Nextera® XT DNA Sample Preparation Kit
The fastest and easiest sample prep workflow for small genomes, PCR amplicons, and plasmids.

**Highlights**

- **Rapid sample preparation**
  Complete sample prep in as little as 90 minutes with only 15 minutes of hands-on time

- **Fastest time to results**
  Go from DNA to data in only 8 hours with the MiSeq® system

- **Optimized for small genomes, PCR amplicons, and plasmids**
  One sample prep kit for many applications

- **Innovative sample normalization**
  Eliminates the need for library quantification prior to sample pooling and sequencing

**Introduction**

The Nextera XT DNA Sample Preparation kit allows researchers to prepare sequencing-ready libraries for small genomes (bacteria, archaea, viruses), PCR amplicons, and plasmids in 90 minutes, with only 15 minutes of hands-on time. The combination of the MiSeq system and Nextera XT sample preparation kits enable you to go from DNA to data in 8 hours (Figure 1). The low amount (1 ng) of input DNA makes this method amenable to precious samples available in limited quantity. Compatible with all Illumina sequencers, Nextera sample preparation can shorten the overall sequencing workflow time for a wide variety of established applications and can be easily automated for greater throughput.

**Fastest and Easiest Sample Prep Workflow**

Using a single “tagmentation” enzymatic reaction, sample DNA is simultaneously fragmented and tagged with adapters. An optimized, limited cycle PCR protocol amplifies tagged DNA and adds sequencing indexes (Figure 1). From start to finish, the complete Nextera XT protocol is over 80% faster than other available sample preparation methods, and requires the least amount of hands-on time.

**Innovative Sample Normalization**

Sample preparation kits for next-generation sequencing result in libraries of varying concentration. In order to pool samples equally and achieve target cluster densities, time-intensive quantitation methods are often used, followed by dilution and pooling of barcoded samples. The Nextera XT Sample Preparation kit eliminates the need for library quantification prior to sample pooling and sequencing by employing a simple bead-based sample normalization step (Figure 2). Prepared libraries are produced at equivalent concentrations enabling simple pooling by volume—simply pool 5 ul of each library to be sequenced.

**Flexible Multiplexing**

The Nextera XT Sample Preparation kit features an innovative indexing solution for processing and uniquely barcoding up to 96 samples in a single experiment. Following the addition of two indices to each DNA fragment, up to 96 uniquely indexed samples can be pooled and sequenced together. After sequencing, the unique combination of the two indices is used to demultiplex the data and assign reads to the proper sample. Using this dual barcode approach, Nextera XT Index kits only require 20 unique index oligos to process up to 96 samples for a

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**Figure 1: Nextera XT Sample Preparation Workflow**

1. **Prepare Input DNA**
2. **Nextera Tagmentation**
3. **Sequencing and Analysis**

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The combination of Nextera XT and rapid sequencing with the MiSeq system provides a complete DNA to data workflow in only 8 hours.
scalable approach. Multisample studies can be conveniently managed using the Illumina Experiment Manager, a freely available software tool that provides easy reaction setup for plate-based processing.

**Simple User Interface for Analysis**

MiSeq Reporter provides automated on-instrument analysis for a variety of applications including small genome de novo or resequencing, PCR amplicon, and plasmid sequencing. Sequencing results and analysis are easy to view and interpret. For example, using the PCR Amplicon workflow in the MiSeq Reporter software, your sequence data is automatically categorized into intuitive tabs: Samples, Regions, and Variants (Figure 3). Within each of these tabs, the variant score, quality (Q) score, and sequencing coverage levels can be determined down to single bases, allowing easy analysis of variants of interest.

**High Coverage, Accurate Calls**

To illustrate the power of amplicon sequencing with Nextera XT and the MiSeq system, nine PCR amplicons of varying sizes were prepared from two different samples of human DNA. Amplicons from each sample were pooled and 1 ng of DNA from each pool was prepared using the Nextera XT kit. Libraries from the two sample pools were combined, sequenced with paired-end 2 × 150 reads on the MiSeq system, and analyzed with MiSeq Reporter using the PCR Amplicon workflow. The approximate mean sequencing coverage values per amplicon and number of variants called (variant score > 99) identified within the amplicons in one of the two samples are shown in Table 1. The output of the MiSeq system supported sequencing of these amplicons to a depth of > 12,000×, enabling confident variant calling. Of the 31 total variants called in this example, 94%
are confirmed within the dbSNP database. These results show that coverage is high and even across a range of amplicon sizes, and that variant calls are accurate.

**Even Coverage Across Large Amplicons**

Large amplicons (> 1 kb) produced by long-range PCR can be easily prepared with the Nextera XT kit and sequenced on any Illumina sequencer. In Figure 4, coverage along amplicon length and position of called variants is shown for a single 5.1 kb amplicon in a highly variable noncoding region of the human genome. The 5.1 kb amplicon was part of a pool of 24 amplicons from human DNA ranging in size from ~300 bp up to 10 kb. Amplicon pools were generated from five different samples, and Nextera XT libraries were made using 1 ng of DNA from each pool. Libraries were combined and sequenced with single read 1 × 150 cycles on MiSeq and analyzed using MiSeq Reporter with the PCR Amplicon workflow.

**De novo Assembly of Small Genomes**

To show the utility of Nextera XT for preparing microbial genomes, 1 ng of genomic DNA from *Escherichia coli* reference strain MG1655 was prepared using Nextera XT, and sequenced using paired-end 2 × 150 reads on the MiSeq system. The data were analyzed using the Assembly workflow on the MiSeq Reporter. Total post-run analysis time for this sample was 28 minutes. Assembly metrics are shown in Table 2. A high-quality assembly was produced, with excellent N50 scores and coverage. This data set is available for analysis in BaseSpace™, the Illumina cloud computing environment.

### Table 1: Amplicon Coverage and Variants Called

<table>
<thead>
<tr>
<th>Amplicon Length (bp)</th>
<th>Mean Coverage (thousands of reads)</th>
<th>Variants Called (SNVs/Indels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>953</td>
<td>15.1</td>
<td>4 SNVs</td>
</tr>
<tr>
<td>1083</td>
<td>27.4</td>
<td>4 SNVs</td>
</tr>
<tr>
<td>1099</td>
<td>22.1</td>
<td>1 SNV</td>
</tr>
<tr>
<td>1800</td>
<td>22.4</td>
<td>7 SNVs</td>
</tr>
<tr>
<td>1809</td>
<td>17.8</td>
<td>1 SNV</td>
</tr>
<tr>
<td>2166</td>
<td>17.6</td>
<td>7 SNVs</td>
</tr>
<tr>
<td>3064</td>
<td>12.5</td>
<td>4 SNVs</td>
</tr>
<tr>
<td>3064</td>
<td>13.3</td>
<td>1 SNV</td>
</tr>
<tr>
<td>3072</td>
<td>14.8 K</td>
<td>1 SNV + 1 indel</td>
</tr>
</tbody>
</table>

### Table 2: De novo Assembly of *E. coli*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Percent of genome covered</td>
<td>98%</td>
</tr>
<tr>
<td>Number of contigs</td>
<td>314</td>
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<tr>
<td>Max contig length</td>
<td>221,108</td>
</tr>
<tr>
<td>Base count</td>
<td>4,548,900</td>
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<tr>
<td>N50</td>
<td>111,546</td>
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<tr>
<td>Avg coverage per base</td>
<td>184.9</td>
</tr>
</tbody>
</table>

Panel A: High sequencing coverage (>1,000×) across a 5.1 kb amplicon

Panel B: Within the same amplicon, the position of 16 variants passing filter (14 SNVs in blue + 2 indels in red) is shown, plotted against variant score (a Phred-scaled measure of variant calling accuracy, maximum = 99). Of the 16 variants, 13 are present in dbSNP.
Summary

Nextera XT DNA Sample Preparation kits are ideal for experiments where speed and ease are of paramount importance. Providing the fastest and easiest sample preparation workflow, Nextera XT enables rapid sequencing of small genomes, PCR amplicons, and plasmids. Combined with the MiSeq system, Nextera XT Sample Preparation kits enable you to go from DNA to data—all in a single day.

References


Nextera XT DNA Sample Prep Kit Specifications

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
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<tbody>
<tr>
<td>Sample DNA input type</td>
<td>Genomic DNA, PCR amplicons, plasmids</td>
</tr>
<tr>
<td>Input DNA</td>
<td>1 ng</td>
</tr>
<tr>
<td>Typical median insert size</td>
<td>&lt; 300 bp</td>
</tr>
<tr>
<td>Available indices</td>
<td>Up to 96</td>
</tr>
<tr>
<td>Compatible sequencers</td>
<td>HiSeq® systems, HiScanSQ™, Genome Analyzer IIx and MiSeq systems</td>
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<tr>
<td>Read lengths supported</td>
<td>Supports all read lengths on any Illumina sequencing system</td>
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Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog No.</th>
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<tbody>
<tr>
<td>Nextera XT DNA Sample Preparation Kit (24 Samples)</td>
<td>FC-131-1024</td>
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<tr>
<td>Nextera XT DNA Sample Preparation Kit (96 Samples)</td>
<td>FC-131-1096</td>
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<td>Nextera XT Index Kit (24 Indices, 96 Samples)</td>
<td>FC-131-1001</td>
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<tr>
<td>Nextera XT Index Kit (96 Indices, 384 Samples)</td>
<td>FC-131-1002</td>
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<td>TruSeq® Dual Index Sequencing Primer Kit, Single Read (single-use kit)*</td>
<td>FC-121-1003</td>
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<tr>
<td>TruSeq Dual Index Sequencing Primer Kit, Paired-End Read (single-use kit)*</td>
<td>PE-121-1003</td>
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*Sequencing primer kits are required for all sequencers except the MiSeq system.