

A Sensitive Method For 5'-Terminal End-group Analysis of RNA Enabled by Terminator™ 5'-Phosphate-Dependent Exonuclease

Terminator™ 5'-Phosphate-Dependent Exonuclease (Terminator Exonuclease) is a processive 5'-3' exonuclease that selectively digests RNA with a 5'-monophosphate. RNA with a 5'-cap, a 5'-triphosphate, or a 5'-hydroxyl is resistant to degradation by Terminator Exonuclease (Table 1). As depicted in FIG 1, Terminator Exonuclease used alone or in conjunction with EPICENTRE Biotechnologies' APex™ Heat-Labile Alkaline Phosphatase (AP) and Tobacco Acid Pyrophosphatase (TAP), enables the user to ascertain the status of the 5'-end of an RNA.

Table 1. Terminator™ 5'-Phosphate-Dependent Exonuclease specifically digests RNA with a 5'-monophosphate. RNA with a 5'-hydroxyl, a 5'-triphosphate or a 5'-cap group are resistant to Terminator Exonuclease digestion.

RNA Structure	Degraded	Examples
^{5'} pN ~~~~~ OH ^{3'}	Yes	- Eukaryotic rRNA - Prokaryotic rRNA - RNA produced by RNase III processing
^{5'} GpppN ~~~~~ OH ^{3'}	No	- Eukaryotic mRNA (capped)
^{5'} pppN ~~~~~ OH ^{3'}	No	- Bacterial primary transcripts
Does not degrade double-stranded RNA or RNA with extensive secondary structure such as tRNA.		

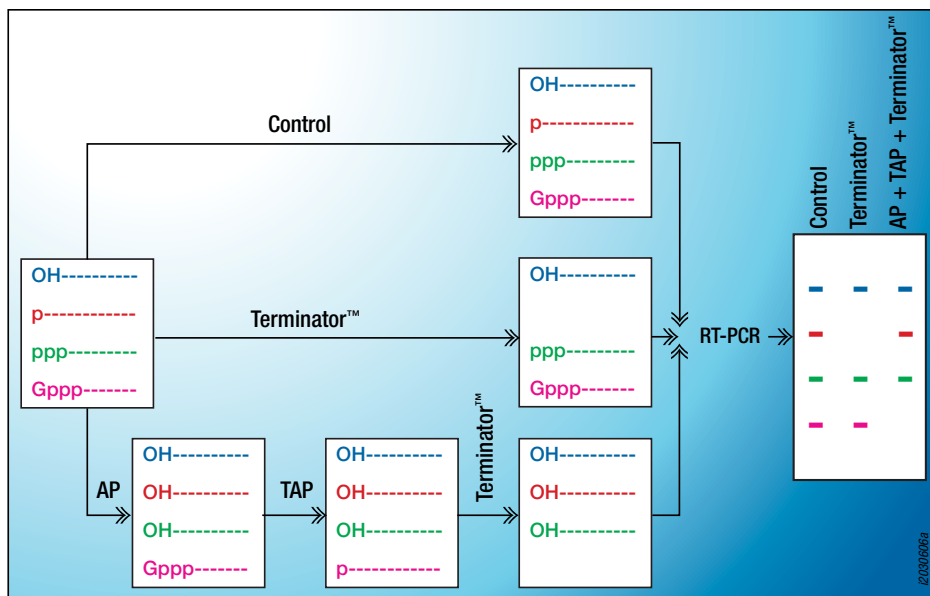


FIG 1. Overview of a process successfully used at EPICENTRE to analyze the 5'-terminal end-group of RNA using Terminator™ 5'-Phosphate-Dependent Exonuclease, AP and TAP. RT-PCR was used to monitor the status of individual transcripts.

Summarized below are two recent publications citing the use of Terminator Exonuclease to analyze the 5'-termini of siRNAs (Pak

and Fire) and miRNA-mediated mRNA degradation process (Wu, Fan, and Belasco).

Distinct populations of primary and secondary effectors during RNAi in *C. elegans*

Pak, J. and Fire, A. (2007) *Science* **315**(5809), 241.

The authors identified "secondary siRNAs" (synthesized by an RNA-directed RNA polymerase) as the majority of small RNAs found during ongoing RNA interference (RNAi) in *C. elegans*. Terminator™ 5'-Phosphate-Dependent Exonuclease was employed as one tool to characterize the 5'-termini of these siRNAs. The authors reported that the siRNAs were resistant to Terminator Exonuclease digestion indicating something other than a monophosphate at their 5'-end. Sequential treatment with alkaline phosphatase (to remove 5'-phosphates), T4 polynucleotide kinase (to add a single 5'-phosphate) and Terminator Exonuclease resulted in degradation of the siRNAs, thus providing evidence that the siRNAs have multiple phosphates at their 5'-ends. Further analysis lead the authors to conclude that these siRNAs have a 5'-triphosphate.

MicroRNAs direct rapid deadenylation of mRNA

Wu, L., Fan, J. and Belasco, J. G. (2006) *Proc. Natl. Acad. Sci.* **103**(11), 4034.

The authors chose miR-125b as a representative micro RNA (miRNA) to study the mechanism of gene expression regulation by miRNAs in eukaryotic cells. They showed that miRNA-mediated reduction of mRNA abundance is a result of deadenylation of the mRNA followed by rapid mRNA decay. During the course of the study they further determined that mRNA undergoing miR-125b-mediated deadenylation retained their 5'-cap structure as evidenced by their resistance to Terminator™ 5'-Phosphate-Dependent Exonuclease treatment. Prior treatment of the mRNAs with tobacco acid pyrophosphatase, to remove the 5'-cap structure, rendered the mRNAs susceptible to Terminator Exonuclease. The authors concluded that mRNA decay triggered by rapid deadenylation is an important mechanism by which miR-125b down-regulates gene expression.

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