

# ThruPLEX® DNA-seq Kit Protocol-At-A-Glance

ThruPLEX DNA-seq builds on the innovative ThruPLEX chemistry to generate DNA libraries with expanded multiplexing capability and with even greater performance. Kits contain either 6, 12, or 24 single-read Illumina®-compatible indexes. ThruPLEX DNA-seq can be used in DNA-seq, RNA-seq, or ChIP-seq and offers robust target enrichment performance with all of the leading platforms. For a detailed protocol, refer to the ThruPLEX DNA-seq Kit User Manual.

## Storage and Handling

Store the kit at –20°C upon arrival. Prior to use, transfer enzymes to ice and centrifuge briefly. Thaw buffers, vortex briefly, and centrifuge prior to use. Keep all enzymes and buffers on ice until used.

## Kit Contents

Name	Cap Color	6S (12) R400523 (12 rxns)	12S (48) R400428 (48 rxns)	48S R400427 (48 rxns)	48D R400406 (48 rxns)	96D R400407 (96 Rxn)
Template Preparation Buffer	Red	1 Tube	1 Tube	1 Tube	1 tube	2 Tubes
Template Preparation Enzyme	Red	1 Tube	1 Tube	1 Tube	1 Tube	2 Tubes
Library Synthesis Buffer	Yellow	1 Tube	1 Tube	1 Tube	1 Tube	2 Tubes
Library Synthesis Enzyme	Yellow	1 Tube	1 Tube	1 Tube	1 Tube	2 Tubes
Library Amplification Buffer	Green	1 Tube	1 Tube	1 Tube	1 Tube	2 Tubes
Library Amplification Enzyme	Green	1 Tube	1 Tube	1 Tube	1 Tube	2 Tubes
Nuclease-Free Water	Clear	1 Tube	1 Tube	1 Tube	1 Tube	2 Tubes
Indexing Reagents	Blue	6 Tubes	12 Tubes	1 Single Index Plate (48S)	1 Dual Index Plate (48D)	1 Dual Index Plate (96D)

## Input DNA Sample Requirements

	Requirement
<b>Nucleic Acid Source</b>	Fragmented double-stranded DNA or cDNA Cells, plasma, urine, other biofluids, FFPE tissues, fresh tissues frozen tissues
<b>Type</b>	Mechanically sheared; enzymatically fragmented; ChIP DNA; low molecular weight cell-free DNA
<b>Molecular Weight</b>	<1,000 bp
<b>Input Amount</b>	50 pg–50 ng
<b>Input Volume</b>	10 µl
<b>Input Buffer</b>	≤10 mM Tris, ≤0.1 mM EDTA

**NOTE:** Please refer to the ThruPLEX DNA-seq Kit User Manual for detailed instructions on preparing DNA samples.

## Notes Before Starting

**Additional materials and equipment needed:** Thermal cycler with 50-µl reaction volume capability and heated lid; centrifuge; PCR tubes or plates; PCR plate seals; low-binding barrier tips; fluorescent dyes; Agencourt AMPure XP (Beckman Coulter, Cat. No. A63880), 80% v/v ethanol (for bead purification), and 70% v/v ethanol.

**Selecting PCR plates/tubes:** Select plates/tubes that are compatible with the thermal cyclers and/or real-time PCR instruments used. Ensure that there is no evaporation during the process by using proper seals/caps during cycling as *evaporation may reduce reproducibility*.

**Positive and negative controls:** If necessary, include a positive control DNA (e.g., Coriell DNA, Covaris sheared, 200–300 bp) and a no-template control (NTC) as a negative control in parallel to ensure that the reaction proceeded as expected.

**Preparation of master mixes:** Keep all enzymes and buffers on ice. The Library Synthesis Master Mix and Library Amplification Master Mix can be prepared during the last 15 min of the previous step's cycling protocol and kept on ice until used.

**Indexing Reagents:** Indexing Reagents can be frozen and thawed no more than four times. Please refer to Appendix 1 of the ThruPLEX DNA-seq Kit User Manual for index plate maps and index sequences.

- ThruPLEX DNA-seq 48S Kit is provided with a Single Index Plate containing 48 Illumina-compatible indexes, each with a unique 8-nt Sanger index sequence. The plate is sealed with pierceable sealing foil; each well has sufficient volume for a single use.
- ThruPLEX DNA-seq 12S (48) Kit are provided with 12 Indexing Reagents pre-dispensed in tubes. They have sufficient reagents for up to eight uses and contain 8-nt Sanger indexes that share the same sequences in the first six bases as the Illumina TruSeq® LT indexes AD001 through AD012.
- ThruPLEX DNA-seq 6S (12) Kit is provided with 6 Indexing Reagents, pre-dispensed in tubes. They have sufficient reagents for up to eight uses and contain 8-nt Sanger indexes that share the same sequences in the first six bases as the Illumina TruSeq LT indexes AD001 through AD006.
- ThruPLEX DNA-seq 48D and 96D kits are provided with Dual Index Plates containing either 48 or 96 Illumina-compatible dual indexes. Each well of the plate has sufficient volume for a single use and contains a unique combination of Illumina's 8-nucleotide TruSeq® HT i5 and i7 index sequences.

**Index plate handling instructions:** Follow the instructions given below to avoid index cross-contamination.

- Thaw the index plate for 10 min on the benchtop prior to use. Once thawed, briefly centrifuge the plate to collect the contents at the bottom of each well. Thoroughly wipe the foil seal with 70% ethanol and allow it to dry completely.
- Pierce the seal above each well containing the specific index combination with a clean 200-µl filtered pipet tip; discard the tip.
- Use a new pipet tip to collect 5 µl of a specific index combination and add it to the reaction mixture at the Library Amplification step. A multichannel pipette may be used if needed. If indexes from the entire plate are not used at the same time, follow the instructions below to avoid contamination:
  - Cover any pierced index wells with tape (e.g., VWR General Scientific Tape 0.5", Cat. No. 89097-920) to mark the index as used.
  - Once the index plate is used, wipe the seal with 70% ethanol and let it dry completely. Replace the plastic lid and return the plate to its sleeve. Store at –20°C.

**Low-level multiplexing:** Select index combinations that meet the Illumina-recommended compatibility requirements. For more information on multiplexing and index pooling, please refer to Appendix 1 of the ThruPLEX DNA-seq Kit User Manual.

**Template Preparation**

1. Add 10 µl of DNA sample to each well of a PCR plate or tube. If necessary, include NTC negative control buffer sample(s) and positive control samples.
2. Depending on the number of reactions, prepare the Template Preparation Master Mix as described in the table below. Mix thoroughly with a pipette. Keep on ice until used.

Template Preparation Master Mix		
Component	Cap Color	Volume/Reaction
Template Preparation Buffer	Red	2 µl
Template Preparation Enzyme	Red	1 µl

3. To each 10-µl sample from Step 1 above, add 3 µl of the Template Preparation Master Mix.
4. Mix thoroughly with a pipette.

**NOTE:** Final volume at this stage will be 13 µl.

5. Seal the PCR plate using proper sealing film or tightly cap the tube(s).
6. Centrifuge briefly to collect contents at the bottom of each well or tube.
7. Place the plate or tube(s) in a thermal cycler with a heated lid set to 101°C–105°C. Perform the Template Preparation Reaction using the conditions in the table below.

Template Preparation Reaction	
Temperature	Time
22°C	25 min
55°C	20 min
4°C	Hold ≤2 hr

8. Remove the plate or tube(s) from the thermal cycler and centrifuge briefly.
9. Continue to Library Synthesis.

**Library Synthesis**

1. Prepare the Library Synthesis Master Mix as described in the table below. Mix thoroughly with a pipette. Keep on ice until used.

Library Synthesis Master Mix		
Component	Cap Color	Volume/Reaction
Library Synthesis Buffer	Yellow	1 µl
Library Synthesis Enzyme	Yellow	1 µl

2. Remove the seal on the plate or open the tube(s).
3. Add 2 µl of the Library Synthesis Master Mix to each well or tube.
4. Mix thoroughly with a pipette.  
**NOTE:** Final volume at this stage is 15 µl.
5. Seal the PCR plate using proper sealing film or tightly cap the tube(s).
6. Centrifuge briefly to collect contents to the bottom of each well or tube.
7. Return the plate or tube(s) to the thermal cycler with a heated lid set to 101°C–105°C. Perform the Library Synthesis Reaction using the conditions in the next table.

Library Synthesis Reaction	
Temperature	Time
22°C	40 min
4°C	Hold ≤30 min

8. Remove the plate or tube(s) from the thermal cycler and centrifuge briefly.
9. Continue to Library Amplification.

**Library Amplification**

1. Remove the Indexing Reagents from the freezer and thaw for 10 min on the bench top. Prior to use, centrifuge the Indexing Reagents to collect the contents at the bottom. For the index plates, wipe the plate foil seal with 70% ethanol and allow to dry.
2. Prepare the Library Amplification Master Mix as described in the table below. Mix thoroughly with a pipette. Keep on ice until used.

Library Amplification Master Mix		
Component	Cap Color	Volume/Reaction
Library Amplification Buffer	Green	25 µl
Library Amplification Enzyme	Green	1 µl
Nuclease-Free Water (plus fluorescent dyes; see NOTES)	Clear	4 µl

**NOTES:**

- Fluorescent dyes (for detection and optical calibration) are added when monitoring amplification in real time during cycling. Please refer to the real-time PCR instrument's user manual for calibration dye recommendations. The volume of detection and calibration dyes plus Nuclease-Free Water should not exceed 4 µl. If a regular thermal cycler is used, there is no need to add the dyes; use 4 µl of Nuclease-Free Water.
- Example: EvaGreen/Fluorescein dye mix. Prepare by mixing 9:1 v/v ratio of EvaGreen Dye, 20X in water (Biotium, Cat. No. 31000-T) and 1:500 diluted Fluorescein Calibration Dye (Bio-Rad Laboratories, Cat. No. 170-8780); add 2.5 µl of this mix and 1.5 µl of Nuclease-Free Water per reaction.

3. Remove the seal on the PCR plate or open the tube(s).
4. Add 30 µl of Library Amplification Master Mix to each well or tube.
5. Add 5 µl of the appropriate Indexing Reagent to each well or tube.  
**NOTE:** For the index plates, follow the index plate handling instructions (on Page 1) to avoid index cross contamination.

6. Mix thoroughly with a pipette. Avoid introducing excessive air bubbles.

**NOTE:** Final volume at this stage is 50 µl.

7. Seal the plate or tube(s) tightly and centrifuge briefly to collect contents at the bottom of each well or tube.
8. Return the plate or tube(s) to the real-time PCR thermal cycler/thermal cycler with a heated lid set to 101°C–105°C. Perform the Library Amplification Reaction using the cycling conditions from the tables below.

**CAUTION:** Ensure that the thermal cycler does not have a denaturing step programmed until Stage 3.

Library Amplification Reaction				
	Stage	Temperature	Time	# Cycles
<b>Extension &amp; Cleavage</b>	1	72°C	3 min	1
	2	85°C	2 min	1
<b>Denaturation</b>	3	98°C	2 min	1
<b>Addition of Indexes</b>	4	98°C	20 sec	4
		67°C	20 sec	
<b>Library Amplification</b>	5	72°C	40 sec	5–16**
		98°C	20 sec	
	6	4°C	Hold	

\*If monitoring in real-time, acquire fluorescence data here.

\*\*See NOTE and table below, Stage 5 Amplification Guide.

**NOTE:** The number of PCR cycles required at Stage 5 of the Library Amplification Reaction is dependent upon the amount of input DNA and the thermal cycler used. We recommend performing an optimization experiment to identify the appropriate number of PCR cycles needed. The table below provides the suggested number of PCR cycles at Stage 5 for different input amounts.

Stage 5 Amplification Guide			
DNA Input (ng)	# of Cycles	DNA Input (ng)	# of Cycles
50	5	2	10
20	6	1	11
10	7	0.2	14
5	8	0.05	16

**NOTES:**

- The amount of amplified library can range from 100 ng to 1 µg, depending on sample type, fragmentation size, and thermal cycler used. When using Covaris-fragmented reference DNA with an average size of 200 bp and following the recommended number of amplification cycles, the typical yield is 300–700 ng.
- Over-amplification could result in a higher rate of PCR duplicates in the library.

9. Remove samples from the thermal cycler and centrifuge briefly.  
**NOTE:** At this stage, samples can be processed for next-generation sequencing (NGS) immediately or stored frozen at –20° and processed later. For instructions on library pooling, purification, quantification, and sequencing, see the ThruPLEX DNA-seq Kit User Manual: [takarabio.com/manuals/](http://takarabio.com/manuals/).

**NOTICE TO PURCHASER**

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at [takarabio.com](http://takarabio.com). It is your responsibility to review, understand and adhere to any restrictions imposed by such statements

© 2017 Takara Bio Inc. All Rights Reserved.

All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Certain trademarks may not be registered in all jurisdictions. Additional product, intellectual property, and restricted use information is available at [takarabio.com](http://takarabio.com).

This document has been reviewed and approved by the Quality Department.