5' End-Seq™

Reveal 5' UTR Landscape and Transcription Start Site Usage Genome-wide

HIGHLIGHTS

Define 5' UTR Landscape

Genome-wide detection of known and novel transcription start sites.

Single Nucleotide End Resolution

Transcription start sites are detected with single nucleotide resolution.

Identify Alternative Promoters

5' End-Seq detects alternative promoters for all endogenous genes.

Introduction

The transcription start site (TSS) is the location of transcription initiation at the 5' end of a gene sequence. Knowledge of the exact position of a TSS of an RNA molecule is crucial for the identification of regulatory regions immediately flanking it. 5' End-Seq facilitates active transcript end detection, enrichment and mapping, and identifies known and novel transcription start sites at single nucleotide resolution in only 2 days.

Define 5' 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. 5' End-Seq can quantify the relative usage of TSS across samples and indicates transcript isoform presence in annotated transcriptomes. Defining the 5' UTR with End-Seq can help predict binding factor motifs more reliably.

5' End-Seq Workflow

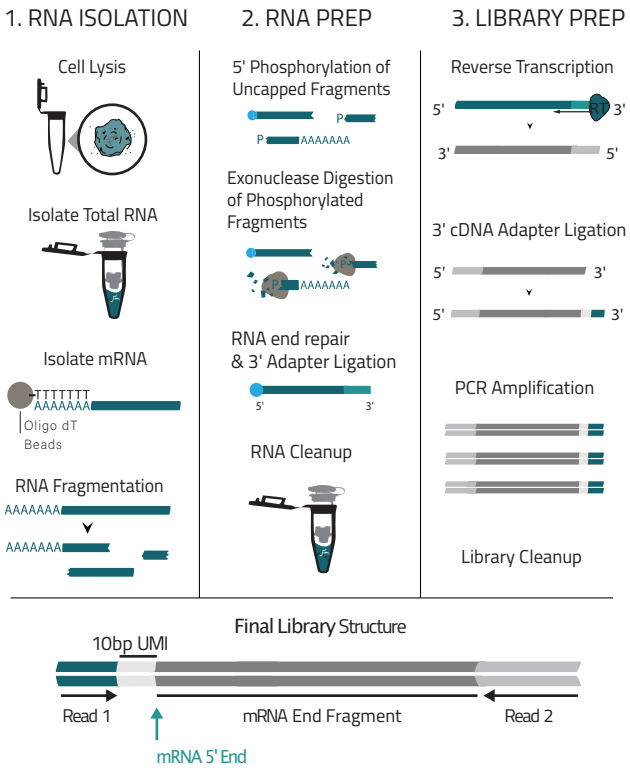

Figure 1. 5' End-Seq Workflow. Total RNA is enriched for polyA-positive mRNA fragments. mRNA is fragmented and uncapped fragments are removed enzymatically. The final library will contain a sense-strand mRNA end fragment, where the 5' end of the read will begin at the 5' end of the mRNA.

Specifications

Input Sample	Total RNA	>3 ug*
	RNA Concentration	>0.1 ug/ul
	RIN	>7
Sequencing Recommendations	Instrument	Illumina
	Sample Depth	10-20M reads
	Run Parameters	SE100

*End-Seq service requires submission of 6ug of total RNA

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5' End Enrichment

5' End-Seq enriches the sample for reads in the 5' UTR 4-fold higher than the coding region. Greater depth of coverage around TSS allows for more precise detection of known and novel TSS.

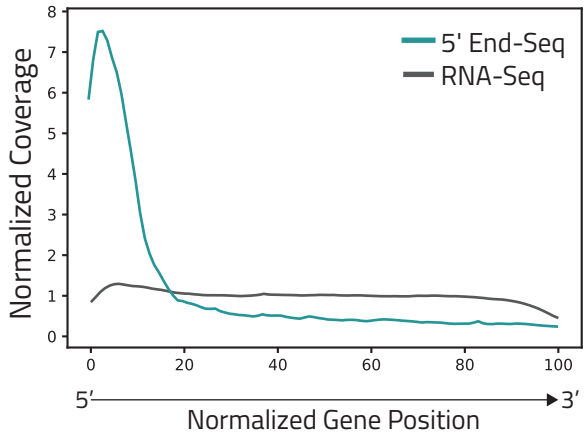

Figure 2. Comparison of read coverage across all hg38 gencode v35 transcripts. RNA-Seq sample shows even coverage across transcripts, while 5' End-Seq library shows enrichment at the 5' end.

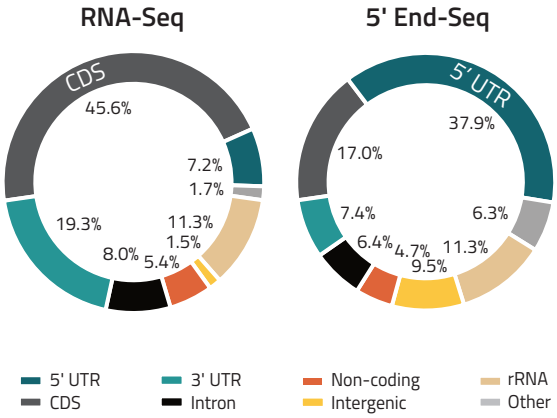

Figure 3. Distribution of reads in RNA-Seq and 5' End-Seq. Majority of reads in RNA-Seq samples are found in the coding region (CDS) of a transcript, while less than 10% of reads are found in the 5' UTR. Reads in the 5' UTR constitute almost 20% of total 5' End-Seq reads.

Ordering information

More information about 5' End-Seq services online at www.eclipsebio.com or contact us at info@eclipsebio.com.

Genome-Wide End Calling

5' End-Seq sequencing data can be used to call TSS genome-wide at single nucleotide resolution. RNA-Seq samples do not allow for calling TSS, as the read coverage does not change dramatically around each TSS. Easily calling TSS at single nucleotide resolution genome-wide may facilitate biomarker and therapeutic target identification.

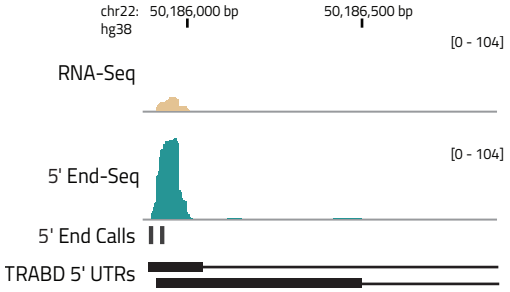

Figure 4. Example of transcription start site calling in RNA-Seq sample and 5' End-Seq sample for the gene TRABD. Transcription start sites are called confidently in 5' End-Seq sample, and not defined in the RNA-Seq sample.

Precise TSS Discovery

5' End-Seq is not only able to identify transcription start sites (TSS) with single nucleotide resolution, but the TSS end calls from 5' End-Seq data are near annotated TSS. RNA-Seq provides data spread across the entire gene but unable to call the 5' ends with the same precision as 5' End-Seq.

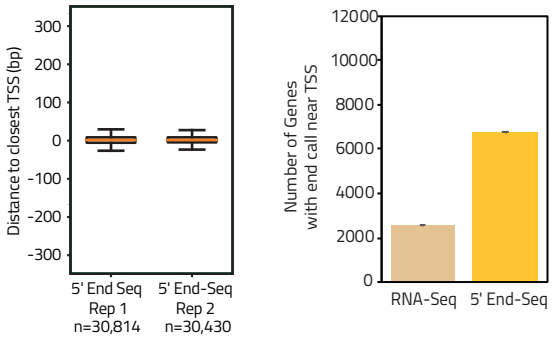

Figure 5. Distance from 5' End-Seq end call to the closest annotated TSS. End calls are found within bases or at annotated TSS. Applying an end call approach to RNA-Seq data identifies less precise 5' ends in fewer genes.