

# Enrich Prokaryotic or Eukaryotic mRNA Using EPICENTRE's Terminator™ 5'-Phosphate-Dependent Exonuclease

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## Introduction

Terminator™ 5'-Phosphate-Dependent Exonuclease\* (Terminator Exonuclease) is a magnesium-dependent 5'-to-3' exonuclease that specifically digests RNA with a 5'-monophosphate, such as prokaryotic 16S & 23S rRNA and eukaryotic 18S and 28S rRNA. It does not digest eukaryotic RNA with a 5'-cap structure, primary prokaryotic mRNA with a 5'-triphosphate or degraded RNA having a 5'-hydroxyl group.

Terminator Exonuclease is not inhibited by proteinaceous RNase Inhibitors. Thus, treatment of intact, undegraded total RNA with Terminator Exonuclease provides an easy and rapid method to enrich for prokaryotic and/or eukaryotic mRNA, without the use of columns, beads or an immobilized oligo(dT) matrix (FIG 1). The success of a Terminator Exonuclease reaction is strongly influenced by the quality of the total RNA sample used in the reaction. Therefore, it is important to prepare and confirm the purity and integrity of the RNA sample prior to beginning the reaction. Here we demonstrate the use of Terminator Exonuclease to enrich for both prokaryotic and eukaryotic mRNA in total cellular RNA samples.

## Methods and Results

Total cellular RNA was purified from *E. coli* MG1655 cells or normal rat kidney (NRK) cells using EPICENTRE Biotechnologies' MasterPure™ RNA Purification Kit, and the integrity of the total RNA preparation confirmed using an Agilent 2100 bioanalyzer. Then 5 µg of total cellular RNA was incubated with 1U of Terminator Exonuclease in a 20 µl reaction for 1 hour at 30°C. The reactions were stopped by adding EDTA to a final concentration of 5 mM. The resulting RNA was purified and concentrated by phenol extraction followed by ethanol precipitation and resuspension in RNase-free water or TE Buffer.

FIG 2 shows Agilent 2100 bioanalyzer profiles of NRK RNA evaluated before and after Terminator Exonuclease treatment. As shown in FIG 2(A), prominent peaks of 18S and 28S rRNA

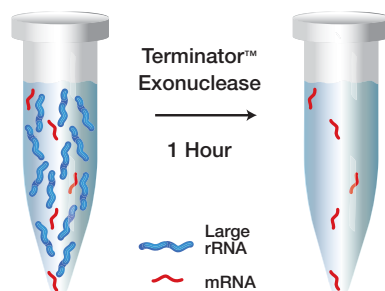


FIG 1. A 1-hour Terminator™ Exonuclease reaction digests the large rRNAs in a eukaryotic or prokaryotic total RNA sample, producing an enriched-mRNA preparation.

are present in the untreated sample. The Terminator Exonuclease-treated sample was concentrated 10-fold in order to detect any remaining 18S or 28S rRNA. However, as shown in FIG 2(B), both 18S and 28S rRNA were undetected following the Terminator Exonuclease treatment leaving a broad peak containing a highly enriched mRNA preparation.

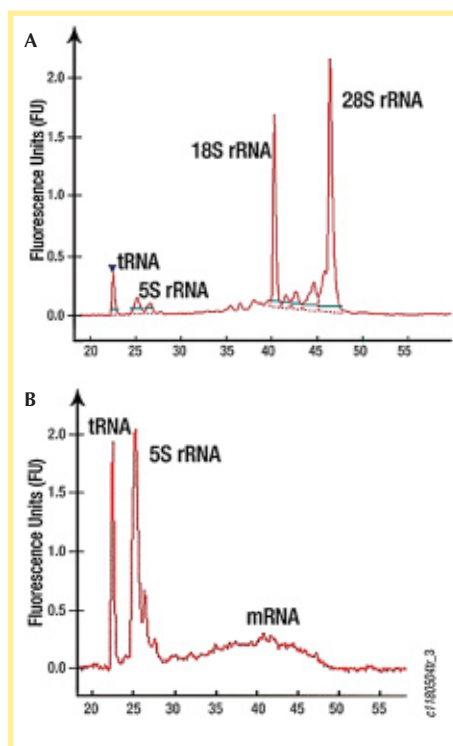


FIG 2. Normal rat kidney (NRK) total RNA before (A) and after (B) Terminator™ Exonuclease treatment. The Terminator Exonuclease-treated RNA was concentrated 10-fold.

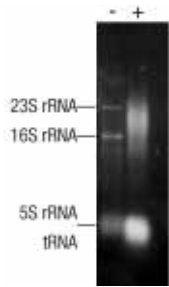
The 5S rRNA, which has a 5'-triphosphate group, and the tRNA, which has a 5'-monophosphate that is inaccessible due to tRNA secondary structure, are not digested by Terminator Exonuclease. If desired, the 5S rRNA and tRNA can be removed by selective precipitation of the mRNA using LiCl. This is accomplished by adding an equal volume of 5M LiCl to the Terminator Exonuclease-treated sample, followed by incubation at -20°C for 30 minutes, and centrifugation at full-speed in a microcentrifuge for 30 minutes at +4°C. The LiCl treatment selectively precipitates the mRNA, while 5S rRNA and tRNA remains in solution.

Two hundred nanograms each of Terminator Exonuclease-treated *E. coli* RNA and untreated *E. coli* RNA were evaluated by denaturing formaldehyde agarose gel electrophoresis. As shown in FIG 3, Terminator Exonuclease treatment removed the 16S and 23S rRNA that is clearly present in the untreated control, yielding a highly enriched prokaryotic mRNA preparation.

## Preservation of relative mRNA abundance levels after Terminator Exonuclease treatment

To make Terminator Exonuclease useful for obtaining mRNA for use in gene expression studies, it is important that the relative abundance of all mRNA transcripts in the sample is maintained. To evaluate the effect of Terminator Exonuclease on the relative abundance of different mRNA transcripts, quantitative real-time RT-PCR (qPCR) was performed on six different mRNA transcripts.

Two micrograms of human reference RNA and human skeletal muscle RNA were treated with 1U of Terminator Exonuclease for 1 hour at 30°C. The RNA was extracted and concentrated by ethanol precipitation and the entire sample was used in a 40 µl reverse transcription reaction using EPICENTRE's MMLV Reverse Transcriptase with random nonamer primers at 37°C for 1 hour. qPCR was performed using EPICENTRE's TAQurate™ GREEN Real-Time PCR MasterMix and optimized concentrations of target-specific primers. The abundance of beta-2-microglobulin



**FIG 3. Denaturing agarose gel analysis of *E. coli* total RNA before (-) and after (+) Terminator™ Exonuclease digestion.** The Terminator Exonuclease-treated RNA was concentrated 10-fold.

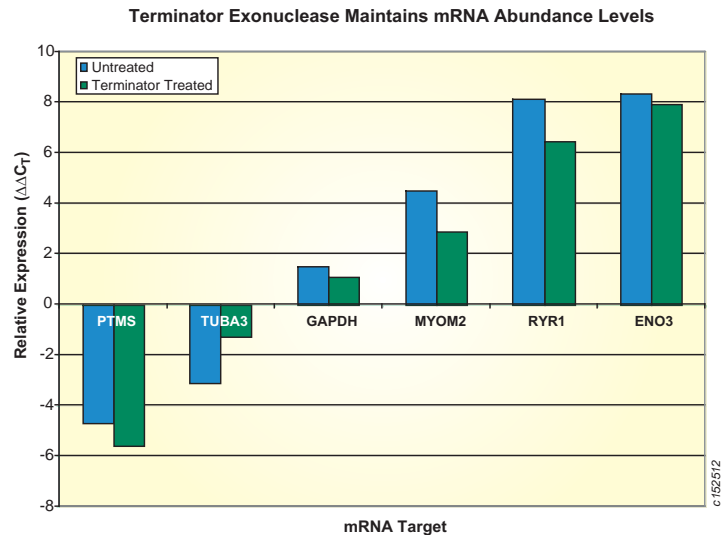
(B2M) in the samples was used for expression level normalization. Duplicate cDNA and qPCR reactions were performed, averaged, and normalized for each comparison. Simultaneous analysis was performed with normalization and test primers, and non-template controls were included. The results shown in FIG 4 are expressed as the difference in threshold cycles ( $C_T$ ) between the target message in one RNA sample (in relation to the normalizer) and that of the other RNA sample (in relation to the normalizer).

**Conclusion**

Terminator™ Exonuclease enables the rapid and simple production of highly-enriched mRNA preparations from total RNA without the use of columns, resins,

beads or immobilized oligo(dT). Terminator Exonuclease acts by selectively digesting the large prokaryotic and eukaryotic rRNAs while conserving the relative abundance of different mRNA species.

\*Patents pending.



**FIG 4. Terminator™ 5'-Phosphate-Dependent Exonuclease treatment of a total RNA sample maintains the relative abundance of the mRNA species in the sample as determined by qPCR (see text for details).**

[www.EpiBio.com/terminator.asp](http://www.EpiBio.com/terminator.asp)

**Terminator™ 5'-Phosphate-Dependent Exonuclease**  
 TER51020 1 U/μl 20 Units  
 Includes Terminator™ Exonuclease 10X Reaction Buffer.

**Make Circular ssDNA without “Bridges” or “Splints”**

EPICENTRE Biotechnologies’ new CircLigase™\* ssDNA Ligase is a thermostable ATP-dependent ligase that catalyzes the circularization of single-stranded DNA (ssDNA) having a 5'-phosphate and a 3'-hydroxyl group. CircLigase efficiently ligates the ends of ssDNA without the need for an oligo “bridge” (also called a “splint”) that is required when circularizing ssDNA using T4 DNA Ligase.

*CircLigase ssDNA Ligase:*

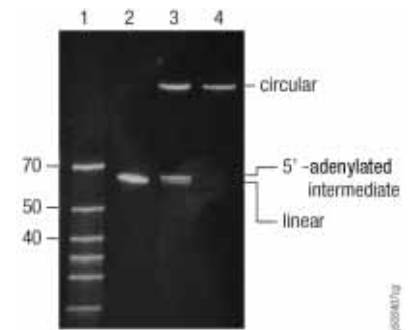
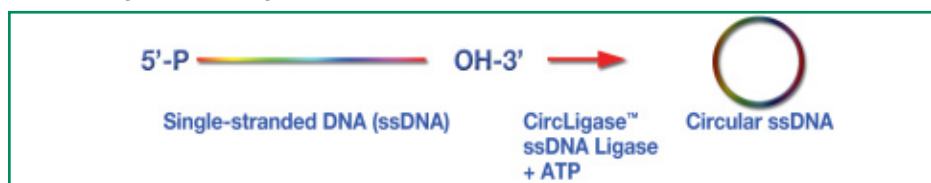
- Circularizes ssDNA of >30 bases.
- Standard reaction conditions produce no detectable ssDNA concatamers or concatameric DNA circles.
- Does not require oligo “bridges” or “splints.”

*Produce circular ssDNA templates for:*

- Rolling circle replication studies.
- Rolling circle transcription studies.
- RNA polymerase activity and inhibitor screening assays.

\*Patents pending.

**FIG 1. CircLigase™ ssDNA Ligase converts linear ssDNA to circular ssDNA.**



**FIG 2. CircLigase™ ssDNA Ligase converts linear ssDNA to circular ssDNA.** A 71-base ssDNA oligo was incubated at 60°C for 1 hour in a reaction containing CircLigase ssDNA Ligase and ATP. **Lane 1**, DNA markers; **Lane 2**, 71-base ssDNA prior to incubation; **Lane 3**, A circular ssDNA and an adenylated intermediate; **Lane 4**, Exonuclease I digested adenylated intermediate and residual starting linear ssDNA oligo, leaving only the circular ssDNA product.

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**CircLigase™ ssDNA Ligase**  
 CL4111K 1,000 Units  
 CL4115K 5,000 Units