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 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[1-9] 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.

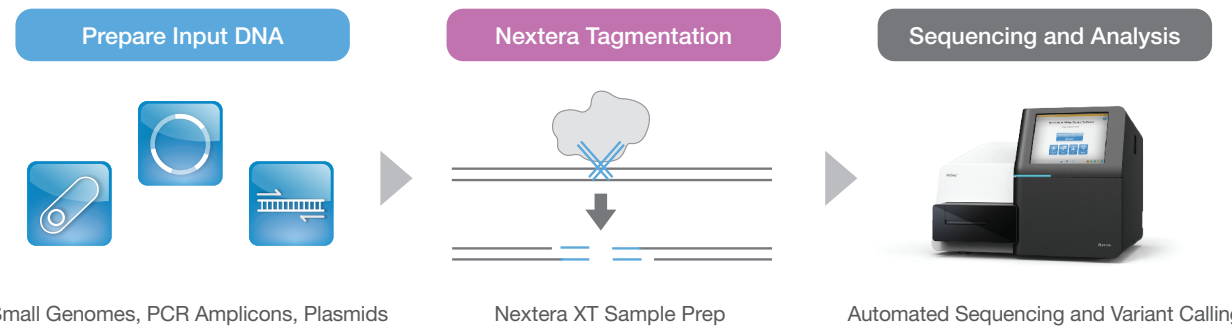
Innovative Sample Normalization

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

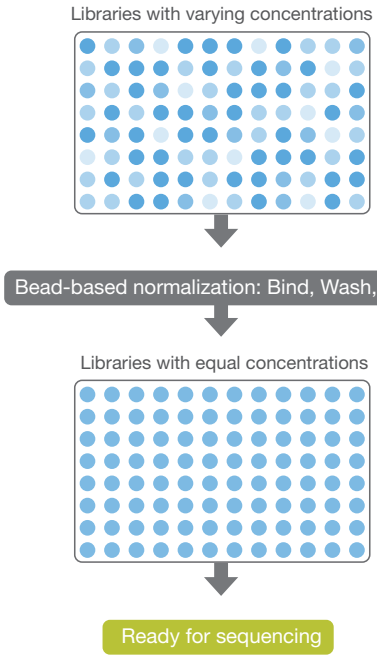
The Nextera XT Sample Preparation kit features an innovative indexing solution for processing and uniquely barcoding up to 96 samples in a single experiment. Following the addition of two indices to each DNA fragment, up to 96 uniquely indexed samples can be pooled and sequenced together. After sequencing, 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

Figure 1: Nextera XT Sample Preparation Workflow



The combination of Nextera XT and rapid sequencing with the MiSeq system provides a complete DNA to data workflow in only 8 hours.

Figure 2: Innovative Sample Normalization



The Nextera XT Sample Preparation kit eliminates the need for library quantification prior to sample pooling and sequencing. Libraries of equivalent concentrations are created by employing bead-based sample normalization, as simple as pipetting 5 µl of each library to be sequenced.

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.

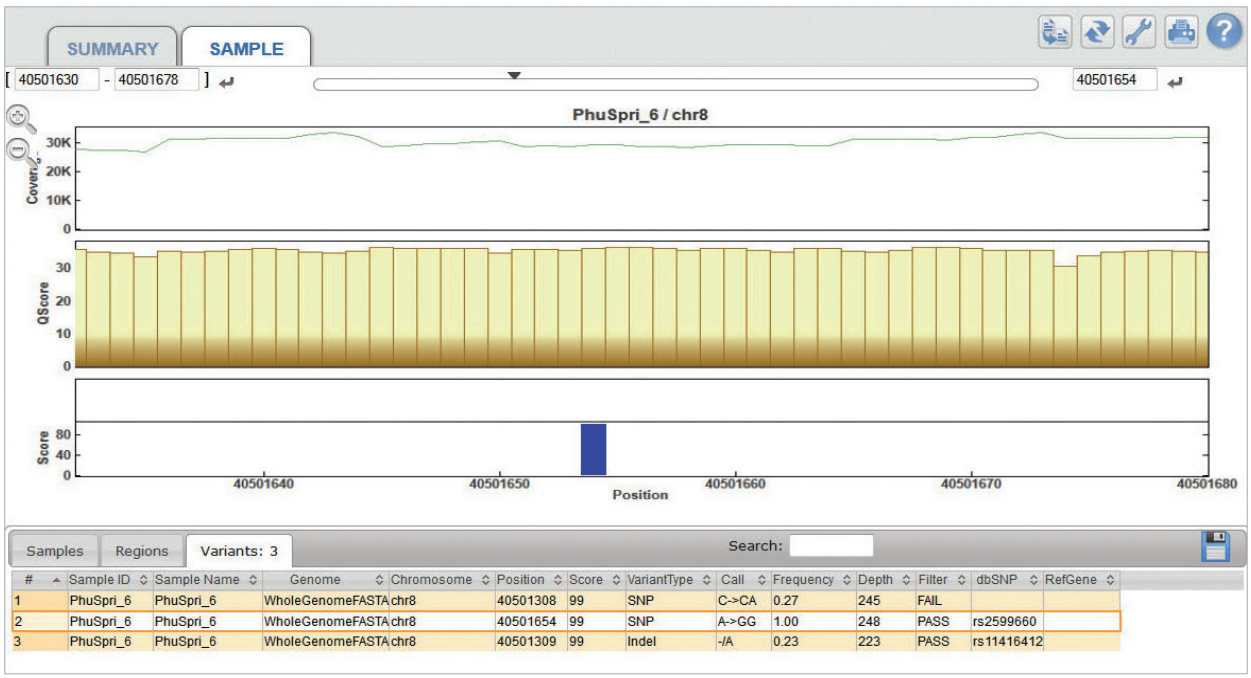
Simple User Interface for Analysis

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%

Figure 3: PCR Amplicon Workflow in MiSeq Reporter



#	Sample ID	Sample Name	Genome	Chromosome	Position	Score	VariantType	Call	Frequency	Depth	Filter	dbSNP	RefGene
1	PhuSpri_6	PhuSpri_6	WholeGenomeFASTA	chr8	40501308	99	SNP	C->CA	0.27	245	FAIL		
2	PhuSpri_6	PhuSpri_6	WholeGenomeFASTA	chr8	40501654	99	SNP	A->GG	1.00	248	PASS	rs2599660	
3	PhuSpri_6	PhuSpri_6	WholeGenomeFASTA	chr8	40501309	99	Indel	-/A	0.23	223	PASS	rs11416412	

MiSeq Reporter provides automated on-instrument analysis for a variety of applications, including PCR amplicon shown here. Samples, regions, and variants are easily accessible, and variant scores, quality (Q) scores, and coverage plots are shown at single nucleotide resolution.

Table 1: Amplicon Coverage and Variants Called

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

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.

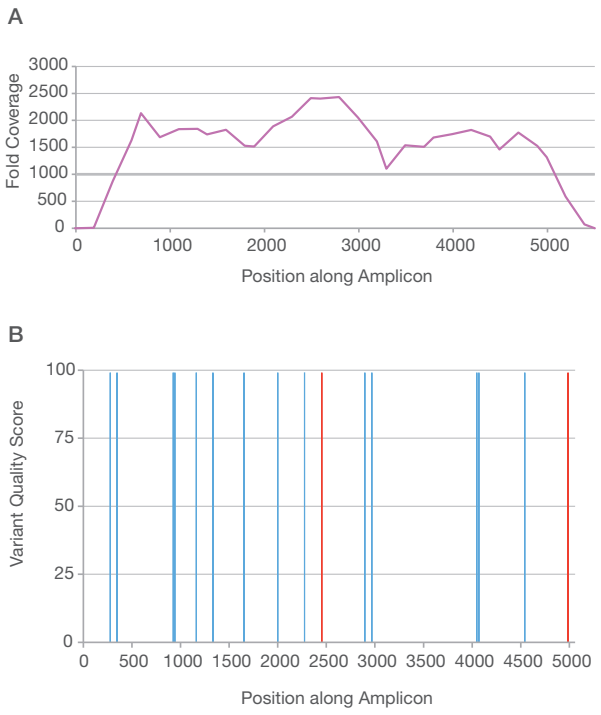
Even Coverage Across Large Amplicons

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[10].

Figure 4: Coverage of Large Amplicons



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.

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

Parameter	Value
Percent of genome covered	98%
Number of contigs	314
Max contig length	221,108
Base count	4,548,900
N50	111,546
Avg coverage per base	184.9

AATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTA
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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

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Nextera XT DNA Sample Prep Kit Specifications

Specification	Value
Sample DNA input type	Genomic DNA, PCR amplicons, plasmids
Input DNA	1 ng
Typical median insert size	< 300 bp
Available indices	Up to 96
Compatible sequencers	HiSeq® systems, HiScanSQ™, Genome Analyzer IIx and MiSeq systems
Read lengths supported	Supports all read lengths on any Illumina sequencing system

Ordering Information

Product	Catalog No.
Nextera XT DNA Sample Preparation Kit (24 Samples)	FC-131-1024
Nextera XT DNA Sample Preparation Kit (96 Samples)	FC-131-1096
Nextera XT Index Kit (24 Indices, 96 Samples)	FC-131-1001
Nextera XT Index Kit (96 Indices, 384 Samples)	FC-131-1002
TruSeq® Dual Index Sequencing Primer Kit, Single Read (single-use kit)*	FC-121-1003
TruSeq Dual Index Sequencing Primer Kit, Paired-End Read (single-use kit)*	PE-121-1003

*Sequencing primer kits are required for all sequencers except the MiSeq system.

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