

Nextera™ DNA Sample Prep Kit (Illumina®-Compatible)

Cat. Nos. GA09115, GA091120, GA0911-50, GA0911-96, and GABC0950

The Nextera™ DNA Sample Prep Kit is designed to prepare genomic DNA libraries compatible with the Illumina® Genome Analyzer I and II and HiSeq™ 2000 sequencers. Nextera technology† employs *in vitro* transposition to simultaneously fragment and tag DNA in a single-tube reaction, and prepare sequencer-ready libraries in under 2 hours. The Nextera library preparation procedure is a significant improvement upon current procedures, which generally consist of distinct DNA fragmentation, end-polishing, and adaptor-ligation steps. The Nextera library preparation procedure combines these steps into one (tagmentation), uses only 50 ng of starting DNA, and allows incorporation of platform-specific tags and optional barcodes.

Product Specifications

Storage: Store the Nextera DNA Sample Prep Kit at –20°C in a freezer without a defrost cycle.

Quality Control: Nextera DNA Sample Prep Kit is function-tested by tagmenting control DNA (lambda) with both Low-Molecular-Weight (LMW) and High-Molecular-Weight (HMW) Buffers. Size distribution following tagmentation and PCR amplification is confirmed by Bioanalyzer profiling.

Contaminating Activity Assays: All components of the Nextera DNA Sample Prep Kit are free of detectable RNase and DNase activities.

Nextera Control DNA: The Nextera Control DNA is unmethylated c1857 *Sam7* Lambda DNA, isolated from infected GM119, an *E. coli* strain lacking both the *dam* and *dcm* methylase activities. GenBank®/EMBL Accession Number J02459, Size: 48.5 kb.

Additional Required Components (Not Provided)

- Zymo DNA Clean & Concentrator™-5 (Cat. No. D4013) or equivalent.
- Nextera™ PCR Enzyme (Cat. Nos. EM091120, EM091150, EM0911-96)

Related Products: The following products are also available:

- Nextera™ Bar Codes (Illumina®-compatible)
- Nextera™ PCR Enzyme

**The 5-, 20-, 50-, and 96-reaction kits contain sufficient sequencing primers (Read 1, Read 2, and Index Read) for 3, 10, 15, and 30 flow cells, respectively. If needed, sequencing primers for additional flow cells can be provided upon request.*

Target DNA is fragmented and tagged with Nextera Enzyme Mix containing transposon ends appended with sequencing primer sites (blue and orange). Limited-cycle PCR with a four-primer reaction adds bridge PCR (bPCR)-compatible adaptors (purple and pink) to the core sequencing library. Optional bar codes (triangle) can be added between the

Nextera™ DNA Sample Prep Kits (Illumina®-compatible) Contents

The Nextera DNA Sample Prep Kit (Illumina®-compatible) is available in four sizes (5, 20, 50, and 96 reactions). All reagents in these kits are in blue-capped tubes.

Component Name	GA091115 5 Reactions	GA091120 20 Reactions	GA0911-50 50 Reactions	GA0911-96 96 Reactions
Nextera™ Enzyme Mix (Illumina®-compatible)	5 µl	20 µl	50 µl	96 µl
5X Nextera™ Reaction Buffer (LMW)	50 µl	200 µl	500 µl	1.0 ml
5X Nextera™ Reaction Buffer (HMW)	50 µl	200 µl	500 µl	1.0 ml
50X Nextera™ Primer Cocktail (Illumina®-compatible)	5 µl	20 µl	50 µl	96 µl
50X Nextera™ Adaptor 2 (Illumina®-compatible)	5 µl	20 µl	50 µl	96 µl
2X Nextera™ PCR Buffer	125 µl	500 µl	1.25 ml	2 x 1.2 ml
Nextera™ Control DNA	10 µl	10 µl	10 µl	10 µl
200X Nextera™ Read 1 Primer*	30 µl	100 µl	150 µl	300 µl
200X Nextera™ Read 2 Primer*	30 µl	100 µl	150 µl	300 µl
200X Nextera™ Index Read Primer*	30 µl	100 µl	150 µl	300 µl

downstream bPCR adaptor (pink) and the core sequencing library adaptor (orange). Alternative sequencing primers are required for the Illumina/Solexa®-compatible libraries: Read 1 Primer (blue/gray arrow); Read 2 Primer (orange/gray arrow); Index Read Primer (gray/orange arrow).

Sequences:

Transposon End Sequence: 5'-AGATGTGATAAGAGACAG-3'

Primer 1*: 5'-AATGATACGGCGACCACCGA-3'

Adaptor 1*:

5'-AATGATACGGCGACCACCGAGATCTACACGCCTCCCTCGGCCATCAG-3'

Primer 2*: 5'-CAAGCAGAAGACGGCATACGA-3'

Adaptor 2 (minus bar code)*:

5'-CAAGCAGAAGACGGCATACGAGATCGGTCTGCCTTGCCAGCCCCTCAG-3'

Nextera Read 1 Primer: 5'-GCCTCCCTCGGCCATCAGAGATGTGTATAAGAGACAG-3'

Nextera Read 2 Primer: 5'-GCCTTGCCAGCCGCTCAGAGATGTGTATAAGAGACAG-3'

Nextera Index Read Primer:

5'-CTGTCTTATACATCTCTGAGCGGGCTGGCAAGGCAGACCG-3'

Note: The kit contains a 50X Nextera Primer Cocktail, which consists of Primer 1 (10 µM),

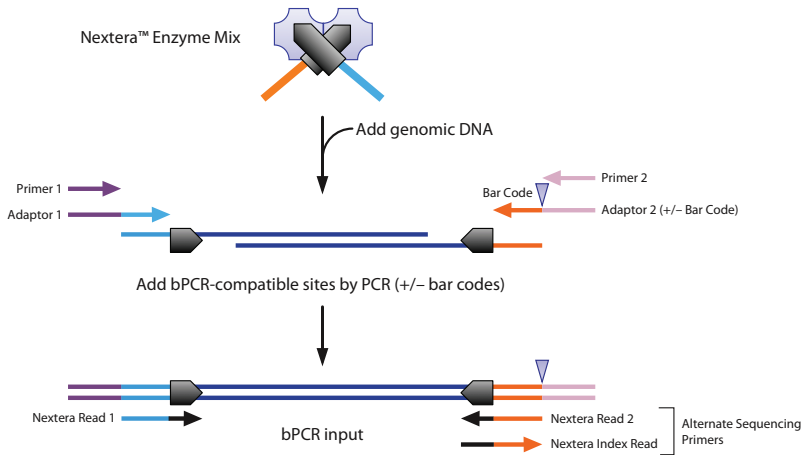


Figure 1. Generating Illumina®-compatible libraries.

Primer 2 (10 μM), and Adaptor 1 (0.5 μM). A single primer, 50X Nextera Adaptor 2 (which does not contain a bar code), is also included in the kit. The 50X Nextera Adaptor 2 (0.5 μM) can be replaced with one of the Bar Coding Primers from the Illumina®-compatible Bar Codes Kit (optional). Nextera Read 1, Read 2, and Index Read Primers are provided at a concentration of 100 μM (200X).

*The sequences of Primer 1 and Primer 2 and portions of Adaptor 1 and Adaptor 2 correspond to Illumina® bPCR sequences and are copyrighted to Illumina, Inc. Oligonucleotide sequences © 2006-2010 Illumina, Inc. All rights reserved.

Important Considerations

- Fragment Size Distribution:** The Nextera DNA Sample Prep Kit contains two buffers, Low-Molecular-Weight Buffer (LMW) and High-Molecular-Weight Buffer (HMW). Refer to table below for approximate fragment size distribution using each buffer.

	Fragment Size (approx.)*		Insert Size (approx.)	
	LMW Buffer	HMW Buffer	LMW Buffer	HMW Buffer
Lambda DNA (Control)	175-400 bp	175-700 bp	40-265 bp	40-565 bp

*Fragment Size (approx.) includes the 135-bp adaptor sequences (67-bp Adaptor 1 + 68-bp Adaptor 2) without bar coding. **For all paired-end sequencing, we recommend using HMW Buffer only.**

Note: These are approximations only, as the actual fragment size distribution will depend on a number of factors including the type and quality of the starting DNA.

*See note, page 4.

- DNA Quality:** The quality of the starting DNA is critical. Contaminants such as protein and DNA may inhibit the Nextera reaction if present in the DNA preparation. If DNA purity is in question, the DNA should be cleaned using Zymo Genomic DNA Clean & Concentrator, Cat. No. D4010 (or equivalent).
- Transposon End Sequence:** The 19-bp transposon DNA sequence is present at the 5' end of all Illumina®-compatible libraries. However, the 19-bp transposon DNA sequence is NOT sequenced on the Illumina® platform. The Nextera Read 1 and Read 2 primers anneal to this sequence so that the first nucleotide sequenced is target DNA.
- Input DNA:** The kit has been optimized to process 50 ng of DNA to the target MW distribution. MW distribution will be lower if using less than 50 ng of DNA.
- Amplicons:** The Nextera DNA Sample Prep Kit can also make libraries from amplicons. Amplicons as small as ~2 kb have been successfully sequenced. However, the distal ~50-100 bp of linear fragments may exhibit a decrease in coverage. Nextera kits can also be used to make libraries from circular DNA samples.
- Bias:** The transposase used in the Nextera system carries mutations and is used under conditions that result in near-random integration. As with any enzymatic system, there is a slight bias in the reaction. However, since the reaction is driven to completion with an excess of the Nextera Enzyme, we have not seen any impact on the distribution of coverage. The coverage across assemblies is comparable to those seen with mechanical methods of shearing.
- Bar Codes:** Platform-specific Bar Coding kits are available to prepare up to 12 bar coded libraries. If additional or alternate Bar Codes are needed, the following template design can be used:

Adaptor 2 (plus Bar Code-optional)

Primer

Sequencing Tag

5'-CAAGCAGAAGACGGCATACGAGAT-[BAR CODE]*-CGGTCTGCCTTGCCAGCCCCTCAG-3'

*Reverse complement of sequencing read.

- Sequencing in the Same Channel:** The Nextera sequencing primers are compatible with the Illumina® sequencing primers, and can be used together.
- Nextera PCR Enzyme:** Use only Nextera PCR Enzyme for limited-cycle PCR (Part B). Other PCR systems have been tested and do not perform as well.

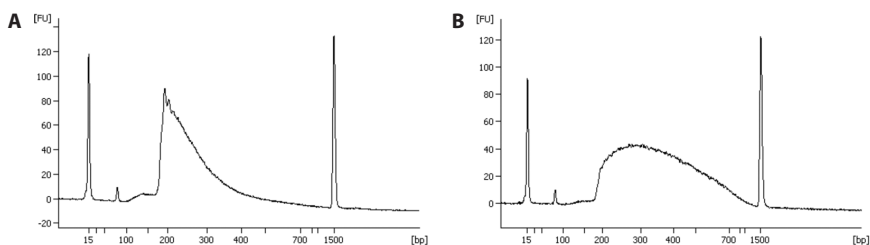
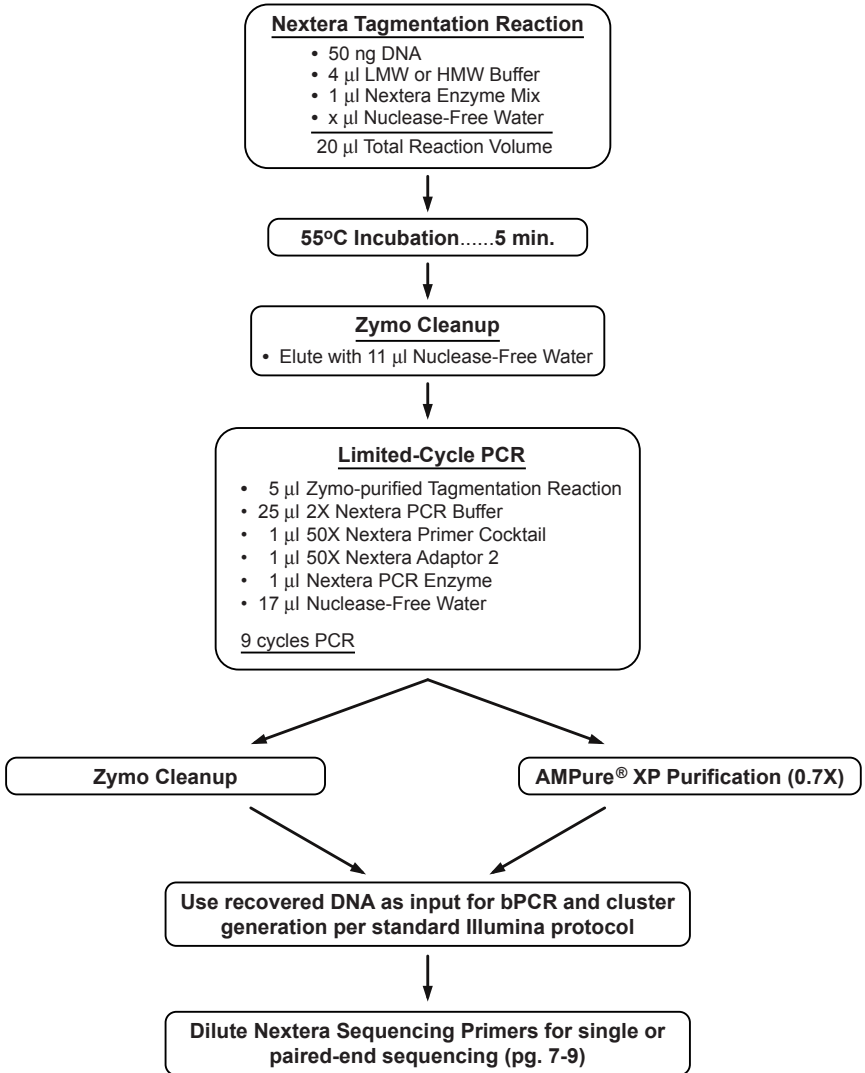


Figure 2. Fragment Size Distribution (approx.) using LMW Buffer (A) and HMW Buffer (B), per standard protocol.

Flowchart for Nextera™ DNA Sample Preparation



Nextera DNA Sample Prep Kit (Illumina®-compatible) Protocols

A. Tagmentation Reaction

1. Prior to assembling the reaction, briefly centrifuge the 5X Nextera Reaction Buffer and Nextera Enzyme Mix tubes to assure that the reagents are at the bottom of the tubes.
2. Assemble the following reaction components on ice, in the order listed:
 - x μ l Nuclease-Free Water
 - 50 ng Target DNA (in T₁₀E₁ Buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA])
 - 4 μ l 5X Nextera Reaction Buffer LMW or HMW
(see Important Considerations, no. 1, p. 4)
 - 1 μ l Nextera Enzyme Mix (Illumina®-compatible)

20 μ l Total reaction volume

3. Mix briefly by vortexing, and incubate at 55°C for 5 minutes.

Notes: To prevent evaporation, the reaction should be carried out in a thermocycler with a heated lid or the reaction should be overlaid with mineral oil.

The tagmentation reaction does occur, although very slowly, at room temperature. We recommend assembling the components on ice and proceeding immediately to the 55°C incubation.

4. Purify the tagmented DNA using a Zymo DNA Clean & Concentrator-5 Kit (or equivalent).

Brief Zymo Protocol (perform at room temperature):

- Add 100 μ l of DNA Binding Buffer to the 20 μ l Tagmentation Reaction from step 2 (above).
- Mix briefly by vortexing, and transfer the mixture to a Zymo-Spin™ Column in a Collection Tube.
- Centrifuge at 10,000 x g for 60 seconds. Discard the flow-through.
- Add 250 μ l of Wash Buffer to the column. Centrifuge at 10,000 x g for 60 seconds. Discard the flow-through.
- Repeat the wash step.
- Centrifuge the column at 10,000 x g for 60 seconds to eliminate any residual Wash Buffer.
- Transfer the column to a clean and sterile 1.5-ml microcentrifuge tube
- Add 11 μ l of Nuclease-Free Water directly to the column and incubate at room temperature for 1-2 minutes. Centrifuge at 10,000 x g for 60 seconds to elute the DNA.
- The final eluted volume should be ~10 μ l. Use 5 μ l as DNA template in Part B, Step 1.

Note: Nextera technology has been validated with the Zymo DNA Clean & Concentrator-5 and Qiagen MinElute® DNA Purification Kits. Equivalent kits can also be used; however, care must be taken when eluting the DNA from the spin columns.

B. Addition of bPCR-Compatible Sites and Library Enrichment

Add bPCR-compatible sites and optional bar coding by PCR.

- Assemble the following reaction components at room temperature:
 - 17 µl Nuclease-Free water
 - 5 µl Recovered DNA Fragment Library (from Part A, Step 3)
 - 25 µl 2X Nextera PCR Buffer
 - 1 µl 50X Nextera Primer Cocktail (Illumina®-compatible)
 - 1 µl 50X Nextera Adaptor 2*
 - 1 µl Nextera PCR Enzyme (sold separately, see Related Products)

 50 µl Total reaction volume

***Note:** For a bar coded library, replace 50X Nextera Adaptor 2 with a bar coded Illumina®-compatible Adaptor 2 from the Nextera Bar Codes (Illumina®-compatible) kit (e.g., GA Adaptor 2 [IDX1]).

- Cycle the samples in a thermocycler under the following conditions:

	followed by 9 cycles of:	
72°C for 3 minutes**	95°C for 10 seconds	
95°C for 30 seconds	→ 62°C for 30 seconds	→ Hold at 4°C
	72°C for 3 minutes	

****Note:** It is critical to perform the 72°C extension step **before** denaturing the DNA templates.

- Purify the tagged DNA fragments using a Zymo DNA Clean & Concentrator-5 kit, or equivalent.

Note: The anticipated yield is ~300 ng of amplified DNA. To remove fragments below 300 bp, we strongly recommend using Agencourt® AMPure® XP beads (0.7X) instead of the Zymo DNA Clean and Concentrator-5 kit.

- Use the recovered DNA as input for bPCR and cluster generation per the standard Illumina protocol.

Note: For cluster sequencing, it is critical to use the provided Nextera sequencing primers.

- Use the Nextera Read 1 Primer for Paired-End Read 1 Sequencing or for Single-Read Sequencing.
- Use the Nextera Index Read Primer for Index Read Sequencing.
- Use the Nextera Read 2 Primer for Paired-End Read 2 Sequencing.

Note: The 5-, 20-, 50-, and 96-reaction kits contain sufficient sequencing primers (Read 1, Read 2, and Index Read) for 3, 10, 15, and 30 flow cells, respectively. If needed, sequencing primers for additional flow cells can be provided upon request.

For Paired-End Reads:

Illumina paired-end sequencing mixes (HP1 and HP2) contain the Illumina® sequencing primers. These primers do not interfere with the Nextera sequencing primers and sequencing can be performed in the presence of HP1 and HP2 primers.

- Paired-End Read 1: Dilute Nextera Read 1 Primer 1:200 into Sequencing Mix HP1.
- Index Read: Dilute Nextera Index Read Primer 1:200 into Hybridization Buffer.
- Paired-End Read 2: Dilute Nextera Read 2 Primer 1:200 into Sequencing Mix HP2.

Alternatively, if it is required to perform sequencing in the presence of only the Nextera primers, the 200X Nextera sequencing primers can be diluted 1:200 into Hybridization Buffer (GA0084204-HT1 or 5X SSC, 0.05% Tween®-20).

For Single Reads:

- Paired-End Read 1: Dilute Nextera Read 1 Primer 1:200 into Sequencing Mix HP4.
- Index Read: Dilute Nextera Index Read Primer 1:200 into Hybridization Buffer.

Appendix A

The Illumina®-compatible Bar Codes Kit contains 12 bar codes. A 50-µl aliquot of each is provided at a concentration of 0.5 µM. This is sufficient for 50 bar coded libraries. All reagents in this kit are in blue-capped tubes. Cat. No. GABC0950

For bar coding, one of these bar codes can be substituted with 50X Nextera Adaptor 2 (Illumina®-compatible) from the Nextera DNA Library Prep Kit (Illumina®-compatible). Use 1 µl in the reaction.

GA Adaptor 2 (IDX1)

5'-CAAGCAGAAGACGGCATAACGAGAT**CGTGAT**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX2)

5'-CAAGCAGAAGACGGCATAACGAGAT**ACATCG**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX3)

5'-CAAGCAGAAGACGGCATAACGAGAT**GCCTAA**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX4)

5'-CAAGCAGAAGACGGCATAACGAGAT**TGGTCA**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX5)

5'-CAAGCAGAAGACGGCATAACGAGAT**CACTGT**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX6)

5'-CAAGCAGAAGACGGCATAACGAGAT**ATTGGC**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX7)

5'-CAAGCAGAAGACGGCATAACGAGAT**GATCTG**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX8)

5'-CAAGCAGAAGACGGCATAACGAGAT**TCAAGT**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX9)

5'-CAAGCAGAAGACGGCATAACGAGAT**CTGATC**CGGTCTGCCTTGCCAGCCCGCTCAG-3'

GA Adaptor 2 (IDX10)

5'-CAAGCAGAAGACGGCATACGAGATAAGCTACGGTCTGCCTTGCCAGCCCCTCAG-3'

GA Adaptor 2 (IDX11)

5'-CAAGCAGAAGACGGCATACGAGATGTAGCCCGGTCTGCCTTGCCAGCCCCTCAG-3'

GA Adaptor 2 (IDX12)

5'-CAAGCAGAAGACGGCATACGAGATTACAAGCGGTCTGCCTTGCCAGCCCCTCAG-3'

Note: Bar Code sequence as read from the Index Read Primer (reverse complement).

IDX1: ATCACG

IDX4: TGACCA

IDX7: CAGATC

IDX10: TAGCTT

IDX2: CGATGT

IDX5: ACAGTG

IDX8: ACTTGA

IDX11: GGCTAC

IDX3: TTAGGC

IDX6: GCCAAT

IDX9: GATCAG

IDX12: CTTGTA

[†]Covered by patents issued and pending.

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