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# Nextera™ DNA Sample Prep Kit

## (Roche FLX-compatible)

Cat. Nos. FL09115, FL091120, and FLBC0950

** Covered by patents issued and pending.*

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## 1. Introduction

The Nextera™ DNA Sample Prep Kit is designed to prepare genomic DNA libraries compatible with the Roche 454™ Genome Sequencer FLX™ System, with standard FLX 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.

## 2. Kit Contents

| Cat. # | Quantity |
|---|---|

**Nextera™ DNA Sample Prep Kit (Roche FLX-compatible) Contents**

The Nextera DNA Sample Prep Kit (Roche FLX-compatible) is available in two sizes (5 and 20 reactions). All reagents in these kits are in green-capped tubes.

**5 Reactions**

| FL09115 | |
|---|---|
| Nextera™ Enzyme Mix (Roche FLX-compatible) | 5 µl |
| 5X Nextera™ Reaction Buffer (LMW) | 50 µl |
| 5X Nextera™ Reaction Buffer (HMW) | 50 µl |
| 50X Nextera™ Primer Cocktail (Roche FLX-compatible) | 5 µl |
| 50X Nextera™ Adaptor 1 (Roche FLX-compatible) | 5 µl |
| 2X Nextera™ PCR Buffer | 125 µl |
| Nextera™ Control DNA | 10 µl |

**20 Reactions**

| FL091120 | |
|---|---|
| Nextera™ Enzyme Mix (Roche FLX-compatible) | 20 µl |
| 5X Nextera™ Reaction Buffer (LMW) | 200 µl |
| 5X Nextera™ Reaction Buffer (HMW) | 200 µl |
| 50X Nextera™ Primer Cocktail (Roche FLX-compatible) | 20 µl |
| 50X Nextera™ Adaptor 1 (Roche FLX-compatible) | 20 µl |
| 2X Nextera™ PCR Buffer | 500 µl |
| Nextera™ Control DNA | 10 µl |

**Additional Required Components**
**(Not Provided)**

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

## 3. 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) with both Low-Molecular-Weight (LMW) and High-Molecular-Weight (HMW) Buffers. Size distribution following fragmentation reaction is confirmed by gel analysis.

**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 cI857 *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.

## 4. Related Products

The following products are also available:

– Nextera™ Bar Codes (Roche FLX-compatible)
– Nextera™ PCR Enzyme

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 FLX-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′-GCCTCCCTCGCGCCATCAG-3′ |
| Adaptor 1(minus bar code): | 5′-GCCTCCCTCGCGCCATCAGAGATGTGTATAAGAGACAG-3′ |
| Primer 2: | 5′-GCCTTGCCAGCCCGCTCAG-3′ |
| Adaptor 2: | 5′-GCCTTGCCAGCCCGCTCAGAGATGTGTATAAGAGACAG-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 FLX-compatible Bar Coding Kit (optional).*

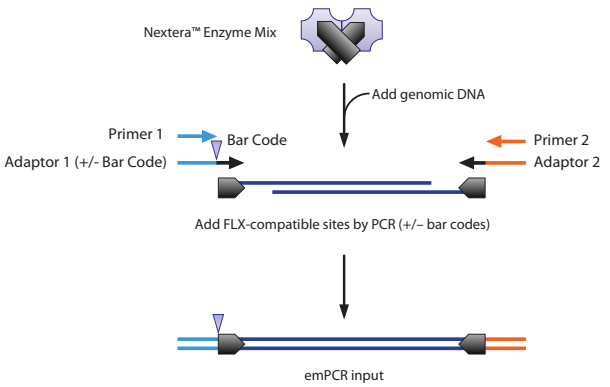


**Figure 1. Generating Roche FLX-compatible libraries.**

## 5. Important Considerations

1. **Expected Fragment Distribution:** The Nextera DNA Sample Prep Kit contains two buffers, Low-Molecular-Weight Buffer (LMW) and High-Molecular-Weight Buffer (HMW). For Roche FLX-compatible libraries, the LMW buffer should yield fragments approximately 200-1,000 bp, and the HMW buffer should yield fragments approximately 200-2,000 bp. These fragment approximations include the 2 x 39-bp adaptor sequence.

   ***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.*

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 FLX-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. If using less than 50 ng of DNA, we recommend analyzing library yield after two to five additional PCR cycles.
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 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 coverage. The coverage across assemblies is comparable to those seen with mechanical methods of shearing.

7. **Larger Fragments:** The current Nextera Enzyme Mix formulation is optimized to generate fragments less than 2 kb when using HMW Buffer. The HMW Buffer could potentially be used to generate fragments greater than 2 kb; however, the conditions for this reaction will need to be optimized separately for individual starting genomic DNA.
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′-GCCTCCCTCGCGCCATCAG-[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 FLX 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.

## 6. Nextera DNA Sample Prep Kit (Roche FLX-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 LMW or HMW (see Important Considerations, no. 1, page 3) |
| 1 | µl | Nextera Enzyme Mix (Roche FLX-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 FLX-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 FLX-compatible Adaptor 1 from the Nextera Bar Codes (Roche FLX-compatible) kit (e.g., FLX 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 PCR amplified DNA.*

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′**

## 7. Troubleshooting Nextera DNA Sample Prep Reactions

**DNA did not fragment**

1) Tagment the control DNA to confirm kit performance. If the control DNA generated a library as expected, confirm input DNA quantity, and clean the DNA to remove any contaminants that may interfere with the Nextera reaction.

**DNA fragments are too small**

1) Confirm that 50 ng of input DNA was used in the tagmentation reaction.
2) Incubating the reaction at 37°C may increase the MW distribution.

**Library did not amplify during emPCR**

1) Confirm PCR yield by gel analysis of fragments. Approximately 20% of the fragments should present a strong smear of the expected size range.

**Library not detected after limited-cycle PCR**

1) Confirm that the initial 72°C extension step was performed.
2) Be sure to use Nextera PCR Enzyme. Other PCR systems have been tested and do not perform as well.

**MW distribution is too broad**

1) The Nextera reaction conditions have been optimized to produce the specified MWs using the two buffers provided. If a more uniform sample is required, we recommend further purification (e.g., gel purification).

**Tagmentation reaction incubated longer than 5 minutes**

1) The reaction is driven to near completion in the initial 5 minutes. If the reaction is allowed to proceed longer, the library will still be functional; however, the average fragment size may be smaller.

**Input DNA is degraded**

1) Nextera technology is capable of making libraries from degraded linear DNA fragments as small as 2 kb. However, the most distal ends of the target DNA will not be incorporated in the library.

**Fragmentation is inconsistent**

1) It is normal for the fragment MW to vary slightly. This is commonly a result of DNA type, quality, and quantitation. The purification procedure used to obtain the template DNA may also affect the fragment size. Template size, source, and G:C content do not have a detectable effect on fragment size.

## 8. Appendix A

The Roche FLX-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 green-capped tubes. Cat. No. FLBC0950

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

**FLX Adaptor 1 (MID1)**
5′-GCCTCCCTCGCGCCATCAG**ACGAGTGCGT**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID2)**
5′-GCCTCCCTCGCGCCATCAG**ACGCTCGACA**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID3)**
5′-GCCTCCCTCGCGCCATCAG**AGACGCACTC**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID4)**
5′-GCCTCCCTCGCGCCATCAG**AGCACTGTAG**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID5)**
5′-GCCTCCCTCGCGCCATCAG**ATCAGACACG**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID6)**
5′-GCCTCCCTCGCGCCATCAG**ATATCGCGAG**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID7)**
5′-GCCTCCCTCGCGCCATCAG**CGTGTCTCTA**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID8)**
5′-GCCTCCCTCGCGCCATCAG**CTCGCGTGTC**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID10)**
5′-GCCTCCCTCGCGCCATCAG**TCTCTATGCG**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID11)**
5′-GCCTCCCTCGCGCCATCAG**TGATACGTCT**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID13)**
5′-GCCTCCCTCGCGCCATCAG**CATAGTAGTG**AGATGTGTATAAGAGACAG-3′

**FLX Adaptor 1 (MID14)**
5′-GCCTCCCTCGCGCCATCAG**CGAGAGATAC**AGATGTGTATAAGAGACAG-3′

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