ThruPLEX® DNA-seq Kit FAQs

Top 10 FAQs

1. **Is ThruPLEX DNA-seq Kit compatible with any target enrichment systems?**

   Yes, ThruPLEX DNA-seq Kit is compatible with the major exome and target enrichment products, including Agilent SureSelect®, Roche NimbleGen® SeqCap® EZ and custom panels. ThruPLEX DNA-seq Kit target enrichment protocols and application notes can be accessed at: http://rubicongenomics.com/applications/enrichment/.

2. **What DNA fragment size do you recommend?**

   ThruPLEX DNA-seq Kit can accommodate a range of 50 – 1,000 bp DNA input. Fragment DNA to the size specified for the Illumina platform you are using, generally between 200 and 500 bp.

3. **What is the recommended elution/resuspension buffer for input DNA sample(s)? Are there any buffer concentration considerations?**

   The DNA sample(s) should be eluted/resuspended in a low-salt and low-EDTA, buffered solution. The preferred buffer is TE with ≤10 mM Tris and ≤0.1 mM EDTA. Avoid phosphate containing buffers.

4. **Do I need to perform cleanup on my DNA sample(s) after shearing?**

   No, simply proceed to the Template Preparation Step (**Section D.I.**) in the ThruPLEX DNA-seq Kit Instruction Manual in order to preserve your total DNA and the overall diversity of the library.

5. **What types of DNA samples can be used with ThruPLEX DNA-seq Kit?**

   Fragmented, double-stranded DNA, such as that from:
   - Mechanically-sheared DNA
   - Enzymatically-fragmented DNA
   - Low molecular weight cell-free DNA from plasma, urine, and other biofluids
   - Double-stranded cDNA
   - Chromatin Immunoprecipitated (ChIP) DNA
   - Sonicated DNA from formalin-fixed material (FFPE)

6. **Can RNA be used with ThruPLEX DNA-seq Kit?**

   Yes, but RNA needs to be converted into double-stranded cDNA and fragmented to proper size prior to preparation with ThruPLEX DNA-seq Kit.

7. **Can plasma, urine, or other biofluids be directly used with ThruPLEX DNA-seq Kit?**

   No, the template DNA must be first extracted with a DNA extraction/purification kit. Since DNA from these sources is normally degraded/fragmented, additional fragmentation is unnecessary. The volume of the sample used in the ThruPLEX DNA-seq Kit must be no more than 10 µL.

8. **Can I prepare samples for both single- and paired-end NGS sequencing?**

   Yes, ThruPLEX DNA-seq Kit is compatible with both single- and paired-end sequencing. The final amplified libraries will contain Illumina-compatible index sequences.

9. **Is there a required DNA sample input volume for ThruPLEX DNA-seq Kit?**

   Yes, the required DNA sample volume is 10 µL.

10. **What is the recommended DNA extraction method for ThruPLEX DNA-seq Kit technology?**

    The DNA extraction method will depend upon the particular sample type. Products and methods are designed specifically for extraction of FFPE tissue, plasma, etc. Regardless of the extraction method used, the DNA sample must be double-stranded and fragmented in order to be used with ThruPLEX DNA-seq Kit.

ThruPLEX® DNA-seq Kit FAQs

Additional FAQ's

General & Sample Preparation Questions

What is the difference between ThruPLEX DNA-seq Kit and other commercially available library preparation kits?

ThruPLEX DNA-seq Kit is the fastest and most sensitive library prep kit available. Its patented repair, ligation, and amplification method is performed in a single tube in three simple steps.

- Input amount: 0.05 ng to 50 ng
- No clean-up steps
- Number of steps to library: 3
- Time to library: less than 2 hours
- Number of sample transfers: 0

Is a high-fidelity enzyme used in the amplification reaction?

Yes, a high-fidelity, high-processivity, low-bias DNA polymerase is used in the ThruPLEX DNA-seq Kit.

Does ThruPLEX DNA-seq Kit's coverage span GC-rich regions?

Yes, ThruPLEX DNA-seq Kit produces uniform coverage in GC-rich regions.

Can ThruPLEX DNA-seq Kit be run on any thermal cycler?

ThruPLEX DNA-seq Kit requires the use of a thermal cycler that can accommodate its 50 µL final reaction volume. Please consult the manual of your PCR instrument.

Can denatured/single-stranded DNA be used with ThruPLEX DNA-seq Kit?

No, DNA must be double-stranded for use with ThruPLEX DNA-seq Kit.

Is DNA fragmentation necessary?

Yes, if the DNA is >1 kb in size, it must be fragmented prior to use with the ThruPLEX DNA-seq Kit.

What are the options available for DNA fragmentation?

DNA fragmentation can be accomplished either mechanically (e.g., Covaris®, Bioruptor®) or enzymatically (e.g., NEBNext® dsDNA Fragmentase® restriction endonucleases).

What total amount of DNA is needed for Covaris shearing, and what type of buffer volumes are used during the shearing?

Successful shearing has been accomplished with 2 – 50 ng of DNA in 50 – 130 µL of TE or low TE buffer.

What Covaris shearing specifications should be used to fragment the DNA prior to ThruPLEX DNA-seq Kit usage?

Covaris recommended settings should be used to obtain the desired template fragment size. Please refer to Covaris website for additional guidance; click on the Protocols tab at the bottom of the page for Quick Guide shearing protocols:

http://covarisinc.com/applications/dnarna-shearing-for-ngs/

Where can I find information about Fragmentase and how do I use it with the ThruPLEX DNA-seq Kit?

Fragmentase can be used with our product following the instructions outlined on the NEB website.

http://www.neb.com/products/m0348-nebnext-dsdna-fragmentase

What reagents not supplied are necessary to generate sequencing-ready ThruPLEX DNA-seq libraries?

Following generation of ThruPLEX DNA-seq libraries, the samples will need to be purified using Agencourt® AMPure® XP (Beckman Coulter, CAT. NO. A63880).

Can all of the ThruPLEX DNA-seq Kit reagents be mixed by vortexing?

No, only buffers and nuclease-free water may be vortexed and then spun down briefly prior to use. Enzymes should be mixed by gentle pipetting and then spun down briefly prior to use. Index Plates should be spun down briefly prior to use.

How should ThruPLEX DNA-seq Kit be stored?

ThruPLEX DNA-seq Kit should be stored at −20°C.

Can ThruPLEX DNA-seq Kit be used for methylation studies?

No.

Can the libraries created by ThruPLEX DNA-seq Kit be used for microarray or PCR analyses?

Yes.

ThruPLEX DNA-seq Kit Protocol

What is the recommended range of DNA input for ThruPLEX DNA-seq Kit?

ThruPLEX DNA-seq Kit has been designed to amplify input range of 50 pg – 50 ng. The input amount depends on the desired level of complexity needed and the application.

What is the recommended fluorescent dye for real-time PCR monitoring?

EvaGreen® fluorescent dye (Biotium, CAT. NO. 31000, EvaGreen Dye, 20X in water) is recommended because of its high sensitivity and low interference with the amplification chemistry of the ThruPLEX DNA-seq Kit.

Is it necessary to use the Indexing Reagents? If so, which indexes should be used together for multiplexing?

Yes, the included Indexing Reagents, supplied in tubes or a microplate, consist of amplification primers containing Illumina-compatible indexes, and therefore must be used in the ThruPLEX DNA-seq Library Amplification Reaction. If using less than the full set of indexes included with the kit, please refer to **Appendix 1** in the ThruPLEX DNA-seq Kit Instruction Manual for low level multiplexing and index pooling guidelines.

For MiSeq RTA v1.17.28 and later, base pair diversity of indexes is no longer checked by the Illumina Experiment Manager because low-plexity index reads can be processed for all applications; therefore, any combination of indexes can be pooled for sequencing on MiSeq.

Can I use indexing oligos prepared by an oligo provider?

No, the indexes are part of the technology developed and patented by Rubicon Genomics.

Can a library be prepared without any PCR amplification?

No, at least two library amplification cycles are required at Stage 5 of the ThruPLEX DNA-seq Library Amplification Reaction. Please refer to **Section D.III.** in the ThruPLEX DNA-seq Kit Instruction Manual for detailed information about the Library Amplification Step.

What is the recommended method to determine the optimal number of PCR cycles needed in the Library Amplification Reaction?

Please refer to **Section D.III.** in the ThruPLEX DNA-seq Kit Instruction Manual for information on selecting the optimal number of cycles for library amplification.

Can ThruPLEX DNA-seq Kit be used without a real time PCR machine? If so, how do I know when to stop my run?

Yes, ThruPLEX DNA-seq Kit can be used even if a lab does not have access to a real time PCR machine. Please refer to **Section D.III.** in the ThruPLEX DNA-seq Kit Instruction Manual and use the table on page 15 as a guide to select the number of PCR cycles based on the DNA input amount used.

How long and at what temperature should amplified libraries be stored before additional amplification?

For best performance, non-purified ThruPLEX DNA-seq libraries should be kept at -20°C for no more than seven days before being additionally amplified and/or purified. Please refer to **Section E. III.** in the ThruPLEX DNA-seq Kit Instruction Manual for additional instructions.

Can I perform additional amplification on libraries prepared with ThruPLEX DNA-seq Kit if the concentrations or yields of the libraries are not sufficient?

Yes, ThruPLEX DNA-seq libraries can be further amplified without adding extra reagents after storage at 4°C for up to 6 hours or –20°C for up to 7 days. Please refer to **Section E.I.** in the ThruPLEX DNA-seq Kit Instruction Manual for the library processing workflow and **Section E.III.** for instructions on performing additional amplification cycles.

Can I perform additional amplification on ThruPLEX DNA-seq libraries after they are purified with AMPure XP?

No.

What is the expected yield for the amplified library from ThruPLEX DNA-seq Kit?

The amount of amplified library can range from 100 ng to 1 µg depending upon many variables including sample type, fragmentation size, and thermal cycler used. When starting with Covaris-fragmented reference DNA with an average size of
200 bp and following the recommended number of amplification cycles (**Section D.III.** in the ThruPLEX DNA-seq Kit Instruction Manual), the typical yields range from 300 ng to 700 ng.

When should library quantification be performed?

Quantification must be performed prior to sequencing. Quantification is normally done after the AMPure XP purification step but libraries can also be quantified immediately following the Library Amplification Reaction to normalize samples prior to pooling. If yield is of concern, the quantification method used must not require purification of the library. Please refer to **Section E.I.** in the ThruPLEX DNA-seq Kit Instruction Manual for the library processing workflow after library preparation and **Section E.II.** for instructions on library quantification.

Is it necessary to perform quantification of ThruPLEX DNA-seq libraries before and after the AMPure XP cleanup step?

No, it is only necessary to quantify your individual or pooled libraries prior to sequencing. If you choose to quantify your libraries after the Library Amplification Reaction, it is recommended to do a final quantification of the purified libraries prior to sequencing because the AMPure XP purification step can result in loss of DNA.

Are there any special considerations that must be taken when performing AMPure XP purification on libraries prepared with ThruPLEX DNA-seq Kit?

Yes, we suggest using a 1:1 bead to sample ratio. Additionally, freshly prepared 80% ethanol solution must be used in all washing steps of the protocol. Please refer to **Section E.V.** in the ThruPLEX DNA-seq Kit Instruction Manual for detailed instructions on AMPure XP purification of ThruPLEX DNA-seq libraries for Next Generation Sequencing.

NGS Using ThruPLEX DNA-seq Kit

Which Illumina NGS systems can I use to sequence the libraries prepared using ThruPLEX DNA-seq Kit?

Libraries prepared with ThruPLEX DNA-seq Kit are compatible with all Illumina sequencing platforms, including the HiSeqs, MiSeq, NextSeq 500, and GAIIx.

What concentration of ThruPLEX DNA-seq library should be loaded onto the flow cell?

Please follow Illumina's recommendations for optimal loading concentration specific to the version of flow cell you are using. For sequencing on the Illumina® MiSeq, v3, we suggest that you load 14 – 15 pM of ThruPLEX DNA-seq libraries.

Are there any special steps that need to be followed when loading a ThruPLEX DNA-seq library onto the flow cell?

No, ThruPLEX DNA-seq libraries should be handled and processed the same as libraries generated using Illumina library preparation kits, such as the TruSeq® Nano DNA HT Sample Prep Kit.

Is it recommended to spike PhiX into the library prior to loading onto the flow cell?

Illumina recommends adding 1% PhiX for most libraries. For low diversity libraries or if experiencing sequencing issues, increase the PhiX control spike-in to at least 5%. Please refer to **Section E.VII.** in the ThruPLEX DNA-seq Kit Instruction Manual for additional sequencing recommendations.

Does ThruPLEX DNA-seq Kit use single indexes or dual indexes?

ThruPLEX DNA-seq 48D and 96D Kits use Illumina-compatible **dual indexes** provided in a 96-well plate.

ThruPLEX DNA-seq 6S (12 Rxn), 12S, 12S (48 Rxn), and 48S Kits use Illumina-compatible **single indexes** pre-dispensed in tubes or a 96-well plate.

Which Illumina dual indexes does ThruPLEX DNA-seq Kit use?

ThruPLEX DNA-seq 48D and 96D Kits use dual indexes that are 8nt long and identical to the **Illumina TruSeq HT** i5 and i7 dual indexes. The i5 indexes have the Illumina D501 to D508 sequences, and the i7 indexes have the D701 to D712 sequences. For more information about the dual indexes, please refer to **Appendix 1** in the ThruPLEX DNA-seq Kit Instruction Manual.

What kind of single indexes does ThruPLEX DNA-seq Kit use?

ThruPLEX DNA-seq 6S (12Rxn), 12S, 12S (48 Rxn), and 48S Kits use Illumina-compatible 8nt single index sequences developed by the Wellcome Trust Sanger Institute in Cambridge, UK. Information about the Sanger index sequences can be found in *Nature Methods* **7**, 111-118 (2010). For more information about the single indexes and setting up a Sample Sheet on the Illumina Experiment Manager, please refer to **Appendix 1** in the ThruPLEX DNA-seq Kit Instruction Manual.

Can I use only one of the indexes of a dual-indexed library?

No, ThruPLEX DNA-seq sample preparation for dual-indexed libraries requires that both indexes be present on the library. To carry out single-indexed sequencing, in which the second index (i5) is not sequenced, pooled libraries must be tagged with different i7 indexes to distinguish the individual samples. A single-indexed sequencing method is supported on Illumina sequencing instruments, where only the Index 1 (i7) is used. See the instrument user guide for more information about setting up an eight-base single-indexed sequencing run.

Note:

If the above FAQs do not address your specific question(s), please email us at **support@rubicongenomics.com** or call at **734-677-4845** (9:00AM – 5:30PM EST).

ThruPLEX® DNA-seq Kit is intended for **Research Use Only**. It may not be used for any other purposes including, but not limited to, use in diagnostics, forensics, therapeutics, or in humans. ThruPLEX DNA-seq Kit may not be transferred to third parties, resold, modified for resale or used to manufacture commercial products without prior written approval of Rubicon Genomics, Inc.

ThruPLEX DNA-seq Kit is protected by U.S. Patents 7,803,550; 8,071,312; 8,399,199; 8,728,737 and corresponding foreign patents. Additional patents are pending.

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