m6A-eCLIP

Efficient and precise identification of m6A modifications on RNA

HIGHLIGHTS

More sites with less input

100-fold less mRNA than previously published methods

Unbiased m6A site specificity

Captures the known m6A DRACH motif and enrichment at stop codons and 3′ UTRs

Precise site resolution

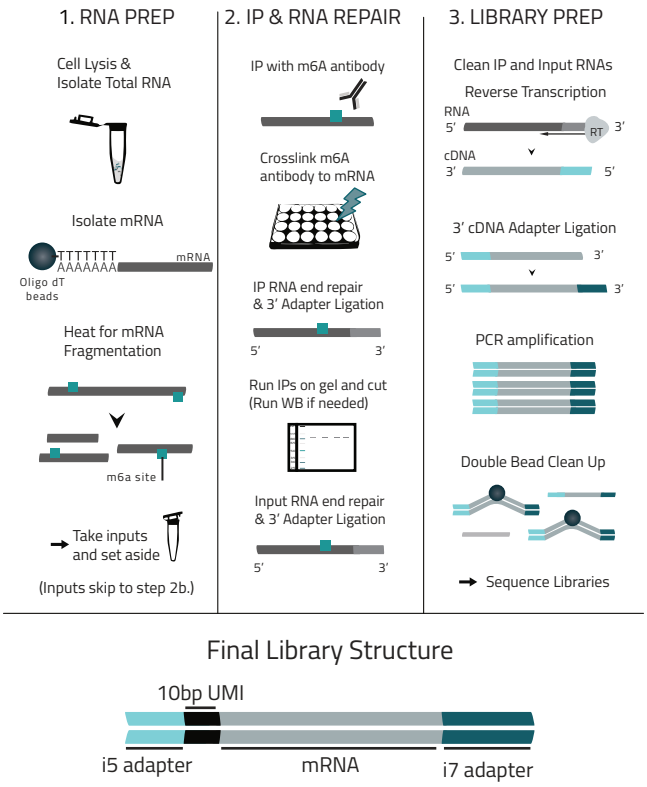
RNA-seq control significantly improves signal/noise to identify true modification sites

Introduction

N6-methyladenosine (m6A) is the most prevalent epitranscriptomic RNA modification found in eukaryotes and has been shown to regulate many aspects of RNA biology including splicing, secondary/tertiary structure, nuclear export, localization, stability, and translation. Levels of m6A modification at specific positions is tuned by writer and eraser enzymes. Modulation of m6A levels has been shown to play a role in stem cell renewal and differentiation and dysregulation of m6A states has been implicated in a wide variety of cancers. Moreover, m6A emerges as a mark of "self" RNA and acquisition of m6A by viral RNA plays a role in evading detection by the innate immune system. Thus, there is a need for methods to robustly profile m6A-modification at a high resolution.

m6A-eCLIP optimizes the enhanced crosslinking and immunoprecipitation (eCLIP) approach to profile m6A RNA modification sites transcriptome-wide with single nucleotide resolution. m6A-eCLIP requires 100-fold less starting material compared to other m6A profiling methods and yields more precise sites.

m6A-eCLIP Workflow



Final Library Structure

Figure 1. m6A-eCLIP transforms RNA analysis by streamlining mRNA isolation, eCLIP sample prep, and library prep, using a robust and reproducible approach to identify and map methylation sites on target RNA. The methylated RNA is immunoprecipitated using a highly specific Eclipse BioInnovations m6A antibody. RNA is chemically fragmented into 100 nucleotides or smaller fragments to generate high quality libraries.

Specifications

Sample Input Requirement	Total RNA	>20-40 ug
	RNA Concentration	>0.1 ug/ul
	RIN	>7
Sequencing Recommendations	Instrument	Illumina
	Sample Depth	40-50M reads
	Run Parameters	SE100

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Unbiased m6A modification sites

Consistent with the well-known biological feature of m6A modifications, peaks of m6A-eCLIP reads are enriched near the stop codons of genes and in the 3' untranslated regions (UTRs).

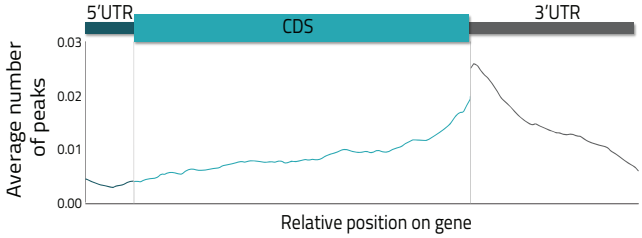

Figure 2. Metagene plot showing density of m6A peaks across a normalized gene transcript. m6A-eCLIP peaks are highest around the stop codon of transcript towards end of coding seqeunce (CDS) or in 3' UTRs.

Enabling single nucleotide m6A sites

Reverse transcriptase (RT) termination at the crosslink sites of the m6A antibody to RNA in m6A-eCLIP, yield fragments that once aligned the reference genome form sharp cliffs of read starts. These sharp cliff formations enable simple single-nucleotide modification site calling with m6A-eCLIP data.

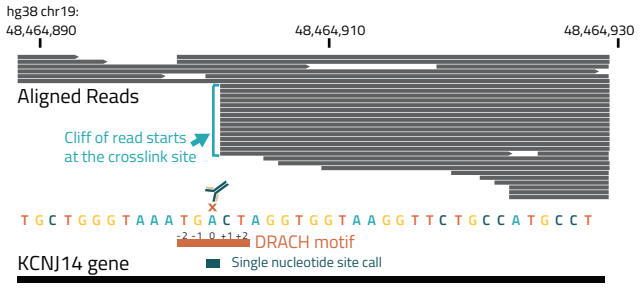

Figure 3. Example of a read cliff formed by aligned read starts at the crosslink site for a region of the KCNJ14 gene enabling the single nucleotide modification site call shown at the bottom. Genomic sequence shows an adenosine at this position centered directly within a DRACH motif. The DRACH motif uses IUPAC nucleotide codes where D = G, A or U, R = G or U, H = U, A or C.

Ordering information

More information about m6A-eCLIP kit and services online at www.nucleusbiotech.com or contact us at info@nucleusbiotech.com.

More precise m6A site resolution

m6A-eCLIP shows very similar read densities to a low-resolution m6A profiling technique m6A-RIP (meRIP), however m6A-eCLIP enables more precise m6A site calls, compared to the broader peak calls of me-RIP. The precise m6A modification site calls correlate with genomic DRACH motifs, a well characterized 5-nucleotide (nt) motif preferred by m6A methyltransferases (the DRACH motif uses IUPAC nucleotide codes where D represents an A, G or U nucleotide, R represents an A or a G nucleotide, and H represents an A, C or U nucleotide).

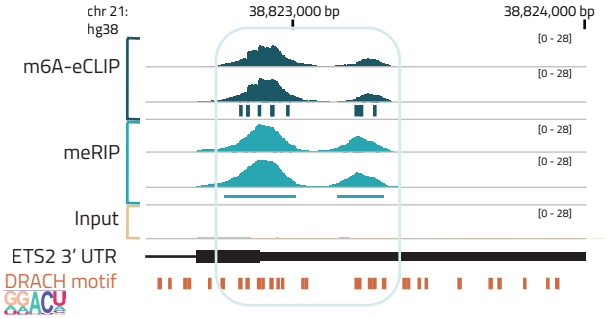

Figure 4. Elevated read densities near stop codon of the ETS2 gene region generated from m6A-eCLIP (darker teal) and comparable method me-RIP (lighter teal). Read cliffs in m6A-eCLIP allow for more specific modification site calls (shown as ticks beneath read density and are in line with genomic DRACH motifs (orange)).

DRACH motif specificity

m6A-eCLIP identifies over 70,000 enriched modification sites per sample of which more than 70% are positioned directly at adenosines and more than 50% are positioned at adenosines in the context of the DRACH motif.

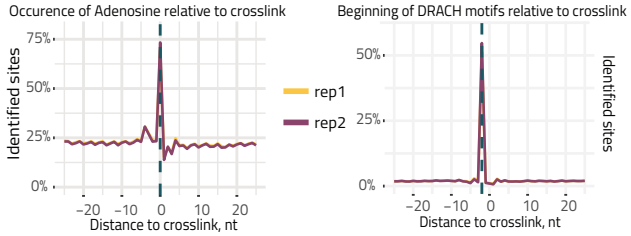

Figure 5. Enrichment of adenosines precisely at the crosslink sites (left). Enrichment of the first nucleotide of the DRACH motif shows the expected maximum 2 nt upstream of the crosslink sites - crosslinks map precisely to the A in DRACH.