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Nextera™ DNA Sample Prep Kit (Roche Titanium-compatible)

Cat. Nos. NT09115, NT091120, NT0911-50, NT0911-96, and NTBC0950

DISCONTINUED

The Nextera™ DNA Sample Prep Kit is designed to prepare genomic DNA libraries compatible with the Roche 454™ Genome Sequencer FLX™ System, with FLX Titanium chemistry. 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, uses only 50 ng of starting DNA, and allows incorporation of platform-specific tags and optional barcodes.

Nextera™ DNA Sample Prep Kits (Roche Titanium-compatible) Contents

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

Component Name	NT09115 5 Reactions	NT091120 20 Reactions	NT0911-50 50 Reactions	NT0911-96 96 Reactions
Nextera™ Enzyme Mix (Roche Titanium-compatible)	5 µl	20 µl	50 µl	96 µl
5X Nextera™ Reaction Buffer (HMW)	50 µl	200 µl	500 µl	2 x 500 µl
50X Nextera™ Primer Cocktail (Roche Titanium-compatible)	5 µl	20 µl	50 µl	96 µl
50X Nextera™ Adaptor 1 (Roche Titanium-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

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 fragmenting/tagging control DNA (lambda) in the High-Molecular-Weight (HMW) Buffer. Size distribution following tagmentation and PCR amplification is confirmed by Bioanalyzer profiling.

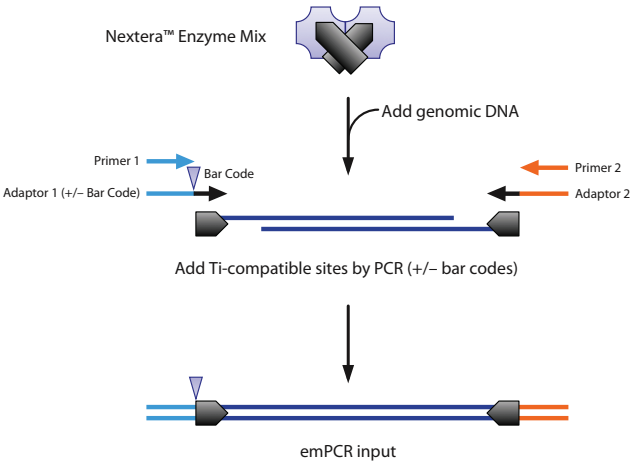


Figure 1. Generating Roche Titanium-compatible libraries.

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 *Sam*7 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 (Roche Titanium-compatible)

– Nextera™ PCR Enzyme

– 10X TA Buffer

Target DNA is fragmented and tagged with the Nextera Enzyme Mix containing free transposon ends. Limited-cycle PCR with a four-primer reaction adds Roche Titanium-compatible adaptor sequences (blue and orange). Optional bar coding (triangle) is added between the upstream emPCR adaptor (blue) and the transposon end (gray).

Sequences:

Transposon End Sequence: 5′-AGATGTGTATAAGAGACAG-3′

Primer 1: 5′-CCATCTCATCCCTGCGTGTCTCCGAC-3′

Adaptor 1(minus bar code):
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAGAGATGTGTATAAGAGACAG-3′

Primer 2: 5′-CCTATCCCCTGTGTGCCTTGGCAGTC-3′

Adaptor 2:
5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGATGTGTATAAGAGACAG-3′

Note: *The kit contains a 50X Nextera Primer Cocktail, which consists of Primer 1 (10 µM), Primer 2 (10 µM), and Adaptor 2 (0.5 µM). A single primer, 50X Nextera Adaptor 1 (0.5 µM) (which does not contain a Bar Code), is also included in the kit. The 50X Nextera Adaptor 1 can be replaced with one of the Bar Coding Primers from the Titanium-compatible Bar Coding Kit (optional).*

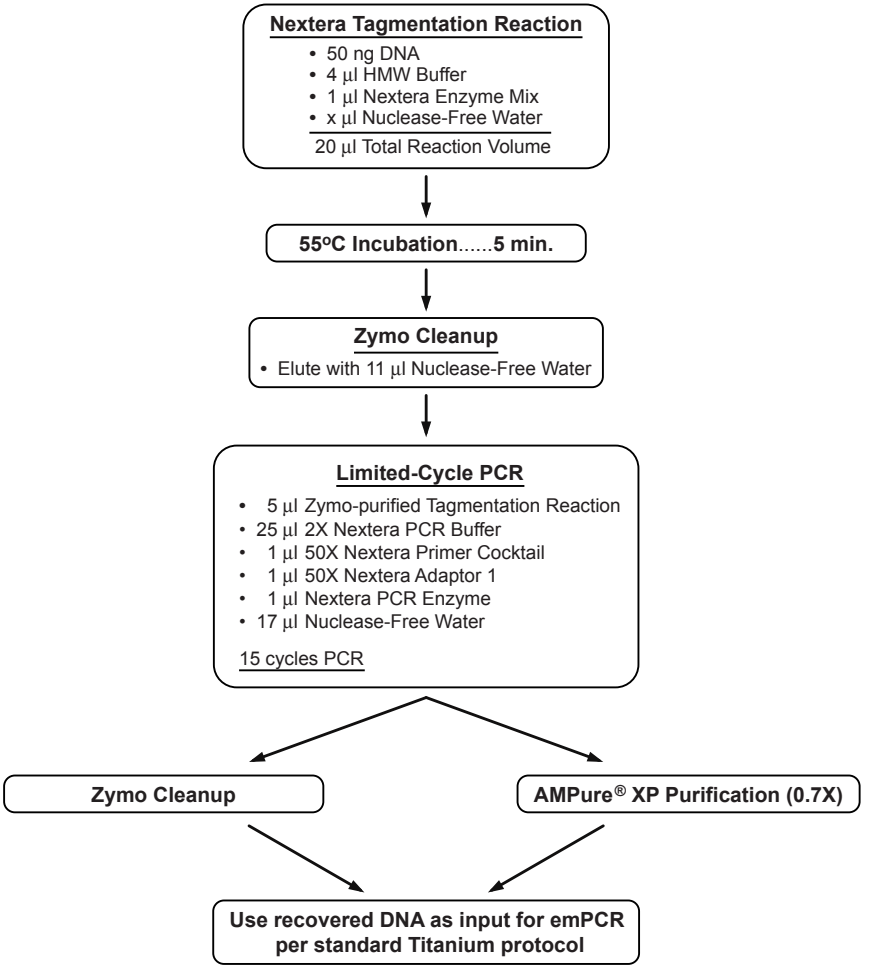
Important Considerations

1. **Fragment Size Distribution:** The Nextera DNA Sample Prep Kit contains a buffer which will yield fragments of approximately 500-1100 bp in size with the Control DNA. Fragment size includes 98-bp adaptor sequences (without bar coding), therefore the actual insert size will be approximately **400-1000 bp**.

   Note: *For different sample types, the fragment/insert size can be adjusted by performing a buffer titration using HMW and TA Buffers (10X TA Buffer: 330 mM Tris-acetate [pH 7.8], 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT; Cat. Nos. TA6115 and TA6160).*
2. **DNA Quality:** The quality of the starting DNA is critical. Contaminants such as protein and RNA 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).
3. **Transposon End Sequence:** The 19-bp transposon DNA sequence is present at the 5′ end of all Roche Titanium-compatible libraries, and will be sequenced by the instrument. This sequence will need to be filtered out prior to assembly and mapping.

   19-bp transposon DNA sequence: 5′-AGATGTGTATAAGAGACAG-3′.
4. **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.
5. **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.
6. **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

Flowchart for Nextera™ DNA Sample Preparation



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.

7. **Nextera PCR Enzyme:** Use only Nextera PCR Enzyme for limited-cycle PCR (Step B, page 6). Other PCR systems have been tested and do not perform as well.
8. **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 1 (plus Bar Code-optional)

5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-[BAR CODE]-AGATGTGTATAAGAGACAG-3′

9. **Plate Reaction Placement:** Nextera-generated and "standard" libraries can be sequenced in the same region of a plate. The Nextera library is compatible with the Roche Titanium emPCR and sequencing primers. The Nextera library can be "binned" based on the presence of the 19-bp transposon sequence or other bar coding sequence.

Flowchart for Nextera DNA Sample Preparation

Nextera DNA Sample Prep Kit (Roche Titanium-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	µl	Nuclease-Free Water
50	ng	Target DNA (in $T_{10}E_1$ Buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA])
4	µl	5X Nextera Reaction Buffer HMW (see Important Considerations, no. 1, page 3)
1	µl	Nextera Enzyme Mix (Roche Titanium-compatible)
20	µl	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 µl of DNA Binding Buffer to the 20 µl 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 µl 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 empty column at 10,000 x g for 60 seconds to dry and to eliminate any residual Wash Buffer.

– Transfer the column to a clean and sterile 1.5-ml microcentrifuge tube.
– Add 11 µl of Nuclease-Free Water directly to the column and incubate at room temperature for 1-2 minutes, and centrifuge at 10,000 x g for 60 seconds to elute the DNA.
– The final eluted volume should be ~10 µl. Use 5 µl as PCR template in Part B, Step 1.

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

B. Addition of emPCR-Compatible Sites and Library Enrichment

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

1. 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 (Roche Titanium-compatible)
1	µl	50X Nextera Adaptor 1*
1	µl	Nextera PCR Enzyme (2.5 U/µl; sold separately, see Related Products
50	µl	Total reaction volume

Note: *For a bar coded library, replace 50X Nextera Adaptor 1 with a bar coded Titanium-compatible Adaptor 1 from the Nextera Bar Codes (Roche Titanium-compatible) kit (e.g., Ti Adaptor 1 [MID1]).*

2. Cycle the samples in a thermocycler under the following conditions:

   72°C for 3 minutes**

   95°C for 30 seconds

   followed by 15 cycles of:

   95°C for 10 seconds

   55°C for 30 seconds

   72°C for 3 minutes

   Hold at 4°C.

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

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

   Note: *The anticipated yield is ~500 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.*

4. Use the recovered DNA as input for emPCR, using the manufacturer's recommended protocol.

Note: *The sequencing reads will contain the 19-base pair transposon end sequence at the 5′ ends. This sequence should be filtered out prior to assembly and analysis.*

19-bp Transposon End Sequence: **5′-AGATGTGTATAAGAGACAG-3′**

Appendix A

The Roche Titanium-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 each. All reagents in this kit are in yellow-capped tubes. Cat. No. NTBC0950

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

Titanium Adaptor 1 (MID1)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**ACGAGTGCGT**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID2)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**ACGCTCGACA**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID3)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**AGACGCACTC**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID4)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**AGCACTGTAG**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID5)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**ATCAGACACG**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID6)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**ATATCGCGAG**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID7)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**CGTGTCTCTA**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID8)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**CTCGCGTGTC**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID10)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**TCTCTATGCG**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID11)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**TGATACGTCT**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID13)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**CATAGTAGTG**AGATGTGTATAAGAGACAG-3′

Titanium Adaptor 1 (MID14)
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG**CGAGAGATAC**AGATGTGTATAAGAGACAG-3′

†*Covered by patents issued and pending.*

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726 Post Road, Madison, WI 53713 (800) 284-8474 (608) 258-3080 Fax (608) 258-3088