RNA Genomics Technologies Enabling RNA Therapeutics and RNA manufacturing quality control

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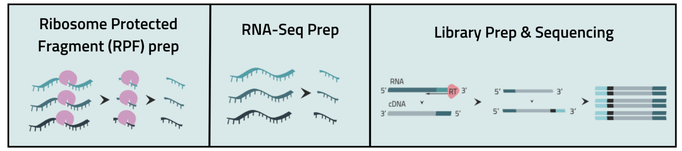
ECLIPSEBIO
THE RNA GENOMICS COMPANY

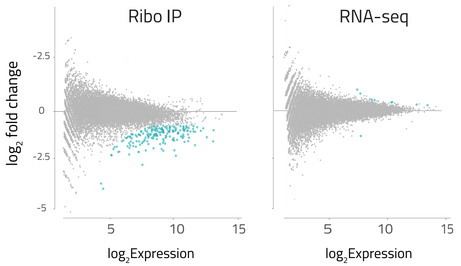
Abstract

The SARS-COV-2 pandemic coupled with the rapid and highly successful development of Covid mRNA vaccines highlight the immense potential of RNA as a therapeutic agent. Hundreds of RNA-based therapeutic programs are now being pursued, and RNA undoubtedly is here to stay as a molecule of the future in genomic medicine. The overall efficacy of an RNA drug relies on multiple features of the RNA molecule introduced during the entire drug development process from concept to manufacturing. This presentation will showcase RNA genomics assays developed at Eclipsebio to enable and accelerate RNA drug development through detailed end-to-end analysis and characterization of RNA. Assays and use case applications will be presented for RBP-eCLIP (to map RNA-binding protein binding sites), miR-eCLIP (to map miR and siRNA binding/off target sites), m6A-eCLIP (to map RNA modifications), End-Seq (to map 5′ translation start sites and 3′ poly-adenylation sites), eSHAPE (to map RNA reactivity/secondary structure), and eRibo to measure ribosome occupancy. Finally, we will discuss how this suite of technologies can be used in combination to provide a comprehensive map of RNA to develop not only tomorrow's RNA medicines but also critical RNA manufacturing quality control standards.

eRibo

eRibo is a suite of technologies that maps and quantitates ribosome occupancy transcriptome-wide. The ability to quantitate the ribosome occupancy on transcripts can yield insight into translational efficiency and provides a means to measure the differences in expressions between samples. One application of these technologies can be to look at differential ribosomal occupancy between compounds, mRNA sequences, or delivery vehicles

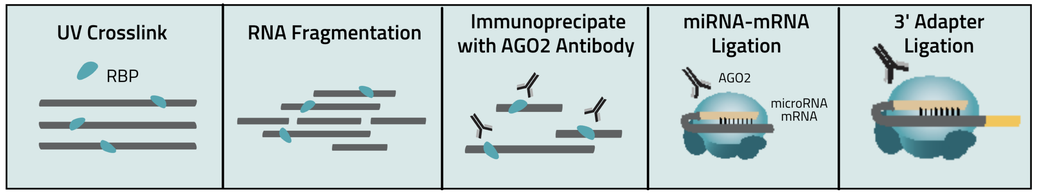


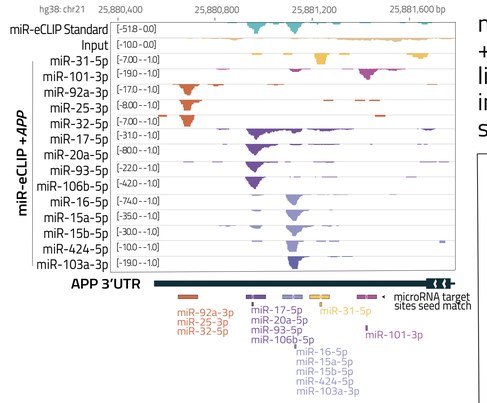


Cells were treated with a translational modifying compound, Torin-1, and a vehicle only control. Teal dots highlight genes specifically targeted at the translation (left) and transcriptional(right) level.

miR-eCLIP

Identification of the mRNA targets of miRNAs remains a critical challenge. miR-eCLIP enables precise mapping of direct miRNA-mRNA interactions transcriptome wide. miR-eCLIP can be used to identify miRNA binding sites on a gene of interest to inform sequenced based optimizations to get the ideal expression profile.

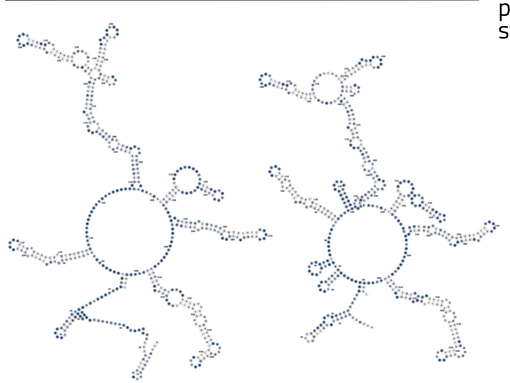




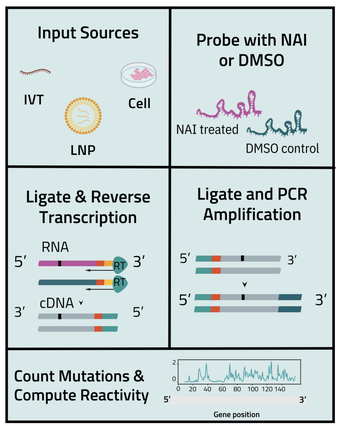
miR-eCLIP miRNA-mRNA chimeric read densities for +*APP* gene enriched and Standard unenriched libraries. miR-eCLIP +*APP* tracks display densities for individual miRNA binding events grouping those with similar seed sequence by color.

eSHAPE

Selective 2′-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) is a chemical probing method that measures RNA flexibility at single nucleotide resolution. As the exclusive licensee of the patented technology, we apply NAI to the RNA probing reaction and forms adducts with the free 2′-OH on the RNA backbone in single stranded regions. RNA strucutre is known to play a key role in RNA stability, immunogencitiy, translation, eSHAPE represents a way to emperically determine the strucutre of the RNA at all stages of development and delivery.

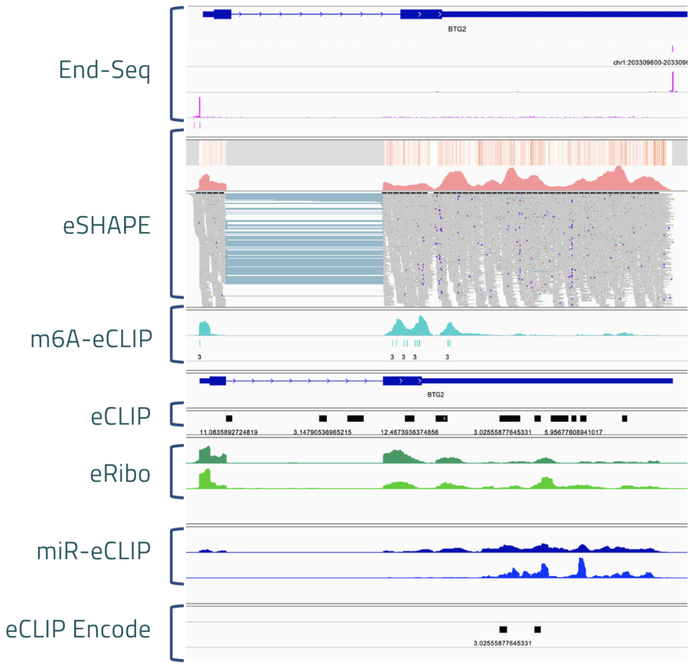


Left RNA folded using eSHAPE reactivity scores vs Right folded using just RNAfold prediction. Differences were observed between the eSHAPE-guided fold prediction and the standard fold prediction.



Comprehensive Map of RNA

RNA is a complex biomolecule that has enabled numerous new therapeutic modalities. However, due to these different complexities understanding the different functions and regulations of any given RNA represents an immense challenge. Eclipsebio's mission is to offer the most complete suite of technologies that can be used to comprehensively characterize how a RNA is regulated and if it is performing its intended therapeutic function.



Here we used six of our different technologies to gain a more complete insite into the target gene BTG2. Above is a genome view of these different technologies. Technologies not described in the poster are:
End-seq - to determine the 5′ and 3′ usage
m6A eCLIP - to identify base level resultion of m6A methylation
miR-eCLIP - to identify which miRNAs are binding RNA

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