3' End-Seq™

Reveal 3' UTR Landscape and Poly-A Usage Genome-wide

HIGHLIGHTS

Define 3' UTR Landscape

Genome-wide detection of known and novel poly-A sites (PAS) to define 3' UTRs.

Single Nucleotide Resolution

Poly-A sites are detected with single nucleotide resolution genome-wide.

High Confidence Poly-A Sites

3' End-Seq data analysis enriches the data sets for high confidence PAS by not calling sites that map to a genomic A-rich region.

Introduction

Poly-A sites (PAS) and the 3' UTR are involved in mRNA regulation and stability. Both PAS and 3' UTRs have been implicated in disease and can function as disease biomarkers and drug targets. 3' End-Seq is a technology for defining 3' ends of transcripts by sequencing from the PAS. 3' End-Seq can identify high confidence, known and novel PAS at single nucleotide resolution in 2 days.

Define 3' UTR Landscape

End-Seq can facilitate precise UTR identification for RNA therapeutics targeting and can be used as a tool for identifying UTR biomarkers associated with disease. 3' End-Seq can quantify the relative usage of PAS across samples and indicate transcript isoform presence in annotated transcriptomes. Defining the 3' UTR with End-Seq helps predict binding factor motifs more reliably.

3' End-Seq Workflow

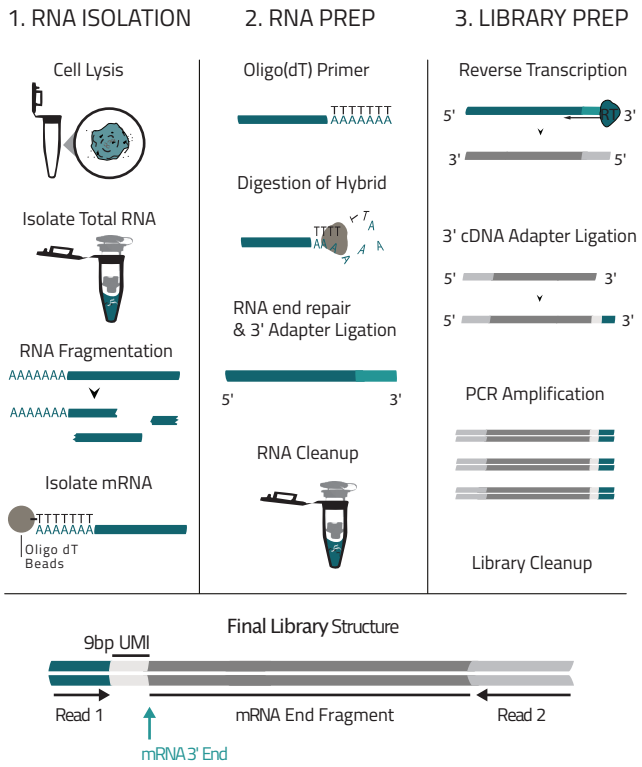

Figure 1. 3' End-Seq Workflow. Total RNA is isolated from cells, tissue or other source of RNA. RNA is enriched for short poly-A-positive mRNA fragments. After the poly-A tail is digested, adapters are ligated to the RNA fragment. The final library will contain an antisense mRNA end fragment, where the 5' end of the read will begin at the 3' end of the mRNA.

Specifications

Input Sample	Total RNA	>3 ug
	RNA Concentration	>0.1 ug/ul
	RIN	>3
Sequencing Recommendations	Instrument	Illumina
	Sample Depth	10-15M reads
	Run Parameters	SE100

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3' End Enrichment

3' End-Seq enriches the sample for reads in the 3' UTR 8-fold higher than the coding region. Greater depth of coverage around poly-A sites allows for more precise detection of known and novel PAS.

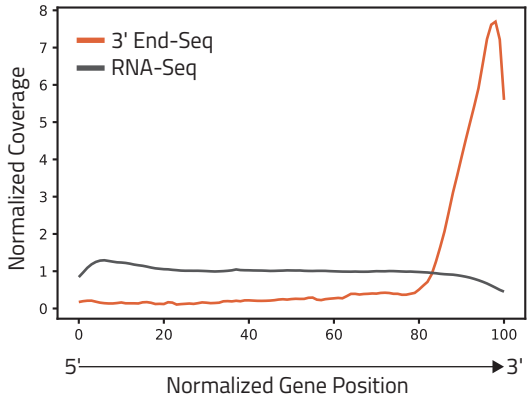

Figure 2. Comparison of read coverage across all hg38 gencode v35 transcripts. RNA-Seq sample shows even coverage across transcripts, while 3' End-Seq library shows enrichment at the 3' end.

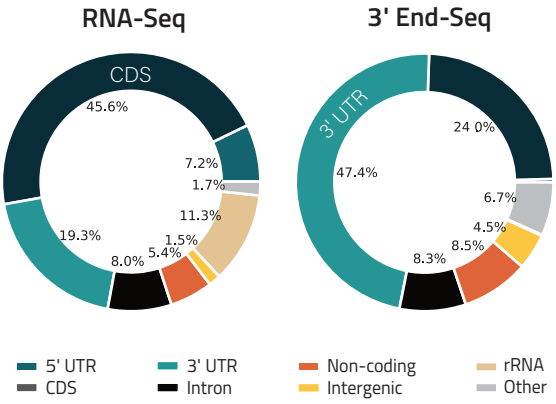

Figure 3. Distribution of reads in RNA-Seq and 3' End-Seq. Majority of reads in RNA-Seq samples are found in the coding region (CDS) of a transcript, while less than 20% of reads are found in the 3' UTR. 3' End-Seq samples constitute almost 50% of reads in the 3' UTR, while 24% are found in the coding region.

Ordering information

More information about 3' End-Seq services online at www.eclipsebio.com or contact us at info@eclipsebio.com.

Genome-Wide End Calling

3' End-Seq calls PAS genome-wide at single nucleotide resolution. Standard RNA sequencing does not allow for easy, genome-wide calling of PAS, as the reads are equally distributed along the transcript from the TSS to the PAS.

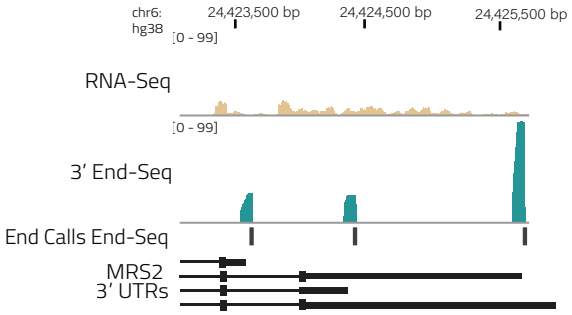

Figure 4. Example of poly-A site calling with 3' End-Seq data (teal) compared to RNA-seq sample (beige) for the gene MRS2. PolyA sites are called at three sites in 3' End-Seq sample, but are not defined in the RNA-Seq sample.

Novel Poly-A Site Discovery

3' End-Seq increases the confidence of detected PAS by removing any PAS reads caused by internal A-rich genomic regions. 3' End-Seq high confidence PAS detection allows the end user to discover novel, non-annotated PAS in their sample on a genome-wide basis.

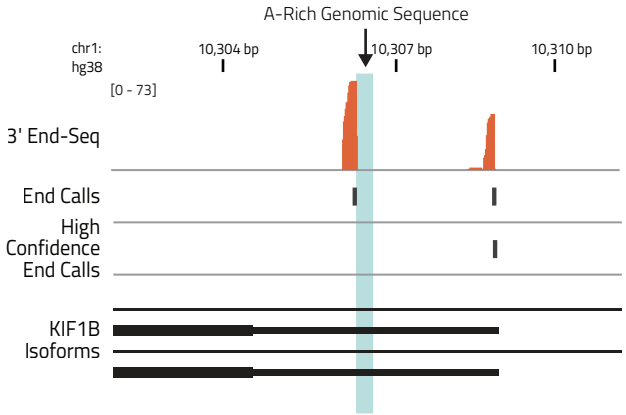

Figure 5. Example of single nucleotide end calls and filtering for high confidence end calls in the KIF1B 3' UTR.