LEXOGEN
The RNA Experts

TeloPrime Full-Length cDNA Amplification Kit V2

- Full-length cDNA generation with high yield
- Exceptional 5′ cap specificity
- Ideal for long-read NGS library generation (PacBio™ and Oxford Nanopore™)
- 1 ng – 2 µg total RNA input
- Flexible protocol enables use of custom primers for reverse transcription

Introduction

The TeloPrime Full-Length cDNA Amplification Kit V2 is an all-in-one protocol for generating full-length cDNA from as little as 1 ng of total RNA. Based on Lexogen´s unique Cap-Dependent Linker Ligation (CDLL) and long Reverse Transcription (long RT) technology, it is highly selective for full-length RNA molecules that are both capped and polyadenylated.

TeloPrime amplified cDNA provides a faithful representation of the mRNA transcriptome, empowering multiple downstream applications such as short- and long-read Next Generation Sequencing (NGS), cloning, or RACE. It enables the detection and correct quantification of splice variants and their true transcription start- and end-sites, for both short and long mRNA molecules.

Workflow

The TeloPrime protocol requires only around 1-1.5 hours of hands-on time. Full-length cDNA synthesis is initiated by oligodT primed long RT. This generates a stable RNA : cDNA hybrid that is maintained throughout post-RT purification and is important for the specificity of the subsequent CDLL reaction.

A double-stranded (ds) adapter with 5′ C overhang allows for atypical base-pairing with the inverted guanosine (G) of the cap structure. Ligation of the 5′ linker to the 3′ end of the cDNA is highly cap-dependent. By using a ds-specific ligase, ligation only takes place if the cap is present and if the RT has extended fully to the 5′ end of the mRNA. No ligation will occur in the absence of a 5′ cap, e.g., for degraded transcripts and at 5′ monophosphates, or if the RT has terminated prematurely because of secondary structures.

In the subsequent second strand synthesis and purification steps all remaining background is eliminated and only 5′-tagged, full-length cDNA is converted into full-length ds cDNA.

The final step is amplification of the full-length ds cDNA using 5′ and 3′ tag-specific primers, and Lexogen's new, optimized TeloPrime V2 PCR protocol.

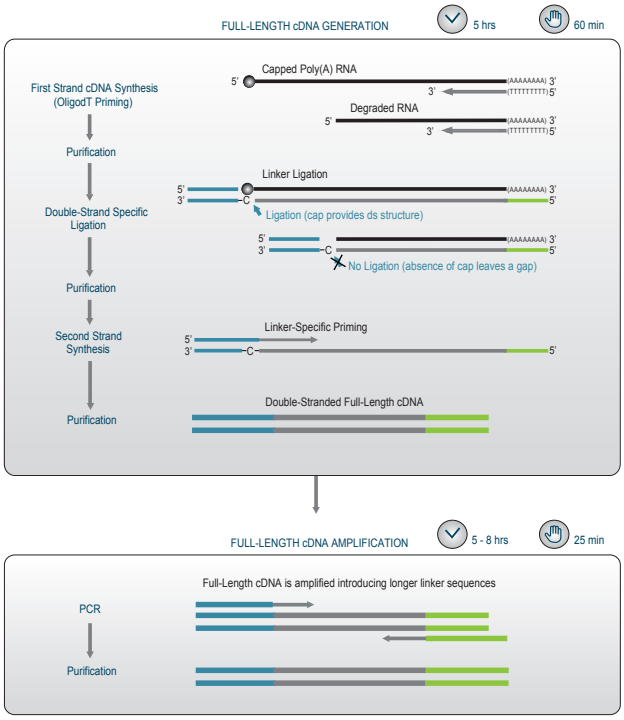

Figure 1 | Schematic overview of the TeloPrime Full-Length cDNA Amplification V2 workflow.

TeloPrime V2 - High Yields for Downstream Applications

The primary 5′ cap specificity of TeloPrime cDNA generation using Lexogen's CDLL technology has not changed in the TeloPrime V2 Kits. However, the V2 Kits feature a new enzyme mix and buffer system for second strand synthesis and PCR, enabling yields of up to 2 µg of full-length cDNA per PCR. This high-efficiency protocol also enhances the amplification of longer full-length cDNAs, which is particularly favorable for long-read sequencing applications.

Find more about TeloPrime at www.lexogen.com.
Contact us at info@lexogen.com or +43 1 345 1212-41.

Exceptional 5' Specificity

TeloPrime delivers superior 5' cap specificity compared to other full-length cDNA preparation methods. Short-read sequencing analyses revealed the highest precision of transcription start site (TSS) mapping for TeloPrime, compared to Template Switch and Oligo Capping methods (Fig. 2).

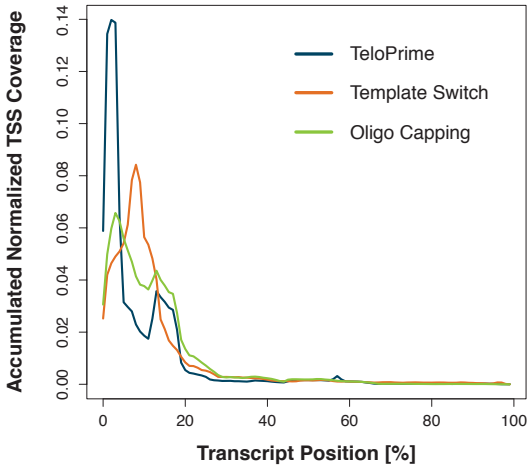

Figure 2 | TeloPrime Full-length cDNA Amplification Kit V2 enables high precision of transcription start sites (TSS) mapping. In an mRNA-Seq experiment, mRNAs were tagged at 5' end using TeloPrime's CDLL, Template-Switch or Oligo Capping technology (500 ng input). Illumina-compatible libraries were then generated from the full-length cDNA and sequenced. TSS were mapped to the human genome. The accumulated TSS read coverage is plotted versus the normalized annotated transcript length to show relative TSS mapping for the top 500 expressed genes.

Long-Read Sequencing Applications on PacBio™ and Oxford Nanopore™ Platforms

The 5' specificity and use of oligodT priming for full-length cDNA generation, together with the high-yield PCR, make TeloPrime V2 the ideal method of choice for long-read NGS applications. TeloPrime cDNA is fully compatible with downstream library preparation for sequencing on both Pacific Biosciences (PacBio™) [1] and Oxford Nanopore Technologies™ [2, 3] instruments. Recommendations for preparation of TeloPrime cDNA for Iso-Seq™ (PacBio™), or cDNA adapter ligation for Oxford Nanopore™ library prep, are provided in the TeloPrime V2 Kit User Guide and online frequently asked questions (FAQs), available on the TeloPrime product page at www.lexogen.com.

TeloPrime V2 cDNA prepared using the Iso-Seq™ protocol for sequencing on the Sequel® (PacBio™) is capable of producing extremely high outputs. Customer-prepared full-length cDNA reached an output of 32.5 GB with a P1 productivity level of 74.6 %, using 6 pM diffusion loading for a 20 hour SMRT cell run and version 2.1 chemistry [4]. Sequencing of TeloPrime cDNA on Oxford Nanopore™ MinION® has already successfully identified full-length transcripts for a number of different viruses [2, 3].

Custom Primers for Flexible cDNA Generation

The TeloPrime V2 Kits are designed for maximum flexibility and support the use of custom primers for reverse transcription (RT) and / or PCR. This approach has been successfully applied to full-length cDNA generation for both plant and vertebrate RNA samples (Fig. 3). Custom RT or PCR primers can also be used to add sample barcodes for multiplexed downstream sequencing approaches. The TeloPrime PCR Add-on Kit V2 (Cat. No. 018) contains additional reagents for PCR to enable further in-depth gene-specific analyses.

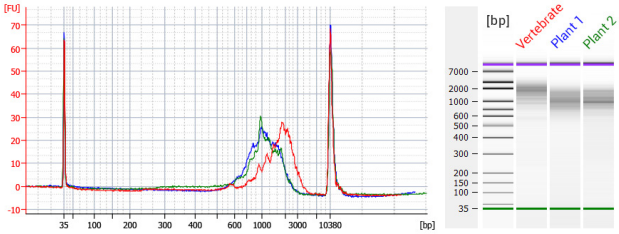

Figure 3 | TeloPrime cDNA generation with custom RT primers. TeloPrime cDNA was generated from 1 µg of total RNA of vertebrate or plant origin, using a custom reverse transcription primer. Endpoint PCR (TeloPrime V2) was performed using a custom PCR reverse primer and the standard TeloPrime Forward PCR primer (FP) with 17 cycles for vertebrate (red trace) or 21 cycles for plant samples (1: blue trace, 2: green trace). PCR products were purified using 1x AMPure® PB beads (PacBio™) and analyzed on a High Sensitivity DNA Chip (Bioanalyzer®, Agilent Technologies). *Customer-provided data reproduced with permission.*

Spike-In Controls

The TeloPrime CDLL technology also recognizes and tags 5' triphosphate structures. Therefore, controls such as Lexogen's Spike-In RNA Variant Control (SIRV) Sets 1, 2, and 3 (Cat. No.'s 025, 050, and 051), can be added to total RNA for TeloPrime cDNA preparation. SIRVs enable the assessment of transcript isoform representation and coverage in final sequencing data.

Customer Testimonials

"Recovering true full-length transcripts with the TeloPrime kit enables our customers to do cutting edge research. Establishing the kit at our facility with help of Lexogen's experts has been a truly satisfying and productive experience."

Laura-Maria Bayer, Sequencing Specialist,
NGS Facility VBCF, Vienna, Austria

"TeloPrime V2 PCRs have significantly improved yields compared to the previous version of chemistry, which makes it possible to construct sequencing libraries from relatively small amounts of input RNA (e.g. 1 µg total RNA for PacBio Iso-Seq)."

Dr. Yuanyuan Cheng, UQ Genomics Initiative,
The University of Queensland, Australia

References

[1] Cartolano M., et al. (2016) cDNA Library Enrichment of Full Length Transcripts for SMRT Long Read Sequencing. *PLoS One*, doi.org/10.1371/journal.pone.0157779.

[2] Moldován N., et. al. (2018) Multi-Platform Sequencing Approach Reveals a Novel Transcriptome Profile in Pseudorabies Virus. *Front. Microbiol.*, doi.org/10.3389/fmicb.2017.02708.

[3] Boldogkői Z., (2018) Transcriptomic study of Herpes simplex virus type-1 using full-length sequencing techniques. *Scientific Data*, 5: 180266, doi: 10.1038/sdata.2018.266.

[4] NGS Facility VBCF, Vienna, (2018) Unpublished data.

Additional TeloPrime publications are featured at www.lexogen.com.

Ordering Information

Catalog Numbers:
013.08 (TeloPrime Full-Length cDNA Amplification Kit V2, 8 preps)
013.24 (TeloPrime Full-Length cDNA Amplification Kit V2, 24 preps)
018.16 (TeloPrime PCR Add-on Kit V2, 16 rxn)

TELO™ PRIME
Full mRNA – precise ends