



TrueLink® DNA Library Preparation Kit

A dual, direct, and independent ligation workflow for preparation of PCR-ready DNA libraries

For Research Use Only. Not for use in diagnostic procedures.

User Guide

Kit Part Numbers

TL0001-24 and TL0001-96

Version

V2.0

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Product Overview

The TrueLink® DNA Library Preparation Kit uses an end-repair-free approach to prepare DNA libraries for Illumina sequencing. Through **dual direct and independent ligation**, two ligases attach distinct single-stranded adapters directly to the 3' and 5' termini of input DNA, without end repair or A-tailing.

By eliminating the nucleotide trimming and fill-in resynthesis associated with conventional end repair, TrueLink preserves native terminal sequence information and avoids artifacts introduced by these activities. The chemistry captures both single-stranded and double-stranded DNA, enabling representation of molecules that may be lost in conventional double-stranded DNA library preparation. These capabilities support research applications in which native fragment ends, sequence fidelity, and representation of diverse DNA molecules are important, including cfDNA analysis, fragmentomics, and rare-variant detection.

The kit contains the enzymes, buffers, and adapters required to convert mechanically or naturally fragmented DNA, such as cfDNA, into adapter-ligated templates ready for indexing PCR. Amplification with compatible user-supplied indexing primers generates libraries for standard Illumina sequencing workflows. Recommended input amounts, fragment sizes, and processing conditions are specified in this guide.

TrueLink® DNA Library Preparation

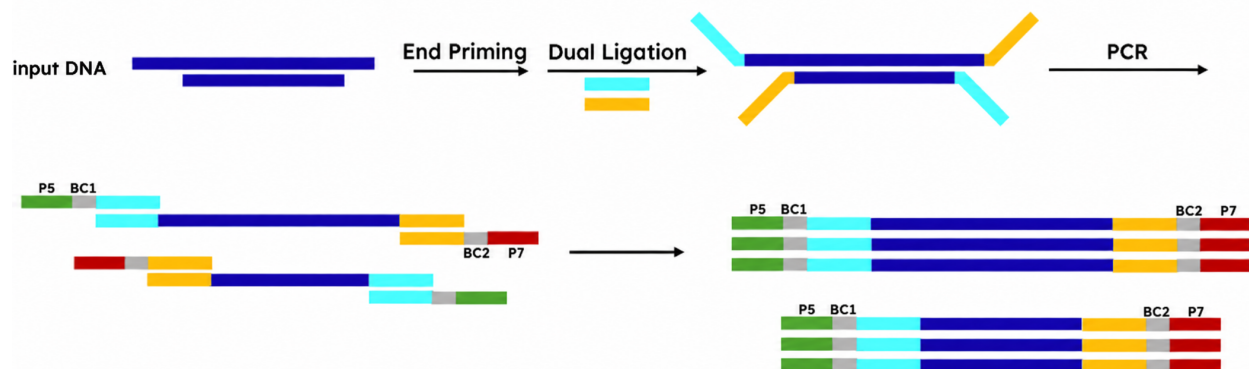


Figure 1. Schematic diagram of TrueLink® DNA library preparation.

Kit Contents

Kit Part Number	Description	Component Part Number	Storage Temperature	Cap Color	Volume (µL)
TL0001-24	TL-D Rxn Buffer	24-100047	2-8°C	Red	50
	EP Enzyme	24-100024	2-8°C	Red	30
	Enhancer	24-100022	2-8°C	Clear	80
	Adapter A	24-100025	2-8°C	Blue	30
	Adapter B	24-100026	2-8°C	Blue	30
	Dual Ligase A	24-100028	2-8°C	Blue	30
	Dual Ligase B	24-100029	2-8°C	Blue	30
	Quench Buffer	24-100048	2-8°C	Clear	300
	Quench Enzyme	24-100049	2-8°C	Clear	25
TL0001-96	TL-D Rxn Buffer	96-100047	2-8°C	Red	140
	EP Enzyme	96-100024	2-8°C	Red	120
	Enhancer	96-100022	2-8°C	Clear	250
	Adapter A	96-100025	2-8°C	Blue	110
	Adapter B	96-100026	2-8°C	Blue	110
	Dual Ligase A	96-100028	2-8°C	Blue	110
	Dual Ligase B	96-100029	2-8°C	Blue	110
	Quench Buffer	96-100048	2-8°C	Clear	1200
	Quench Enzyme	96-100049	2-8°C	Clear	70

Shipping, Storage and Stability

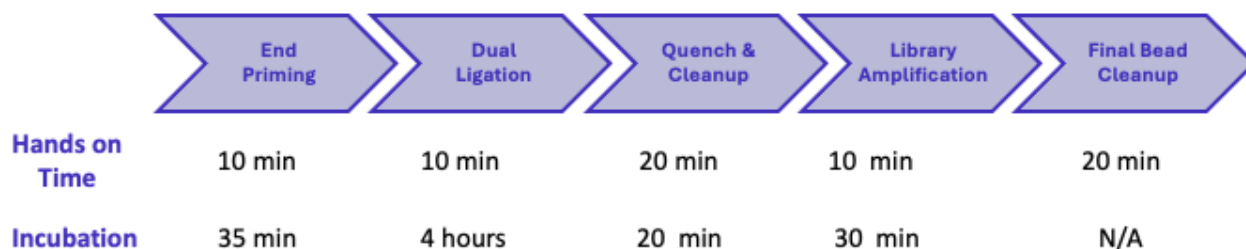
- TrueLink® DNA Library Preparation Kits are shipped with cooling gel packs.
- Store kit components at **2°C to 8°C** in a constant-temperature refrigerator.
- TrueLink® reagents have limited stability data at this time; shelf life is limited to 4 months unless otherwise specified on the product label.
- Keep enzymes, adapters, and reaction components cold during setup. Briefly spin down tubes before opening. Mix enzymes by gentle pipetting unless otherwise specified. Return reagents to **2°C to 8°C** promptly after use.
- Do not freeze kit components unless specifically instructed by Canal Biosciences.

Required Materials (not included)

- Nuclease-free water
- 80% ethanol (freshly prepared)
- 100% isopropanol (molecular biology grade)
- AMPure XP size selection beads (Beckman Coulter Life Sciences, #A63880)
- Agilent D1000 or HS D1000 ScreenTape® or equivalent (Agilent, #5067-5582, #5067-5584)
- xGen™ UDI Primers 10 µM each (IDT, #10005975, #10005922, #10008052)
- Roche KAPA HiFi HotStart ReadyMix amplification mix (Roche Sequencing, KK2602)
- Elution buffer 10 mM Tris-HCl, pH 8.0
- Magnetic stand for 0.2 mL PCR tubes, strips or plate
- Thermocycler with heated lid
- Thin-wall 200 µL PCR tubes, strips or plates

Workflow/Quick Reference

The TrueLink® Workflow



Quick Reference Guide

Use this table as a bench-side summary only. Follow the full protocol sections below for detailed instructions, reagent-handling notes, and troubleshooting guidance.

Detailed Workflow Summary

Workflow Step	Bench Action	Input	Add / Reagent	Ratio / Volume	Program / Incubation	Output / Next step
End Priming	Set up End Priming reaction	30 µL pre-sheared DNA or cfDNA in water	3 µL End Priming Master Mix	33 µL final reaction volume	37°C 30 min; 65°C 5 min; hold 4°C	End-primed sample.
Dual Ligation	Set up Dual Ligation reaction	33 µL end-primed sample	7 µL Dual Ligation Master Mix	40 µL final reaction volume	70°C 240 min; hold 4°C	Dual-ligated sample. Hold for up to 24 hours at 4°C.
Quench & Cleanup	Quench Dual Ligation reaction	40 µL dual ligation reaction	10 µL Quench Buffer + 0.5 µL Quench Enzyme	50.5 µL quenched reaction volume	37°C 15 min; 65°C 5 min; hold 4°C	Proceed immediately to post-ligation bead cleanup.
Quench & Cleanup	Post-ligation bead cleanup	50.5 µL quenched ligation reaction	100 µL AMPure XP beads + 50 µL Isopropanol	~2.0X AMPure ~1.0X isopropanol	5 min bead bind; 2 × 200 µL 80% ethanol washes; air dry up to 5 min	Elute in 22 µL Elution Buffer; transfer 22 µL cleared eluate. SAFE STOP: -20°C.
Library Amplification	Set up PCR amplification	22 µL purified post-ligation eluate	25 µL 2X KAPA HiFi ReadyMix + 3 µL indexed PCR primer mix	50 µL final PCR volume	98°C 2 min; 5–10 cycles of 98°C 15 s / 55°C 15 s / 72°C 30 s; 72°C 2 min; hold 4°C	Amplified library. Select PCR cycles based on input amount and application requirements.
Final Bead Cleanup	Post-amplification bead cleanup	50 µL PCR reaction	45 µL AMPure XP beads	0.9X bead ratio	5 min bead bind; 2 × 200 µL 80% ethanol washes; air dry up to 5 min	Elute in 30 µL Elution Buffer; transfer 30 µL cleared library. SAFE STOP: -20°C.
Library QC	Library QC	Final purified library	Dilute 1:10 as needed for TapeStation or equivalent analysis	Assay-dependent	Follow instrument or assay instructions	Assess library size distribution and yield. Proceed to pooling and sequencing.

Protocol

Starting Material: Use 1–50 ng of mechanically fragmented DNA or naturally fragmented DNA, such as cell-free DNA (cfDNA), in a maximum input volume of 30 µL. The protocol supports low-input library preparation, including 1 ng inputs, and is optimized for DNA fragment lengths of 100–300 bp.

For optimal performance, use DNA templates in nuclease-free water. Compatibility with other template buffers has not been fully characterized. Where possible, elute DNA into nuclease-free water during sample preparation, and use nuclease-free water for all subsequent template dilutions (see troubleshooting section for further guidance).

For samples requiring fragmentation, mechanical shearing (e.g., Covaris) is recommended to preserve native sequence information and avoid DNA resynthesis associated with some enzymatic fragmentation workflows. Naturally fragmented samples do not require additional shearing if their fragment size distribution is appropriate for the intended application.



Indicates a point where samples may be stored as described before continuing the protocol.

Before You Begin:

Create the following thermocycler programs:

End Priming (EP) Program

End Priming (EP) Program			
Step	Block Temperature	Lid Temperature	Duration
1	37°C	100°C	30 min
2	65°C	100°C	5 min
3	4°C	N/A	Hold

Dual Ligation (DL) Program

Dual Ligation (DL) Program			
Step	Block Temperature	Lid Temperature	Duration
1	70°C	100°C	240 min
2	4°C	N/A	Hold

Quenching Program

Quenching Program			
Step	Block Temperature	Lid Temperature	Duration
1	37°C	100°C	15 min
2	65°C	100°C	5 min
3	4°C	N/A	Hold

Library Amplification (KAPA HiFi 2X HotStart ReadyMix)

Library Amplification Program				
Step	Block Temperature	Lid Temperature	Duration	# of Cycles
1	98°C	100°C	2 min	1
2	98°C	100°C	15 sec	5 -10*
3	55°C	100°C	15 sec	
4	72°C	100°C	30 sec	
5	72°C	100°C	2 min	1
6	4°C	N/A	Hold	1

***Note:** See Section 5, Library Amplification, for recommended cycle number (based on input and yield requirements).

TrueLink® Library Preparation:

1. TrueLink® End Priming

- 1.1 Transfer up to 30 µL of DNA template, preferably supplied in nuclease-free water, into a 0.2 mL PCR tube. If the template volume is less than 30 µL, bring the total volume to 30 µL with nuclease-free water. Do not use Tris-based elution buffer or TE buffer to adjust the template volume.

Note: For DNA shearing, we recommend conventional mechanical shearing (e.g., Covaris) to avoid DNA resynthesis activities that may occur in some enzymatic fragmentation methods.

- 1.2 Prepare End Priming Master Mix on ice by combining the components listed below in a sterile, nuclease-free tube.

Component	1X Reaction Volume
TL-D Rxn Buffer	1.0 µL
Enhancer	1.0 µL
EP Enzyme	1.0 µL
Total Volume	3.0 µL

Note: For multi-sample master mixes, multiply each “1X Reaction Volume” by the number of samples and add 10% to ensure sufficient total volume.

- 1.3 Mix the components thoroughly by gently pipetting the master mix up and down at least 10 times.

- 1.4 Add 3.0 µL of End Priming Master Mix to each Template DNA tube.

Component	1X Reaction Volume
Template DNA	30.0 µL
End Priming Master Mix	3.0 µL
Total Volume	33.0 µL

- 1.5 Pipette up and down at least 10 times with P200 pipettor set to 20 µL to mix thoroughly.

- 1.6 Place the capped tubes in a thermocycler, with the heated lid on, and run the **End Priming** program.

End Priming (EP) Program			
Step	Block Temperature	Lid Temperature	Duration
1	37°C	100°C	30 min
2	65°C	100°C	5 min
3	4°C	N/A	Hold

2. Dual Ligation

- 2.1 Prepare the Dual Ligation Master Mix on ice by combining the following components, **in the order listed below**, in a sterile, nuclease-free tube.

Note: Prepare the Dual Ligation Master Mix when the End Priming Program is complete and the end-primed template is ready for addition of the master mix.

Component	1X Reaction Volume
Enhancer	1.0 µL
Adapter A	1.0 µL
Adapter B	1.0 µL
Dual Ligase A	1.0 µL
Dual Ligase B	1.0 µL
Nuclease-free water	2.0 µL
Total Volume	7.0 µL

Note: For multi-sample master mixes, multiply each “1X Reaction Volume” by the number of samples and add 10% to ensure sufficient total volume.

- 2.2 Mix the components thoroughly by gently pipetting the master mix up and down at least 10 times.

- 2.3 Add 7.0 µL of the Dual Ligation Master Mix to each end primed sample reaction and mix thoroughly by gently pipetting up and down at least 10 times with a P200 set to 30 µL.

Component	1X Reaction Volume
End Primed Sample Reaction	33.0 µL
Dual Ligation Master Mix	7.0 µL
Total Volume	40.0 µL

- 2.4 Place the capped tubes in a thermocycler and run the **Dual Ligation** program.

Dual Ligation (DL) Program			
Step	Block Temperature	Lid Temperature	Duration
1	70°C	100°C	240 min
2	4°C	N/A	Hold

Note: The recommended Dual Ligation incubation is 4 hours to maximize conversion. A shortened 2-hour incubation may be used when a shorter workflow is preferred. In internal testing, the 2-hour incubation achieved more than 75% of the conversion obtained with the 4-hour incubation.



Completed Dual Ligation reactions may be held at 2-8°C for up to 24 hours before proceeding.

3. Quenching Reaction

- 3.1 Prepare the Quench Master Mix on ice by combining the following components, in the order listed below, in a sterile, nuclease-free tube.

Component	1X Reaction Volume
Quench Buffer	10.0 µL
Quench Enzyme	0.5 µL
Total Volume	10.5 µL

Note: For multi-sample master mixes, multiply each “1X Reaction Volume” by the number of samples and add 10% to ensure sufficient total volume.

- 3.2 Mix the components thoroughly by gently pipetting the master mix up and down at least 10 times.
- 3.3 Add 10.5 µL of the Quench Master Mix to each dual ligation reaction and mix thoroughly by gently pipetting up and down at least 10 times with a P200 set to 40 µL.

Component	1X Reaction Volume
Dual Ligation Reaction	40.0 µL
Quench Master Mix	10.5 µL
Total Volume	50.5 µL

- 3.4 Place the quenching reaction tube(s) in a thermocycler and run the **Quenching Program**.

Quenching Program			
Step	Block Temperature	Lid Temperature	Duration
1	37°C	100°C	15 min
2	65°C	100°C	5 min
3	4°C	N/A	Hold

- 3.5 Proceed immediately to post-ligation bead cleanup step.

4. Post-Ligation Bead Cleanup

Note: The post-ligation cleanup uses a modified AMPure XP procedure that includes the addition of 100% isopropanol to improve DNA recovery. Follow the reagent volumes and order of addition specified below.

- 4.1 Prepare fresh 80% ethanol with molecular biology grade 100% ethanol and nuclease free water.
- 4.2 Bring AMPure XP beads to room temperature and vortex to fully resuspend.
- 4.3 Add 100 µL of resuspended AMPure XP beads to each 50.5 µL quenched dual ligation reaction.
- 4.4 Add 50 µL of 100% Isopropanol to dual-ligation reaction/AMPure bead mixture.
- 4.5 Pipette up and down for at least 10 times with P200 pipettor set to 100 µL to mix thoroughly, then incubate at room temperature for 5 minutes.
- 4.6 Place reaction tube(s) on an appropriate magnetic rack to separate the beads from the supernatant.
- 4.7 After 5 minutes (or when the supernatant is clear), remove and discard the supernatant, taking care not to disturb the pelleted beads.
- 4.8 Leave tube(s) on the magnetic rack and add 200 µL of freshly prepared 80% ethanol. Incubate at room temperature for 30 seconds, and then remove and discard the supernatant, taking care not to disturb the pelleted beads.
- 4.9 Repeat step 4.8 once, for a total of 2 ethanol washes.
- 4.10 With tube(s) remaining on the magnetic rack, carefully remove residual ethanol from the bottom of the sample tube(s) using a P20 pipette.

- 4.11 Allow the beads to air dry for up to 5 minutes. Do not over-dry beads; beads should appear matte but not cracked.
- 4.12 Remove the tube(s) from the magnetic rack and resuspend the beads by adding 22 µL of Elution Buffer (10 mM Tris buffer pH 8.0). Rinse down the beads on the tube wall.
- 4.13 Incubate at room temperature for 2–5 minutes.
- 4.14 Place the tube(s) back on the magnetic rack. After 5 minutes (or when the solution is clear), with tube(s) still in contact with the magnet, carefully transfer 22 µL of eluted samples into a fresh PCR tube.



Samples can be stored at -20°C at this point.

5. Library Amplification

- 5.1 Thaw KAPA HiFi PCR ReadyMix and Illumina TruSeq-compatible indexing PCR primers on ice.
- 5.2 Set up the Amplification reaction in a sterile, nuclease-free PCR tube as follows:

Component	1X Reaction Volume
2X KAPA HiFi PCR ReadyMix	25.0 µL
Indexed PCR primer mix (10 µM each)	3.0 µL
Eluted dual ligation reaction	22.0 µL
Total Volume	50.0 µL

Note: When using 2X KAPA HiFi PCR ReadyMix, the Library Amplification Program listed below is recommended. Use only indexing primers compatible with the TrueLink® adapter architecture and the intended Illumina platform. Confirm index read configuration before sequencing.

- 5.3 Perform PCR amplification using the recommended thermocycler program. Select the number of PCR cycles based on the input DNA amount and desired library yield, using the table below as a guide.

Input DNA Amount	Recommended Cycle Number
1–5 ng	7 - 10 cycles
>5–20 ng	6 - 8 cycles
>20–50 ng	5 - 7 cycles

Note: Following indexing PCR, the expected library size distribution corresponds to the input DNA fragment lengths plus approximately 150 bp contributed by the full-length sequencing adapters

KAPA HiFi Amplification Program				
Step	Block Temperature	Lid Temperature	Duration	Cycles
1	98°C	100°C	2 min	1
2	98°C	100°C	15 s	5 -10
3	55°C	100°C	15 s	
4	72°C	100°C	30 s	
5	72°C	100°C	2 min	1
6	4°C	N/A	Hold	1

6. Post-Amplification Bead Cleanup

- 6.1 Prepare fresh 80% ethanol with molecular biology grade 100% ethanol and nuclease free water.
- 6.2 Bring AMPure XP beads to room temperature and vortex to fully resuspend.
- 6.3 Add 45 µL (0.9X volume) of resuspended AMPure XP beads to each completed 50 µL PCR reaction.
- 6.4 Pipette up and down for at least 10 times with pipette set to 50 µL to mix thoroughly, then incubate at room temperature for 5 minutes.
- 6.5 Place reaction tube(s) on an appropriate magnetic rack to separate the beads from the supernatant.
- 6.6 After 5 minutes (or when the supernatant is clear), remove and discard the supernatant, taking care not to disturb the pelleted beads.
- 6.7 Leave samples on the magnetic rack and add 200 µL of freshly prepared 80% ethanol. Incubate at room temperature for 30 seconds, then remove and discard the supernatant, taking care not to disturb the pelleted beads.
- 6.8 Repeat step 6.7 once, for a total of 2 ethanol washes.
- 6.9 With tubes remaining on the magnetic rack, remove any residual ethanol from the bottom of the sample tube(s) using a P20 pipette.
- 6.10 Allow the beads to air dry for up to 5 minutes. Do not over-dry beads; beads should appear matte but not cracked.
- 6.11 Remove the tube(s) from the magnet and resuspend the beads by adding 30 µL of elution buffer (10 mM Tris buffer pH 8.0). Rinse down the beads on the tube wall.
- 6.12 Incubate at room temperature for 2–5 minutes.

- 6.13 Place the tube(s) back on the magnetic stand. After 5 minutes, or when the solution is clear, carefully transfer 30 µL into a final PCR tube.
- 6.14 Assess library size distribution and amplification yield using an Agilent D1000 ScreenTape or High Sensitivity D1000 ScreenTape, or equivalent fragment analysis method. If using High Sensitivity D1000 ScreenTape, dilute the library 1:10 in nuclease-free water or elution buffer before analysis



Samples may be stored at -20°C at this point.

Downstream Processing and Sequencing Guidance

TrueLink® DNA libraries are structurally equivalent to standard Illumina TruSeq-compatible libraries, containing the Illumina P5 and P7 flow-cell adapter sequences.

Libraries prepared with the TrueLink® kit may be used with common enrichment systems such as hybrid-capture probe panels and with standard Illumina sequencing platforms.

Library Quantification

- qPCR-based library quantification kits are recommended because they selectively quantify adapter-ligated molecules capable of cluster amplification.
- Fluorometric assays, such as Qubit dsDNA HS, may be used to estimate total dsDNA concentration but may overestimate sequenceable library molecules.
- Fragment analysis confirms size distribution but does not determine sequenceable molarity.
- Assess library size distribution using a fragment analysis instrument such as Agilent TapeStation, Fragment Analyzer, or equivalent capillary electrophoresis system.
- The expected size distribution should correspond to the input fragment distribution plus approximately 150 bp from standard Illumina full-length adapters.

Library Pooling

- Pool libraries based on molar concentration to ensure balanced sequencing representation.
- Use unique index combinations for each sample.
- Confirm index compatibility and index read configuration with the selected Illumina sequencing platform.
- Follow standard Illumina index-balancing guidelines for optimal sequencing performance.

Sequencing

TrueLink® libraries can be sequenced using standard Illumina sequencing primers and run configurations. Read length selection should be compatible with the insert size distribution of the library.

Data Pre-Processing/Trimming

Remove adapter sequences before downstream analysis. Select trimming settings according to the intended application, as below.

Rare variant detection:

When the use case for TrueLink® is rare variant detection, read trimming of the first and last 3 bases is recommended for pre-processing TrueLink® raw sequencing data, as below:

```
fastp --in1 {input.fastq_r1} \
      --in2 {input.fastq_r2} \
      --out1 {output.fastq_r1} \
      --out2 {output.fastq_r2} \
      --unpaired1 {output.unpaired_r1} \
      --unpaired2 {output.unpaired_r2} \
      --failed_out {output.failed} \
      --trim_front1 {params.n} --trim_tail1 {params.n} \
      --trim_front2 {params.n} --trim_tail2 {params.n} \
      --adapter_sequence {params.adapter_1} \
      --adapter_sequence_r2 {params.adapter_2} \
      --trim_poly_g \
      --poly_g_min_len 10 \
      --html {output.report_html} \
      --json {output.report_json} \
      --report_title {params.title} \
      --thread {threads}
```

For parameters:

- adapter_1: AGATCGGAAGAGCACACGTCTGAACTCC
- adapter_2: AGATCGGAAGAGCGTCGTGTAGGGAAAG
- n = 3, for # of bases to trim on each end

This step removes adapter sequences and trims three bases from both ends of each read to reduce the contribution of terminal sequencing errors.

All other applications:

For all other applications, adapter trimming is recommended, as below:

```
fastp --in1 {input.fastq_r1} \
      --in2 {input.fastq_r2} \
      --out1 {output.fastq_r1} \
      --out2 {output.fastq_r2} \
      --unpaired1 {output.unpaired_r1} \
      --unpaired2 {output.unpaired_r2} \
      --failed_out {output.failed} \
      --adapter_sequence {params.adapter_1} \
      --adapter_sequence_r2 {params.adapter_2} \
```

```
--trim_poly_g \  
--poly_g_min_len 10 \  
--html {output.report_html} \  
--json {output.report_json} \  
--report_title {params.title} \  
--thread {threads}
```

For parameters:

- adapter_1: AGATCGGAAGAGCACACGTCTGAACTCC
- adapter_2: AGATCGGAAGAGCGTCGTGTAGGGAAAG

Troubleshooting

Observation	Possible Cause	Recommended Action
Adapter dimer observed as low-molecular-weight peak	Low input DNA or excess adapter carryover	Verify DNA input, adapter volumes, and cleanup steps. If adapter dimers persist, perform an additional bead cleanup after pooling the libraries to help minimize sample loss. Additional cleanup may reduce yield and short-fragment recovery. Reassess the pool's size distribution and concentration before sequencing.
Low library yield	Insufficient input, sample degradation, bead loss, over-dried beads, or suboptimal amplification cycles	Confirm input amount and fragment distribution. Ensure AMPure XP beads are fully resuspended and not over-dried. Review PCR cycle number and reagent handling.
Low library yield	Components of the template buffer may interfere with the reaction	Tris buffer, pH 7.0–8.5, is tolerated at a final concentration below 5 mM in the 33 µL End Priming reaction, however we strongly recommend the use of DNA templates in nuclease-free water where possible. For templates in other buffers, consider a suitable buffer-exchange method that preserves recovery of the intended DNA fragment sizes. Or, to assess compatibility, compare equal DNA inputs from the same sample prepared in the original template buffer and in nuclease-free water, using identical library preparation conditions.
Broad or high-molecular-weight smear	Over-amplification or sample carryover	Reduce PCR cycle number when possible. Confirm bead cleanup performance and avoid disturbing bead pellets during supernatant removal.
Unexpected library size distribution	Input fragment size distribution differs from expected or bead ratio selected for adapter dimer mitigation	Confirm input DNA size profile. Confirm correct bead ratios. Consider whether reduced bead ratios may have depleted smaller fragments.

If additional technical support is required, please email info@canalbiosciences.com. Or, visit the TrueLink® DNA Library Prep Kit product page on the canalbiosciences.com website for a list of Frequently Asked Questions.

Revision History

V1.1	Formatting updates
V1.2	Updated protocol conditions and cleanup ratios to improve recovery of smaller library fragments, modified dual ligation temperature, added adapter dimer mitigation guidance, incorporated PCR programs inline with the protocol, added Appendix text, adjusted recommended PCR cycle ranges, updated use statement to “For Research Use,” corrected 96-reaction kit fill volumes, removed duplicate cleanup instructions, and completed formatting cleanup.
V2.0	Introduced TL-D Rxn Buffer for end priming and dual ligation; reduced dual-ligation reaction volume and adapter/ligase additions; added a quench enzyme, quench master mix, and revised quenching program; incorporated isopropanol into post-ligation bead cleanup to improve DNA recovery; updated PCR cycle recommendations and kit component fill volumes.

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