wide in both normal and diseased skeletal muscle using miR-eCLIP with PCR-based miR-486 amplification*.

miR-eCLIP identified 18 direct miR-486 target transcripts with binding primarily in 3' UTRs and coding regions (Figure 2B). The target genes are involved in muscle-related pathways (Figure 2F), including contractile fiber, muscle contraction, myofibril, sarcomere and actin filament binding. A unique aspect of this study was the combination of the miR-486 knockout RNA-Seq expression changes and the wild-type miR-eCLIP targets, revealing a complex network of directly regulated miR-486 targets. Many of the miR-486 targets revealed by miR-eCLIP have been shown to be involved in dystrophic disease progression, further validating the role that miR-486 has in dystrophic pathologies.





ID	Source	Term ID	Term Name	Adjusted P-value
1	GO:CC	GO:0043292	Contractile fiber	9.26×10^{-10}
2	GO:BP	GO:0006936	Muscle contraction	2.99×10^{-8}
3	GO:CC	GO:0030016	Myofibril	4.76×10^{-8}
4	GO:BP	GO:0003012	Muscle system process	4.52×10^{-7}
5	GO:CC	GO:0030017	Sarcomere	1.55×10^{-6}
6	GO:MF	GO:0051015	Actin filament binding	3.21×10^{-6}
7	GO:CC	GO:0099080	Supramolecular complex	3.13×10^{-5}
8	GO:CC	GO:0099081	Supramolecular polymer	6.52×10^{-5}
9	GO:CC	GO:0005865	Striated muscle thin filament	9.73×10^{-5}
10	GO:BP	GO:0006941	Striated muscle contraction	1.97×10^{-4}

Figure 2. A reproduction of Figure 5B and F from Samani *et al.* In B, all mir-486 eCLIP peaks are shown against the mouse genome. One such peak, for the *Mt2* transcript, is expanded showing the high resolution of the miR-eCLIP method. In F, the top 10 pathways enriched by g:Profiler for the 18 miR-486 targets identified using miR-eCLIP.

While antisense and siRNA therapeutics have been well accepted due to their specificity, miRNA-based therapeutics have lagged, possibly due to their multi-target mode of action. However, this biology could be leveraged to create a more potent drug - one that alters the expression of several related genes simultaneously. In order to advance miRNAs as a therapeutic, miRNA:target networks need to be better understood. Samani *et al.* provides a great example of this sort of groundwork. Eclipsebio's miR-eCLIP assay and other orthogonal technologies were used to unambiguously map miR-486 to its target mRNAs. This study provides a nice foundation for future efforts aimed at optimizing miR-486 as a therapeutic, for example, by causing overexpression of miR-486 to restore dystrophic muscle phenotypes.

"We are excited to integrate the power of miR-eCLIP technology towards identifying additional skeletal muscle microRNAs that influence other neuromuscular diseases to both identify new biomarkers and new miRNA targets for potential therapies." said University of Alabama's Associate Professor of Pediatric Neurology & Genetics, Matthew S. Alexander, PhD.

**This study used a previous version of miR-eCLIP whereby miRNAs are amplified using PCR. The current method uses probe-based enrichment prior to sequencing.*

References

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