LEXOGEN

Enabling complete transcriptome sequencing

NEW VERSION

TELO™ PRIME

Full mRNA – precise ends

Full-Length cDNA Amplification Kit V2

User Guide

Catalog Numbers:
013 (TeloPrime Full-Length cDNA Amplification Kit V2)
018 (TeloPrime PCR Add-on Kit V2)
022 (Purification Module with Magnetic Beads)

013UG022V0200

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NEW VERSION

013UI188V0100

The TeloPrime Full-Length cDNA Amplification and PCR Add-on Kits have been updated to Version 2!

The TeloPrime V2 Kit update incorporates changes to the Kit Components and Protocols for second strand synthesis, qPCR, and endpoint PCR (steps 19 – 42). The primary cap-dependent ligation protocol for 5'-specific full-length cDNA generation has not changed.

The following kit components were updated:

- Enzyme Mix 3 (**E3** ○) – The V2 kits include a new enzyme mix which offers increased length distribution and yields for TeloPrime full-length cDNA.
- Second Strand Mix (**SS** ○) & TeloPrime PCR Mix (**Telo PCR** ○) – updated buffer formulations for new **E3** ○.
- PCR Forward and Reverse Primers (**FP** ○, **RP** ○) and DNA Buffer formulations were also changed to enhance PCR efficiency.

The detailed and short protocol steps were updated for second strand synthesis, qPCR and PCR, including:

- Reduced elution volume (step 19).
- Increased **SS** ○ volume (step 21).
- Reduced volume for **FP** ○ and **RP** ○ primers for qPCR and PCR.
- PCR cycle temperatures and extension times.

Additional changes to User Guide and General Recommendations:

- Column Binding Buffers 1 and 2 should be stored at -20 °C until first use and thereafter at room temperature.
- New information on Downstream applications for Iso-Seq™ (Pacific Biosciences), and Oxford Nanopore long-read sequencing, and use of custom primers for reverse transcription and PCR were added in Appendix D.

The TeloPrime PCR Add-on Kit V2 now contains 16 reactions and has a new product number: Cat. No. 018.16.

For further details of TeloPrime V2 kit updates please see the Revision History in the TeloPrime Full-Length cDNA Amplification Kit V2 User Guide (013UG022, Appendix E, p.27).

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When describing a procedure for publication using this product, please refer to it as Lexogen's TeloPrime Full-Length cDNA Amplification Kit V2.

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1. Overview

The TeloPrime Full-Length cDNA Amplification Kit V2 is an all-in-one protocol for generating full-length cDNA from 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 downstream applications such as Next Generation Sequencing (NGS), cloning, and RACE (Appendix D, p.25). It enables the detection and correct quantification of full-length splice variants and their true transcription start- and end-sites, for both short and long mRNA molecules.

In a first step, full-length cDNA synthesis is initiated by oligodT-primed long reverse transcription. This generates a stable RNA : cDNA hybrid that is maintained throughout post RT purification, which immediately preserves the complete RNA sequence information in the cDNA for cap selection.

This double stranded RNA : cDNA hybrid is also important for the specificity of the subsequent Cap-Dependent Linker Ligation reaction. A double-stranded adapter with a 5' C overhang allows for an atypical base-pairing with the inverted G of the cap structure. By using a double-strand-specific ligase, the ligation will only take place if the cap is present and if the RT has really reached the 5' end of the mRNA. No ligation will take place if no cap is present e.g., in degraded RNA (low RIN) or if the RT has terminated prematurely because of secondary structures.
Therefore the ligation of the 5' linker tag to the 3' end of the cDNA takes place in a highly cap-dependent manner. 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 double-stranded (ds) cDNA.

The full-length ds cDNA is then globally amplified by PCR, using 5' and 3' tag-specific PCR primers. To determine the Optimal Endpoint PCR (OEP) cycle number, additional PCR reagents are included for each sample, to enable both qPCR and endpoint PCR to be performed for each sample. For in depth gene-specific analysis, Lexogen offers also a TeloPrime PCR Add-on Kit V2 with 16 additional PCR reactions (Cat. No. 018). Custom primers can also be used for reverse transcription and/or cDNA amplification (see Appendix D, p.25).

Quantification and quality control of the final amplification product should be done using standard methods and is further discussed in Appendix C (p.24). The finished ds cDNA can be further used for (5') RACE, cloning, sequencing, and library or probe generation.

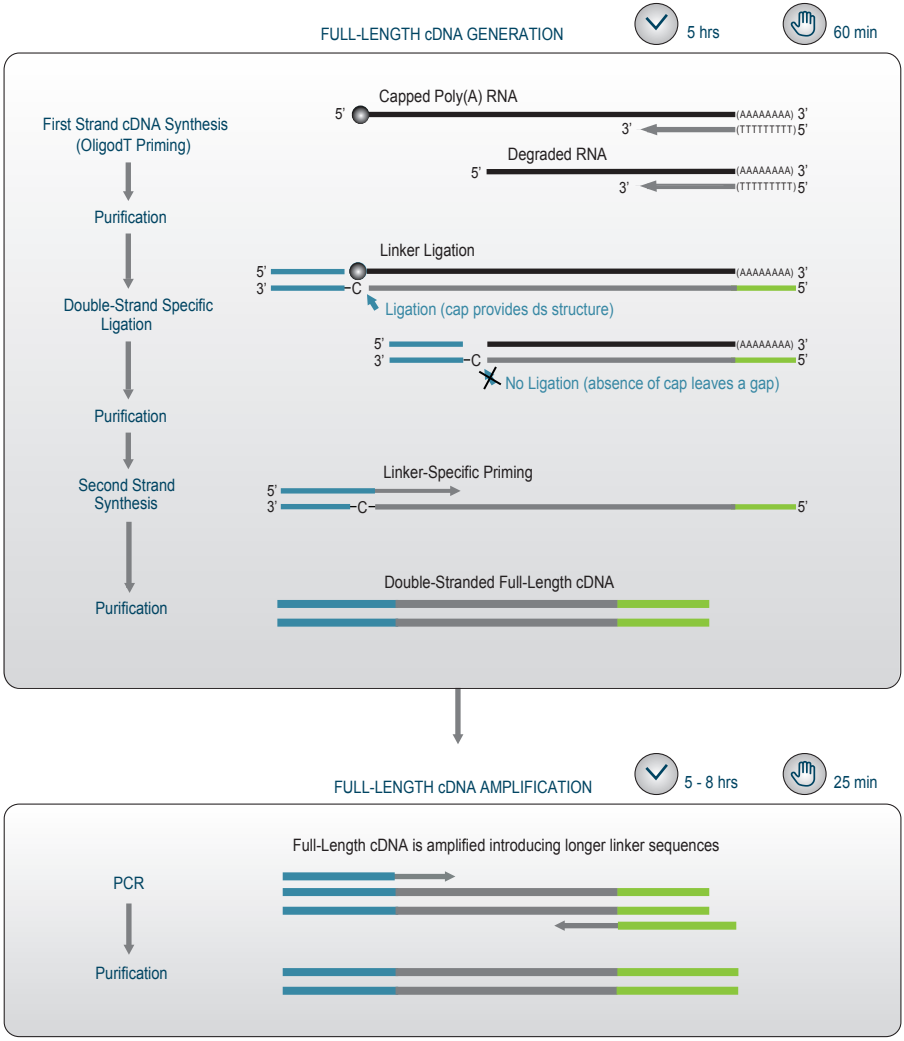

Figure 1. Schematic overview of the TeloPrime workflow.

2. Kit Components and Storage Conditions

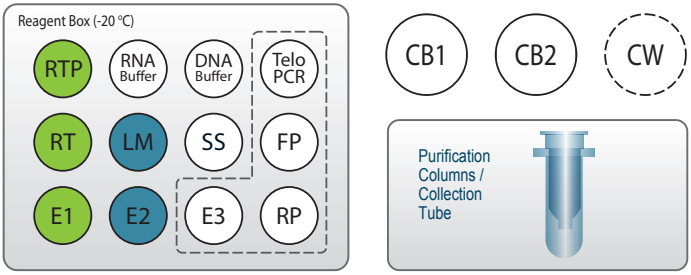

Figure 2. Location of kit components for Teloprime Full-Length cDNA Amplification Kit V2 (Cat. No. 013). The 24 prep kit contains two bottles of Column Wash Buffer (CW, dashed line). Kit components included in the Teloprime PCR Add-on Kit V2 (Cat. No. 018) are outlined with a dashed line in the Reagent Box.

Kit Components	Tube Label	Volume [1]			Storage
		4 preps *	8 preps [2]	24 preps	
Reverse Transcription Primer	**RTP** ●	8.8 µl	17.6 µl	52.8 µl	-20 °C
Reverse Transcription Mix	**RT** ●	17.6 µl	35.2 µl	105.6 µl	-20 °C
Enzyme Mix 1	**E1** ●	8.8 µl	17.6 µl	52.8 µl	-20 °C
Ligation Mix	**LM** ●	88 µl	176 µl	528 µl	-20 °C
Enzyme Mix 2	**E2** ●	8.8 µl	17.6 µl	52.8 µl	-20 °C
Second Strand Synthesis Mix	**SS** ○	35.2 µl	70.4 µl	211.2 µl	-20 °C
Enzyme Mix 3	**E3** ○	13.2. µl	26.4 µl[2]	79.2 µl	-20 °C
TeloPrime PCR Mix	**Telo PCR** ○	70.4 µl	140.8 µl[2]	422.4 µl	-20 °C
PCR Forward Primer	**FP** ○	8.8 µl	17.6 µl[2]	52.8 µl	-20 °C
PCR Reverse Primer	**RP** ○	8.8 µl	17.6 µl[2]	52.8 µl	-20 °C
RNA Buffer	**RNA Buffer** ○	220 µl	440 µl	1,320 µl	-20 °C
DNA Buffer	**DNA Buffer** ○	220 µl	440 µl	1,320 µl	-20 °C
Column Binding Buffer 1	**CB1** ○	2.11 ml	4.22 ml	12.67 ml	-20 °C/RT
Column Binding Buffer 2	**CB2** ○	1.41 ml	2.82 ml	8.45 ml	-20 °C/RT
Column Wash Buffer	**CW** ○	1.76 ml[3]	3.52 ml[3]	2x 5.28 ml[3]	RT

* Trial kit volumes. [1] Including 10 % surplus. [2] Volume included in TeloPrime PCR Add-on Kit V2 (Cat. No. 018.16).
[3] Excluding ethanol (to be added by user - see bottle for volume to add).

Upon receiving the TeloPrime Kit V2, store the Reagent Box, **CB1** ○, and **CB2** ○ in a -20 °C freezer. The PCR Add-on Kit V2 is also stored at -20 °C . The rest of the kit components including the purification columns should be stored at room temperature (RT). After the **CB1** ○, and **CB2** ○ are thawed the first time these buffers should be stored further at room temperature and protected from light at all times during storage.

Please refer to the information on the Column Wash Buffer (**CW** ○) bottles, for the amount of absolute ethanol to be added before use. Shake well to mix.

3. User-Supplied Consumables and Equipment

Check to ensure that you have all of the necessary materials and equipment before beginning the protocol. All reagents, equipment, and labware must be free of nucleases and nucleic acid contamination.

Reagents

- Absolute Ethanol (100 %) to add to Column Wash Buffer (**CW** ○).
- SYBR Green I (Sigma-Aldrich, Cat.No. S9430) 10,000x in DMSO) for qPCR.

Equipment

- Benchtop centrifuge (3,000 x g, rotor compatible with 96-well plates)
- Benchtop centrifuge (12,000 x g, rotor compatible with 1.5 ml and 2.0 ml micro-tubes).
- Calibrated single-channel pipettes for handling 1 µl to 1,000 µl volumes.
- Thermocycler.
- Vortex mixer.
- qPCR machine.
- Ice bath or ice box, ice pellets, benchtop cooler (-20 °C for enzymes).

Optional Equipment

- Automated microfluidic electrophoresis station (Agilent Technologies 2100 Bioanalyzer).
- Agarose gels, dyes, and electrophoresis rig.

Labware

- Suitable certified ribonuclease-free pipette tips (pipette tips with aerosol barriers recommended).
- 1.5 ml and 2 ml tubes with cap, low binding, certified ribonuclease-free.
- 200 µl PCR tubes or 96-well plates, with caps or sealing foil.

The complete set of materials, reagents, and labware necessary for quality control is not listed.

4. Guidelines

RNA Handling

- RNases are ubiquitous and special care should be taken throughout the procedure to avoid RNase contamination.
- Use commercial ribonuclease inhibitors (i.e., RNasin, Promega Corp.) to maintain RNA integrity when storing samples.
- Use a sterile and RNase-free workstation or laminar flow hood if available. Please note that RNases may still be present on sterile surfaces, and that autoclaving does not completely eliminate RNase contamination. Before starting cDNA synthesis, clean your work space, pipettes, and other equipment with RNase removal spray (such as RNaseZap, Ambion Inc.) as per the manufacturer's instructions.
- Protect all reagents and your RNA samples from RNases on your skin by wearing a clean lab coat and fresh gloves. Change gloves after making contact with equipment or surfaces outside of the RNase-free zone.
- Avoid speaking above opened tubes. Keep reagents closed when not in use to avoid airborne RNase contamination.

General

- Mastermixes are required for steps 2, 12, 21, 30 and 34 of the protocol. When preparing mastermixes and when using multi-channel pipettes, always include a 10 % surplus per reaction in order to have enough solution available for all reactions.
- Unless explicitly mentioned, all steps should be carried out at room temperature (RT) between 20 °C and 25 °C. Results may be negatively impacted if the protocol is performed at temperatures outside of this range. While reaction set-up is often performed at RT, incubation temperatures are explicitly defined, and must be strictly adhered to.
- To further increase reproducibility and to avoid cross contamination a centrifugation step should be performed after incubations at elevated temperatures and before removing the sealing foil from PCR plates or opening tubes.
- Ensure that adequate volumes of all reagents and the necessary equipment are available before beginning the protocol.
- Perform all pipetting steps with calibrated pipettes and always use fresh tips. Pipette carefully to avoid foaming as some solutions contain detergents.
- Thaw all necessary buffers at room temperature (RT), or as indicated in the preparation tables at the beginning of each step of the detailed protocol. Mix reagents well by vortexing or pipetting repeatedly and centrifuge briefly with a benchtop centrifuge to collect contents before use.

- Keep enzyme mixes at -20 °C until required or store in a -20 °C benchtop cooler.
- When mixing by pipetting, set the pipette to a larger volume. For example after adding 6 µl in step 4 use a pipette set to 15 µl or 20 µl to ensure proper mixing.
- The purification buffers **CB1** ○, and **CB2** ○ should be stored at -20 °C until first use. After thawing, store the buffers at room temperature. **ATTENTION:** Ensure that **CB1** ○ and **CB2** ○ are protected from light during storage.
- Before you start, check all solutions for the formation of precipitate, in particular: **CB1** ○, **CB2** ○, and **CW** ○. If necessary, incubate at 37 °C until buffer components dissolve completely, then equilibrate to room temperature again before use.
- If necessary, the protocol can be stopped at the indicated ☞ safe stopping points, where samples can be stored at -20 °C.
- When restarting the protocol after storing samples at -20 °C, ensure these are equilibrated to room temperature before proceeding to the next step. This is particularly crucial for restarting at purification (steps, 24 and 37). Cooler temperatures may affect the purification efficiency and result in reduced yields.

Pipetting and Handling of (Viscous) Solutions

- Enzyme mixes and Ligation Mix (**LM** ●) are viscous solutions that require care to pipette accurately. Quickly spin down the tubes to collect all liquid at the bottom of the tube. Be sure to pipette slowly and check the graduation marks on your pipette tips when removing aliquots.
- When drawing up liquid, the tip should be submerged 3 to 5 mm below the surface of the liquid, always at a 90 degree angle. Do not submerge the tip any further as viscous solutions tend to stick to the outside of pipette tips.
- Any residual liquid adhering to the tip should be removed by sliding the tip up the wall or edge of the tube from which the liquid was taken. Spin down the tube after use to ensure that all liquid is collected at the bottom of the tube for further storage.
- When dispensing, the pipette should be held at a 45 degree angle, and the tip placed against the side of the receiving vessel.
- When pipetting liquids from bottles, take special care that only the sterile pipette tip touches the bottle opening to prevent introducing RNases or other contaminants. Tips are sterile, whereas the pipette itself is not. If necessary, tilt the bottle to bring the liquid closer to the opening and facilitate pipetting

Safety Information

Column Binding Buffer 1 (**CB1** ○) and Column Binding Buffer 2 (**CB2** ○) contain guanidine isothiocyanate, an irritant, which might also be present in the flow-through fractions. This chemical is harmful, contact with acids liberates very toxic gas (hydrogen cyanide).

ATTENTION: Important Notes regarding Column Binding Buffers!

- Do not add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidine isothiocyanate.
- Solutions containing isopropanol or ethanol are considered flammable. Use appropriate precautions when using these chemicals.
- For your protection, always wear a laboratory coat, gloves, and safety glasses when handling these chemicals.
- Dispose of the buffers and chemicals in appropriate waste containers.
- Consult the appropriate Material Safety Data Sheets (MSDS), available at www.lexogen.com, and contact your Environmental Health and Safety department for proper work and disposal guidelines.

5. Detailed Protocol

5.1 Full-Length cDNA Synthesis

Preparation

First Strand Synthesis	Ligation	Second Strand Synthesis	Purification
RNA Buffer ○ – thawed at RT **RT** ● – thawed at RT **RTP** ● – thawed at RT **E1** ● – keep on ice or at -20 °C	**LM** ● – thawed at RT **E2** ● – keep on ice or at -20 °C	**SS** ○ – thawed at RT **E3** ○ – keep on ice or at -20 °C	**CB1** ○ – thawed at RT **CB2** ○ – thawed at RT **CW** ○ – stored at RT **100% Ethanol – provided by user!** **RNA Buffer** ○ – thawed at RT **DNA Buffer** ○ – thawed at RT
Thermocycler 70 °C, 30 sec 37 °C, 1 min hold at 37 °C to add MM 37 °C, 2 min 46 °C, 50 min 10 °C, ∞	Thermocycler 25 °C, 3 hrs / overnight 10 °C, ∞	Thermocycler 95.8 °C, 90 sec 62 °C, 60 sec 72 °C, 5 min 4 °C, ∞	**Purification Columns Collection Tubes** **2 ml tubes - provided by user** Thaw and equilibrate all reagents to room temperature before use

First Strand cDNA Synthesis – Reverse Transcription

RNA samples and the Reverse Transcription Primer (**RTP** ●) are briefly heated to resolve secondary structures and promote efficient hybridization of the primer before a reverse transcription is performed. For information on total RNA input see Appendix A, p.20.

OPTIONAL: To enhance yields for downstream Iso-Seq™ library prep and long-read sequencing (Pacific Biosciences), duplicate reactions for each starting RNA sample (1-2 µg total RNA per reaction) can be prepared and pooled prior to PCR amplification.

1. Dilute 1 ng - 2 µg of total RNA to a volume of 12 µl with **RNA Buffer** ○ and add 2 µl of Reverse Transcription Primer (**RTP** ●). Mix well. **ATTENTION:** Keep the RNA sample at room temperature after adding **RTP** ●. **REMARK:** A custom reverse transcription primer may also be used instead of the standard **RTP** ●. For details see Appendix D, p.25.
2. Prepare a mastermix containing 4 µl Reverse Transcription Mix (**RT** ●) and 2 µl Enzyme Mix 1 (**E1** ●) per reaction. Mix well. Keep the mastermix at room temperature before use.
3. Denature the RNA / **RTP** mix for 30 seconds at 70 °C in a thermocycler, then cool down to 37 °C, and incubate for 1 minute. Spin down briefly then place the samples back onto the thermocycler at 37 °C.
4. Leave the reaction on the thermocycler at 37 °C and add 6 µl of **RT** / **E1** mastermix to each reaction. Mix by pipetting, and seal the tubes or plate. Spin down briefly then place the samples back onto the thermocycler at 37 °C.

5 Incubate for 2 minutes at 37 °C, followed by 50 minutes at 46 °C, hold at 10 °C. Spin down before proceeding to the next step.

Purification

The cDNA : RNA hybrid is purified using silica columns to remove all reaction components.

ATTENTION: Important information for purification!

- Thaw Column Binding Buffer 1 (**CB1** ○) and equilibrate to room temperature before use. Incubate briefly at 37 °C to dissolve any precipitates if required, then cool to room temperature.
- Samples must be equilibrated to room temperature before beginning the purification.
- Add 100 % Ethanol to the Column Wash Buffer (**CW** ○) before use. The volume of ethanol to add is indicated on the bottle. Shake well to mix.
- The centrifuge temperature must stay between 18 - 25 °C (i.e., room temperature). Temperatures outside this range may affect purification efficiency.
- The flow-through does not need to be discarded between column washing steps.

6 Add a total of 160 µl Column Binding Buffer 1 (**CB1** ○) to the reaction. Mix well, and transfer the solution to a Purification Column placed in a 2 ml Collection Tube. Centrifuge for 1 minute at 12,000 x g. **REMARK:** Remove the flow-through using a pipette, or exchange the collection tube for a fresh 2 ml tube.

7 Apply 200 µl of Column Wash Buffer (**CW** ○) to the column and centrifuge for 1 minute at 12,000 x g.

8 Repeat this washing step once (for a total of two washes).

9 Discard the flow-through. Centrifuge for 2 minutes at 12,000 x g to dry the column.

10 Transfer the column to a new 1.5 ml tube and apply 19 µl of **RNA Buffer** ○ to the column. Incubate for 1 minute at room temperature and centrifuge for 2 minutes at 12,000 x g to elute the cDNA : RNA hybrid.

11 Transfer 18 µl of the eluted sample to a fresh PCR tube or plate.

Ligation

During this step the adapter is ligated to the cDNA in the hybrid by base-pairing of the 5' C to the cap structure of the RNA, using a double-strand specific ligase.

ATTENTION: Ligation Mix (**LM** ●) is a viscous solution and needs to be mixed thoroughly before use.

12 Prepare a mastermix containing 20 µl of Ligation Mix (**LM** ●) and 2 µl of Enzyme Mix 2 (**E2** ●) per reaction. Mix well. Keep the mastermix at room temperature before use.

13 Add 22 µl of **LM** / **E2** mastermix to each reaction. Mix well.

14 Incubate the reaction for 3 hours at 25 °C, then hold at 10 °C. **REMARK:** Ligation can also be performed overnight at 25 °C.

Purification

The ligated cDNA : RNA hybrid is purified using silica columns to remove all reaction components, including excess adapter.

ATTENTION: Important information for purification!.

- Samples must be equilibrated to room temperature before beginning the purification.
- The centrifuge temperature must stay between 18 - 25 °C (i.e., room temperature). Temperatures outside this range may affect purification efficiency.
- The flow-through should be discarded between column washing steps.

15 Add a total of 320 µl Column Binding Buffer 1 (**CB1** ○) to the reaction, mix well, and transfer the solution to a Purification Column placed in a 2 ml Collection Tube. Centrifuge for 1 minute at 12,000 x g. **REMARK:** Remove the flow-through using a pipette, or exchange the collection tube for a fresh 2 ml tube.

16 Apply 400 µl of Column Wash Buffer (**CW** ○) to the column and centrifuge for 1 minute at 12,000 x g. Discard the flow-through.

17 Repeat this washing step once (for a total of two washes).

18 Discard the flow-through. Centrifuge for 2 minutes at 12,000 x g to dry the column.

19 Transfer the column to a new 1.5 ml tube and apply 12 µl of **RNA Buffer** ○ to the column. Incubate for 1 minute at room temperature and centrifuge for 2 minutes at 12,000 x g to elute the cDNA : RNA hybrid.

20 Transfer 11 µl of the eluted sample to a fresh PCR tube or plate. ☞ Safe stopping point. The purified ligation product can be stored at -20 °C at this point.

Second Strand Synthesis

The full-length cDNA is converted to ds cDNA using the PCR Forward Primer (**FP** ○), which is included in the Second Strand Mix (**SS** ○).

21 Prepare a mastermix containing 8 µl of Second Strand Mix (**SS** ○) and 1 µl of Enzyme Mix 3 (**E3** ○) per reaction. Mix well. Keep the mastermix at room temperature before use.

22 Add 9 µl of **SS** / **E3** mastermix to each reaction. Mix well.

23 Conduct one cycle of thermocycling with the following program: 90 seconds at 95.8 °C, 60 seconds at 62 °C, 5 minutes at 72 °C, then hold at 4 °C.

Purification

The double-stranded cDNA is purified to remove all reaction components and any potential single-stranded template switch background of the Reverse Transcription Primer.

ATTENTION: Important information for purification!

- Thaw the Column Binding Buffer 2 (**CB2** ○) and equilibrate to room temperature before use. Incubate briefly at 37 °C to dissolve any precipitates if required, then cool to room temperature.
- Samples must be equilibrated to room temperature before beginning the purification.
- The centrifuge temperature must stay between 18 - 25 °C (i.e., room temperature). Temperatures outside this range may affect purification efficiency.
- The flow-through does not need to be discarded between column washing steps.

24	Add a total of 160 µl Column Binding Buffer 2 (**CB2** ○) to the reaction, mix well, and transfer the solution to a Purification Column placed in a 2 ml Collection Tube. Centrifuge for 1 minute at 12,000 x g. **REMARK:** Remove the flow-through using a pipette, or exchange the collection tube for a fresh 2 ml tube.
25	Apply 200 µl of Column Wash Buffer (**CW** ○) to the column and centrifuge for 1 minute at 12,000 x g.
26	Repeat this washing step once (for a total of two washes).
27	Discard the flow-through. Centrifuge for 2 minutes at 12,000 x g to dry the column.
28	Transfer the column to a new 1.5 ml tube and apply 20 µl of **DNA Buffer** ○ to the column. Incubate for 1 minute at room temperature and centrifuge for 2 minutes at 12,000 x g to elute the ds cDNA.
29	Transfer 18 µl of the eluted sample into a fresh PCR tube or plate. ☞ Safe stopping point. The purified full-length cDNA can be stored at -20 °C at this point. **OPTIONAL:** If preparing cDNA for Iso-Seq™ library prep (Pacific Biosciences), pool duplicate, purified cDNA samples before proceeding to qPCR and library amplification.

5.2 Full-Length cDNA Amplification

Preparation

PCR		Purification	
FP ○	– thawed at RT	**CB2** ○	– thawed at RT
RP ○	– thawed at RT	**CW** ○	– stored at RT
Telo PCR ○	– thawed at RT	**100% Ethanol**	– **provided by user!**
E3 ○	– keep on ice or at -20 °C	**DNA Buffer**	– thawed at RT
SYBR Green I (Sigma-Aldrich, Cat. No. S9430), 10,000 x in DMSO for qPCR - **provided by user!**			
Thermocycler	95.8 °C, 30 sec 50 °C, 45 sec 72 °C, 20 min 95.8 °C, 30 sec 62 °C, 30 sec 72 °C, 20 min 72 °C, 20 min 10 °C, ∞	**xx-1** cycle number according to qPCR.	**Purification Columns** **Collection Tubes** **2 ml tubes - provided by user** Thaw and equilibrate all reagents to room temperature before use

qPCR

An aliquot of the full-length ds cDNA is used for a qPCR assay to determine the Optimal Endpoint PCR (OEP) cycle number for full-length cDNA amplification. If desired, the standard PCR Forward Primer (**FP** ○) and Reverse Primer (**RP** ○) can be exchanged for custom primers (see Appendix D, p.25).

ATTENTION: Important information for qPCR assays!

- Ensure that SYBR Green I (user-provided) is added to a **final concentration of 0.1x**. Higher concentrations will inhibit full-length amplification!
- The PCR program includes a **20 minute extension time** and takes in total ~15 hours. We recommend running the qPCR overnight.
- If custom primers will be used for the endpoint PCR, these should also be used for the qPCR assay instead of the PCR Forward and Reverse Primers (**FP** ○ and **RP** ○, see Appendix B, p.22).

30 Prepare a mastermix containing: 8 µl of TeloPrime PCR Mix (**Telo PCR** ○), 1 µl of PCR Forward Primer (**FP** ○), 1 µl of PCR Reverse Primer (**RP** ○), 1 µl of Enzyme Mix (**E3** ○), 1 µl of a 2x SYBR Green I dilution (dilute 10,000x stock 1:5,000 in DMSO). The final concentration of SYBR Green I in the 20 µl PCR reaction must be 0.1x).

31 Add 12 µl of **Telo PCR** / **FP** / **RP** / **E3** / SYBR Green I mastermix to up to 8 µl of purified cDNA. If less sample is used add molecular-biology grade water (H_2O) or **DNA Buffer** ○ to a final volume of 20 µl per reaction. Mix well, seal the plate or tubes and spin down briefly. **REMARKS:** If the template should be amplified in more than one PCR reaction use a smaller aliquot for qPCR, e.g., 1 - 2 µl. The remaining cDNA can be stored at -20 °C.

32 Conduct 40 cycles using a real-time PCR machine, with the following program: one cycle of 95.8 °C for 30 seconds, 50 °C for 45 seconds, 72 °C for 20 minutes; then 39 cycles of 95.8 °C for 30 seconds, 62 °C for 30 seconds, 72 °C for 20 minutes, and a final extension at 72 °C for 20 minutes, hold at 10 °C.

33 Determine the fluorescence value of the plateau on the qPCR amplification curve. Calculate at which cycle the fluorescence is at 80 % of the maximum. This is the Optimal Endpoint PCR (OEP) cycle number. If the same volume of cDNA is used for final cDNA amplification then this is the cycle number to use for the endpoint PCR. If a larger volume of cDNA is used for the endpoint PCR the cycle number should be adjusted to account for the relative increase in template. **EXAMPLE:** if 2 µl cDNA was used for qPCR but 9 µl will be used for endpoint, subtract 2 cycles to account for using ~4x more template used for endpoint PCR.

Endpoint PCR

The endpoint PCR is performed to generate enough material for downstream applications, including long-read sequencing (see Appendix D, p.25). The number of PCR cycles should be determined using the qPCR assay, to prevent over- or undercycling (see also Appendix B, p.22).

ATTENTION: Important information for Endpoint PCR!

- SYBR Green I is not included in the endpoint PCR!
- The qPCR assay determines total number of cycles (xx) to use for endpoint PCR: e.g. for 18 cycles, perform 1 initial cycle with 50 °C annealing temperature then 17 cycles (xx-1) with 62 °C annealing temperature.
- The PCR program includes a 20 minute extension time, therefore total run time is 5 - 8 hours, or longer, depending on the number of cycles. This PCR can be run overnight.

REMARK: When preparing cDNA for downstream Iso-Seq™ library prep, PCR products can instead be purified using the PacBio Ampure Beads, e.g., according to the Iso-Seq™ Template Preparation protocol for the Sequel® Systems (PN 101-070-200, see also Appendix D, p.25).

34 Prepare a mastermix containing 8 µl of TeloPrime PCR Mix (**Telo PCR** ○), 1 µl of the PCR Forward Primer (**FP** ○), 1 µl of the PCR Reverse Primer (**RP** ○), and 1 µl of Enzyme Mix 3 (**E3** ○), per sample. **REMARK:** When using custom primers for PCR please refer to Appendix D, p.25.

35 Add 11 µl of **Telo PCR** / **FP** / **RP** / **E3** mastermix to up to 9 µl of cDNA. If less cDNA is used add molecular biology grade water (**H_2O**) or **DNA Buffer** ○ to a final volume of 20 µl per reaction. Mix well, seal the plate and spin down.

36 Conduct xx PCR cycles (as determined by qPCR) of thermocycling with the following program: one cycle of 95.8 °C for 30 seconds, 50 °C for 45 seconds, 72 °C for 20 minutes, then xx-1 cycles of 95.8 °C for 30 seconds, 62 °C for 30 seconds, 72 °C for 20 minutes, and a final extension at 72 °C for 20 minutes, hold at 10 °C. **REMARK:** One cycle more than the OEP cycle number calculated by qPCR can typically be added to increase yield, without risking overcycling (see Appendix B, p.22). ☛ Safe stopping point. The amplified full-length cDNA can be stored at -20 °C at this point.

Purification

The amplified transcripts are purified from PCR components that can interfere with quantification and other downstream applications.

ATTENTION: Important information for purification!

- Ensure the samples are equilibrated to room temperature before beginning the purification.
- Thaw the Column Binding Buffer 2 (**CB2** ○) and equilibrate to room temperature before use. Incubate briefly at 37 °C to dissolve any precipitates if required.
- Ensure the temperature of the centrifuge remains between 18 - 25 °C. Temperatures outside this range may affect purification efficiency.
- The flow-through does not need to be discarded between column washing steps.

37	Add a total of 160 µl Column Binding Buffer 2 (**CB2** ○) to the reaction, mix well, and transfer the solution to a Purification Column placed in a 2 ml Collection Tube. Centrifuge for 1 minute at 12,000 x g. **REMARK:** Remove the flow-through using a pipette, or exchange the collection tube for a fresh 2 ml tube.
38	Apply 200 µl of Column Wash Buffer (**CW** ○) to the column and centrifuge for 1 minute at 12,000 x g.
39	Repeat this washing step once (for a total of two washes).
40	Discard the flow-through. Centrifuge for 2 minutes at 12,000 x g to dry the column.
41	Transfer the column to a new 1.5 ml tube and apply 21 µl of **DNA Buffer** ○ to the column. Incubate for 1 minute at room temperature and centrifuge for 2 minutes at 12,000 x g to elute the ds cDNA.
42	At this point, the full-length cDNA amplification is finished and the ds cDNA is ready for quality control (Appendix C, p.24). ☞ Safe stopping point. The purified full-length cDNA can be stored at -20 °C at this point.

6. Short Procedure

All centrifugation steps are at 12,000 x g and 18 - 25 °C.

5 hrs		Full-Length cDNA Synthesis
☐	Dilute 1 ng to 2 µg total RNA in 12 µl **RNA Buffer** ○.	First Strand cDNA Synthesis
☐	Add 2 µl of **RTP** ● and mix well.	
☐	Pre-mix 4 µl **RT** ● and 2 µl of **E1** ● per reaction.	
☐	Denature RNA / **RTP** mix for 30 sec at 70 °C, cool down to 37 °C and incubate for 1 min, hold at 37°C.	
☐	Add 6 µl **RT** / **E1** mix per reaction, mix well.	
☐	Incubate for 2 min at 37 °C, then 50 min at 46 °C, hold at 10 °C.	
☐	Add 160 µl of **CB1**○, mix and apply to column, centrifuge 1 min.	Purification
☐☐	Add 200 µl of **CW** ○, centrifuge 1 min, repeat once.	
☐	Discard flow-through, centrifuge 2 min.	
☐	Exchange Collection Tube with 1.5 ml tube.	
☐	Add 19 µl **RNA Buffer** ○, incubate 1 min at RT, centrifuge 2 min.	
☐	Transfer 18 µl of sample to new PCR tube/plate.	
☐	Pre-mix 20 µl **LM** ● and 2 µl **E2** ● per reaction.	Ligation
☐	Add 22 µl of **LM / E2** mix to each reaction.	
☐	Incubate for 3 hrs at 25 °C, hold at 10 °C. **OPTIONAL:** Ligate overnight at 25 °C.	
☐	Add 320 µl of **CB1** ○, mix and apply to column, centrifuge 1 min.	Purification
☐☐	Add 400 µl of **CW** ○, centrifuge 1 min, discard flow-through, repeat once.	
☐	Discard flow-through, centrifuge 2 min.	
☐	Exchange Collection Tube with 1.5 ml tube.	
☐	Add 12 µl **RNA Buffer** ○, incubate 1 min at RT, centrifuge 2 min.	
☐	Transfer 11 µl of sample to new PCR tube/plate. ☞ Safe stopping point.	
☐	Pre-mix 8 µl of **SS** ○ and 1 µl of **E3** ○ per reaction.	2nd Strand Synthesis
☐	Add 9 µl of **SS / E3** mix to each reaction.	
☐	Incubate: 95.8 °C / 90 sec, 62 °C / 60 sec, 72°C / 5 min, 4 °C hold.	
☐	Add 160 µl of **CB2** ○, mix and apply to column, centrifuge 1 min.	Purification
☐☐	Add 200 µl of **CW** ○, centrifuge 1 min, repeat once.	
☐	Discard flow-through, centrifuge 2 min.	
☐	Exchange Collection Tube with 1.5 ml tube.	
☐	Add 20 µl **DNA Buffer** ○, incubate 1 min at RT, centrifuge 2 min.	
☐	Transfer 18 µl of sample to new PCR tube/plate. ☞ Safe stopping point.	

20 hrs Full-Length cDNA Amplification

- ☐ Pre-mix 8 µl of **Telo PCR** ○, 1 µl of **FP** ○, 1 µl of **RP** ○, 1 µl of **E3** ○, and 1 µl of SYBR Green I 1:5000 dilution (i.e., 2x).
- ☐ Add 12 µl of **Telo PCR / FP / RP / E3/** SYBR Green I (1:5000) mix with up to 8 µl ds cDNA sample. Adjust the total volume to 20 µl with H_2O.
- ☐ qPCR:

		95.8 °C, 30 sec	qPCR
		50 °C, 45 sec	
		72 °C, 20 min	
39 x		95.8 °C, 30 sec	
		62 °C, 30 sec	
		72 °C, 20 min	
		72 °C, 20 min	
		10 °C, ∞	

- ☐ Take the cycle number where fluorescence is at 80 % of the maximum for endpoint PCR.

- ☐ Pre-mix 8 µl of **Telo PCR** ○, 1 µl of **FP** ○, 1 µl of **RP** ○, and 1 µl of **E3** ○.
- ☐ Add 11 µl of **Telo PCR / FP / RP / E3** mix to up to 9 µl ds cDNA sample. Adjust the total volume to 20 µl with H_2O.
- ☐ Endpoint PCR:

		95.8 °C, 30 sec	Endpoint PCR
		50 °C, 45sec	
		72 °C, 20 min	
xx-1 (see Appendix B, p.22)		95.8 °C, 30 sec	
		62 °C, 30 sec	
		72 °C, 20 min	
		72 °C, 20 min	
		10 °C, ∞ ☞ Safe stopping point.	

- ☐ Add 160 µl of **CB2** ○, mix and apply to column, centrifuge 1 min.
- ☐☐ Add 200 µl of **CW** ○, centrifuge 1 min, repeat once.
- ☐ Discard flow-through, centrifuge 2 min.
- ☐ Exchange Collection Tube with 1.5 ml tube.
- ☐ Add 21 µl **DNA Buffer** ○, incubate 1 min at RT, centrifuge 2 min. ☞ Safe stopping point.

Purification

7. Appendix A: RNA Requirements

RNA Integrity

Full-length cDNA synthesis relies on high quality input RNA (RIN 9-10) with intact 5' and 3' ends. In lower quality RNA samples some transcripts may lack a cap due to partial degradation, hence full-length cDNA synthesis is not possible, resulting in lower yields. However, using TeloPrime, which tags the poly(A) tail and the cap, full-length mRNAs will be preferentially amplified. The integrity of an RNA sample can be assessed with a variety of methods. We recommend the use of a microfluidics assay such as the RNA6000 series for the 2100 Bioanalyzer (Agilent Technologies), though RNA quality can also be assessed with denaturing agarose gel electrophoresis if such a device is not available. Most microfluidics platforms will carry out an automated peak analysis and generate a quality score (RIN or RQN), in addition to the 28S / 18S rRNA ratio.

RNA Amount

The input range for the TeloPrime full-length cDNA Amplification Kit V2 protocol is 1 ng – 2 µg of total RNA. The amount of total RNA required depends on the poly(A) RNA content and the integrity of the sample in question. TeloPrime cDNA generation was extensively tested with Universal Human Reference RNA (UHRR). For examples of TeloPrime library generation from other RNA sources please refer to the TeloPrime online Frequently Asked Questions page: www.lexogen.com/teloprime-full-length-cdna-amplification/#teloprimefaq. Library size profiles and yields may vary according to the RNA input amount and quality. PCR cycle numbers for endpoint PCR must be adjusted accordingly (see Appendix B, p.22).

Potential Contaminants

RNA samples should be free of salts, metal ions, and organic solvents, which can be carried over from the RNA extraction. Several sources of contamination can be detected with an UV-Vis spectrophotometer. An acceptably pure RNA sample should have an A260 / A280 ratio between 1.8 and 2.1. The A260 / A230 ratio should also be approximately 2. Several common contaminants including proteins, chaotropic salts and phenol absorb strongly between 220 and 230 nm and can often be identified as peaks in this region. Contamination with any of these generates a lower A260 / 230 ratio. Phenol also has an absorption maximum between 250 and 280 nm, which overlaps that of nucleic acid. Hence, high 230 nm absorbance combined with a biphasic or broad peak between 250 and 280 nm may indicate contamination with phenol rather than chaotropic salts.

Genomic DNA Contamination

Depending on the RNA extraction protocol used, samples may also contain significant amounts of gDNA, which is indistinguishable from RNA on a spectrophotometer. Furthermore, as many of the dyes used in RNA microfluidics assays stain single-stranded nucleic acids much more intensely than double-stranded, low to moderate amounts of gDNA may not be readily visible with an RNA-specific microfluidics assay. We highly recommend examining all RNA samples on a denaturing agarose gel or using a fluorometric assay with DNA- and RNA-specific dyes to check samples for DNA contamination. On an agarose gel, gDNA can appear as either a dark mass which remains in the well if relatively intact or as a high molecular weight smear if it has been sheared during extraction.

The best way to avoid gDNA contamination is to use an RNA extraction protocol that does not co-isolate gDNA such as Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008). However, DNA can be removed from irreplaceable samples by acidic phenol extraction or DNase I digestion. We do not recommend DNase treatment with heat inactivation, as the extended incubation with divalent cations can lead to RNA hydrolysis and reduced RNA quality. If samples must be DNase treated, heat inactivation should be avoided and the enzyme deactivated by other means such as phenol/chloroform extraction or silica column purification.

Spike-in RNA Variant Controls

Lexogen's Spike-in RNA Variant Controls (SIRVs, Cat. No. 025, 050, 051) can be added to total RNA for TeloPrime full-length cDNA generation. The SIRVs are a set of 69 synthetic sequences, designed to model the isoform complexity of 7 human genes. The 7 SIRV genes contain between 6 and 18 artificial transcript variants each, which comprehensively reflect isoform complexity, including alternative splicing, alternative transcription start- and end-sites, overlapping genes, and antisense transcripts. Hence, SIRVs are ideal controls for full-length cDNA amplification, particularly for NGS applications aimed at isoform detection for transcriptome annotation or *de novo* assembly.

Three SIRV-Sets are available from Lexogen, providing different options for SIRV spike-in. SIRV-Set 1 (Cat. No. 025) contains three SIRV Isoform Mixes: E0, E1, and E2, which provide different concentrations ratios of the SIRV isoform transcripts. SIRV-Set 2 (Cat. No. 050) contains only the Isoform Mix E0, with all SIRVs present at equimolar concentrations. SIRV-Set 3 (Cat. No. 051) contains the Isoform Mix E0 in combination with the 92 monoexonic ERCC Spike-in Controls, providing an enhanced total number of spike-in transcripts, covering both isoform complexity and a large dynamic range of concentration.

Although SIRVs (and ERCCs) do not possess a canonical 7-methylguanosine (m^7G) cap, these transcripts feature a 5'tri-phosphate linker, which does allow for Cap-Dependent Linker Ligation and full-length TeloPrime cDNA generation from SIRV RNA. For more information on Lexogen's available SIRV Sets, or advice on SIRV spike-in amounts please visit www.lexogen.com/sirvs/, or email info@lexogen.com.

8. Appendix B: Calculation of Optimal Endpoint PCR Cycle Number

The endpoint PCRs are performed to generate enough material for any subsequent application such as NGS sample prep, microarrays, or cDNA cloning. The optimal number of cycles for endpoint PCR depends on the quality of your RNA, the total RNA input, and the mRNA content, which varies between cell types, tissues, and organisms. It is also important to avoid overcycling while ensuring you generate sufficient amounts of cDNA for downstream applications. Overcycling may result in reduced overall cDNA yield and enrichment of shorter versus longer cDNAs (Figure 3).

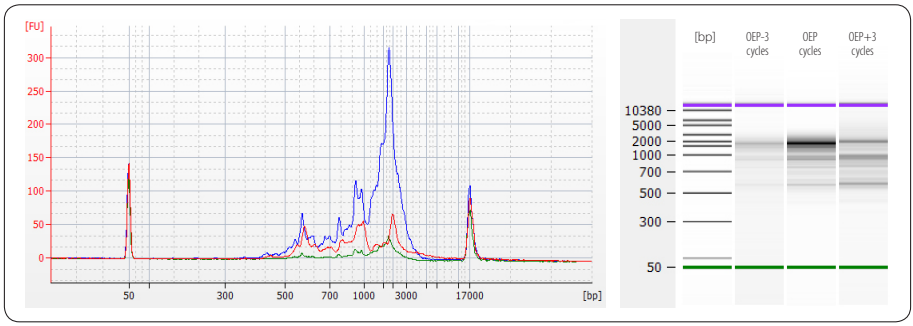

Figure 3. TeloPrime cDNA generated with different PCR cycles. Endpoint PCR was performed using 2 µl of pooled TeloPrime cDNA with Optimal Endpoint PCR (OEP) cycle number (as calculated from qPCR, i.e., 80 % of maximum fluorescence), or using OEP ± 3 cycles. The amplified cDNA was analyzed on a Bioanalyzer DNA 12000 Chip (Agilent Technologies). Undercycling (green, OEP-3) produces low yields, while overcycling by 3 cycles (red, OEP +3) results in enrichment of shorter versus longer products and also reduces the yield. The optimal cycle number (blue, OEP) gives highest yield for longer cDNAs.

A qPCR assay is included as a recommended step for all samples. All TeloPrime Full-Length cDNA Amplification Kits V2 (Cat. No. 013) contain sufficient reagents to perform a qPCR and endpoint PCR for each prep. Additional SYBR Green I (Sigma-Aldrich, Cat. No. S9430) is needed for the qPCR assay, to enable fluorescent read-out of amplification using a real-time PCR machine. SYBR Green I must be used at a final concentration of 0.1x only. Higher concentrations may inhibit amplification.

Run the qPCR for 40 cycles and determine the maximum value at which the fluorescence reaches a plateau. Calculate where the fluorescence is at 80 % of this maximum to give the number of cycles to use for the endpoint PCR (assuming equal volume of cDNA template used for qPCR and endpoint PCR). If the volume of cDNA template is adjusted for endpoint PCR, the cycle number should also be adjusted accordingly.

NOTE: The PCR program for amplification is a two stage PCR with the initial cycle using a lower annealing temperature (Ta) of 50 °C. The resulting endpoint cycle number calculated by qPCR includes this cycle. For example, if the Optimal Endpoint PCR (OEP) cycle number determined by

qPCR is 18 (xx, i.e., 80 % of max FU), perform one cycle with 50 °C Ta and 17 cycles (xx-1) with 62 °C Ta, with an equal volume of cDNA as template. Once the cycle number is established for a certain kind of sample, the same can be used for further experiments.

EXAMPLE: if 2 µl cDNA was used for qPCR but 9 µl will be used for endpoint PCR, subtract 2 cycles to account for using ~4x more template for the endpoint PCR.

REMARK: Using one cycle more than the OEP cycle number calculated by qPCR can typically be added to increase yield, without risking overcycling.

9. Appendix C: Quality Control

Quality control of the amplified full-length cDNA is highly recommended and can be carried out with various methods depending on the available equipment. A thorough quality control procedure should include the analysis of concentration, size distribution, and banding pattern of the amplified products.

Quality Control Methods

The concentration of the PCR products can be measured with an UV-Vis spectrophotometer. Visual control of the banding pattern and the size distribution as well as detection of side-products can be done by analyzing a small volume of sample with microcapillary electrophoresis. For low-to medium-throughput applications, we recommend the Bioanalyzer 2100 with Agilent High Sensitivity DNA, DNA 7000, or DNA 12000 chips (Agilent Technologies). Typically 1 ng of the amplified sample is sufficient for analysis. Very basic quality control can also be performed by separating about 100 ng of sample on a 0.7 % agarose gel. An example of TeloPrime full-length cDNA generated from 500 ng of Universal Human Reference RNA (UHRR, Agilent Technologies) total RNA input is depicted in Figure 4.

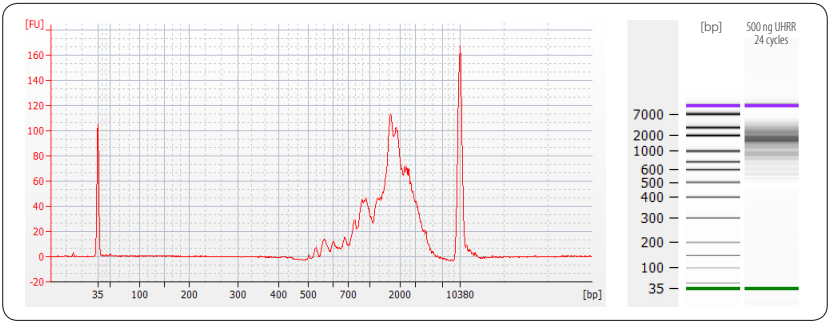

Figure 4. TeloPrime cDNA Quality Control. TeloPrime cDNA was prepared from 500 ng of Universal Human Reference RNA (UHRR). Amplification was performed using 1 µl of cDNA with 24 cycles of PCR. Final cDNA was run at 1:10 dilution on a Bioanalyzer High Sensitivity DNA Chip (Agilent Technologies). The optimal endpoint PCR (OEP) cycle number was determined by qPCR as described in Appendix B, p.22.

10. Appendix D: Downstream Applications

TeloPrime cDNA can be used for various downstream applications such as Next Generation Sequencing (NGS) - including long-read sequencing on Pacific Biosciences and Oxford Nanopore instruments - as well as 5'RACE, cloning, microarray probes, and normalization.

For additional full-length or gene-specific PCRs, Lexogen offers a TeloPrime PCR Add-on Kit V2 (Cat. No. 018). This kit includes the PCR Forward Primer (**FP** ○) and Reverse Primer (**RP** ○) separately. Hence, these can be replaced by custom primers.

The PCR primers included in all TeloPrime Kits V2 (Cat. No. 013, 018) have the following sequence:

```
FP: 5' – TGGATTGATATGTAATACGACTCACTATAG – 3'
RP: 5' – TCTCAGGCGTTTTTTTTTTTTTTTTT – 3'
```

If custom primers are used for PCR, calculate the melting temperature (Tm) at a final concentration of 5.1 mM $MgCl_2$. Each primer should be added at a final concentration of 2 µM in the PCR reaction mix. A gradient PCR is recommended, to identify the optimal annealing temperature (Ta) for qPCR and endpoint PCR.

Use of Custom Reverse Transcription Primers

Custom primers may be also used instead of the standard Reverse Transcription Primer (**RTP** ●) for the reverse transcription (RT) steps of the TeloPrime Full-Length cDNA Amplification Kit V2 protocol.

Please note the following considerations when designing and using custom RT primers:

- One or more custom RT primers may be used in a single reaction instead of the standard Reverse Transcription Primer (**RTP** ●, **ATTENTION:** Do not mix custom primers with **RTP** ● !).
- Custom primers should bind at least 18 - 20 nt of the target transcript (Max. length 25 nt).
- The primer sequences should be the same as the first strand cDNA (i.e., reverse complement to the target mRNA sequence).
- The concentration of custom RT primer(s) required for reverse transcription may require optimization. We recommend testing final primer concentrations between 12.5 nM - 1.5 µM for the first strand cDNA synthesis reaction. The maximum primer concentration should not exceed 2 µM.
- Custom RT primer(s) are added to the reaction in a maximum volume of 2 µl, unless the total RNA input volume is reduced.
- A custom PCR Reverse Primer (custom RP) must also be designed and used in place of the provided PCR Reverse Primer (**RP** ○). The full or partial custom RP primer binding site should be included at the 5' end of the custom RT primer. The Tm of the custom RP primer should be similar to the PCR Forward Primer (**FP** ○) to ensure efficient annealing and amplification.

Iso-Seq™ Library Preparation

The full-length cDNA generated from the TeloPrime protocol is fully compatible with downstream Iso-Seq™ library preparation and long-read sequencing on Pacific Biosciences Instruments. To enhance the yield and minimize the number of PCR cycles required, TeloPrime full-length cDNA can be generated in two parallel reactions from the same starting RNA sample (1-2 µg total RNA input per reaction is recommended). The ds cDNA can then be pooled after second strand synthesis and purification, before PCR amplification. Depending on the starting RNA quality and input amount, up to 2 µg of TeloPrime full-length cDNA can be generated from a single PCR. If even higher yields are required, replicate PCRs can be performed using multiple aliquots of the same TeloPrime ds cDNA.

The TeloPrime PCR Add-on Kit V2 (Cat. No. 018) can be used for additional PCRs and provides reagents for 16 reactions.

As an alternative to the column-based purification outlined in this user guide, TeloPrime cDNA intended for Iso-Seq™ library preparation can be purified after PCR amplification using Ampure® PB Beads (Pacific Biosciences). Please refer to the protocol details for purification in the Procedure & Checklist - Iso-Seq™ Template Preparation for Sequel® Systems (PN 101-070-200 version 6, Pacific Biosciences).

Lexogen's Purification Module with Magnetic Beads (Cat. No. 022) can also be used for post-PCR purification of TeloPrime ull-length cDNA. Please contact info@lexogen.com for further protocol details.

11. Appendix E: Revision History

Revisions made to this User Guide are indicated from 2016 onwards. The most recent updates are highlighted in green. The complete User Guide Revision History is available from the Support Tools page at www.lexogen.com.

Publication No. / Revision date	Change	Page
013UG022V0200 (V2) Nov. 22, 2018	**Version 2 Kit Update**: The following kit components were updated: Second Strand Mix (SS), DNA Buffer, TeloPrime PCR Mix (Telo PCR), Enzyme Mix 3 (E3), PCR Forward Primer (FP), and PCR Reverse Primer (RP).	
	Detailed protocol was updated for: second strand synthesis, qPCR and endpoint PCR (steps 19 - 42). Elution volume reduced to 12 µl (steps 19 - 20), Second Strand Mix (SS) and Mastermix 4 volumes increased (steps 21 - 22), denaturing temperature for step 23 changed to 95.8 °C, PCR Primer volumes reduced to 1 µl, each PCR program, temperatures, and timing was changed. Notes added before purification sections.	11-17
	Kit workflow timing, kit components figures, and volume table updated.	5, 6
	Store Column Binding Buffers 1 (CB1) and 2 (CB2) at -20 °C until first use, then afterwards at room temperature.	6
	Short protocol steps and volumes updated.	18-19
	Safe stopping points indicated at steps 20, 29, 36, and 42.	13, 15
	Updated text and figures in Appendices A-D.	20-26
013UG022V0106 (V1) Mar. 13, 2017	Added note that additional reagents are included for qPCR.	6
	Adjustment of reagent volumes.	6
	Addition of ethanol to CW is indicated on the tube label only.	6
013UG022V0105 (V1) Apr. 1, 2016	Tables reformatted.	
013UG022V0104 (V1) Jan. 12, 2016	Label color of PCR, RP, FP, SS, E3 changed to white.	
013UG022V0100 (V1) Aug. 27, 2014	Initial Release.	

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Enabling complete transcriptome sequencing

TeloPrime Full-Length cDNA Amplification Kit V2 · User Guide

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