illumina

Nextera XT DNA Library Prep

Overview

This guide explains how to prepare up to 384 dual-indexe
Nextera XT DNA Library Prep workflow.

The Nextera XT workflow:

- Uses tagmentation, an enzymatic reaction, to fragmer
  sequences in only 15 minutes.
- Reduces reagent containers, pipetting, and hands-on
  reagents.
- Requires only 1 ng input DNA.
- Supports genomes that are less than 5 Mb.

Table 1: Example Applications

Nextera XT (FC-131-1024, FC-131-1096)	**Illumina DN**
Small genomes, amplicons, plasmids	Human ger genomes
PCR amplicons (> 300 bp)*	Small geno plasmids, PCR amplic
Plasmids	Nonhuman mouse, rat,
Microbial genomes (eg, Prokaryotes, Archaea)	Plant geno rice)
Concatenated amplicons	Invertebrat
Double-stranded cDNA	
Single-cell RNA-Seq	

* Using > 300 bp amplicon size ensures even coverage acros
information, refer to *PCR Amplicons*.

How the Nextera XT Assay Works

DNA Input Recommendations

The Nextera XT protocol is optimized for 1 ng of input DNA preparing libraries.

Assess DNA purity to make sure that the initial DNA sampl free of organic contaminants, such as phenol and ethanol. Nextera XT tagmentation reaction and result in assay failu

Input DNA Quantification

The enzymatic DNA fragmentation used for this protocol i mechanical fragmentation. Success depends on accurate

Use a fluorometric-based method to quantify input DNA. F Assay system, use 2 µl of each DNA sample with 198 µl of that measure total nucleic acid, such as NanoDrop or othe

Assess DNA Purity

UV absorbance is a common method used for assessing t absorbance at 260 nm to absorbance at 280 nm provides protocol is optimized for DNA with 260/280 absorbance ra pure DNA sample.

For a secondary indication of sample purity, use the ratio 230 nm. Target a 260/230 ratio of 2.0–2.2. Values outside contaminants. For a complete list of contaminants, includi library preparation, refer to *Nextera XT Library Prep: Tips 015)*.

Dilute the starting material in 10 mM Tris-HCl, pH 7.5–8.5. contaminants can cause library preparation failure, poor c

PCR Amplicons

When starting with PCR amplicons, the PCR amplicon mus protocol depletes libraries < 500 bp. Therefore, Illumina re undergo a 1.8 x Illumina Purification Beads volume normal *Libraries*. Shorter amplicons can otherwise be lost during

Tagmentation cannot add an adapter directly to the distal coverage of ~50 bp from each distal end is expected. To e target region, design primers to extend beyond the target

Consumables & Equipme

The protocol described in this guide assumes that you hav
confirmed protocol contents, and obtained all required cor

Illumina-Supplied Consumabl

Completing the Nextera XT protocol requires library prep r

Component	Kit Options
Library prep reagents	Nextera XT DNA Library Preparati (24 Samples)
	Nextera XT DNA Library Preparati (96 Samples)
Index adapters	IDT for Illumina DNA/RNA UD Inde A, Tagmentation (96 Indexes, 96 Samples)
	IDT for Illumina DNA/RNA UD Inde B, Tagmentation (96 Indexes, 96 Samples)
	IDT for Illumina DNA/RNA UD Inde C, Tagmentation (96 Indexes, 96 Samples)
	IDT for Illumina DNA/RNA UD Inde D, Tagmentation (96 Indexes, 96 Samples)
	IDT for Illumina Nextera DNA Uniq Indexes Set C (96 Indexes, 96 Samples)
	IDT for Illumina Nextera DNA Uniq Indexes Set D (96 Indexes, 96 Samples)
	Nextera XT Index Kit v2 Set A (96 Indexes, 384 Samples)
	Nextera XT Index Kit v2 Set B (96 Indexes, 384 Samples)
	Nextera XT Index Kit v2 Set C (96 Indexes, 384 Samples)
	Nextera XT Index Kit v2 Set D (96 Indexes, 384 Samples)
	Nextera XT Index Kit (24 Indexes, 96 Samples)
Accessory Products for Nextera XT	Illumina Purification Bead, 100 ml
	Illumina Purification Bead, 400 ml

Nextera XT Library Prep Kit Contents

Index Kit Contents

User-Supplied Consumables

Make sure that you have the required consumables and eq

The protocol has been optimized and validated using the i
not guaranteed when using alternate consumables and eq

Consumables

Equipment

Thermal Cyclers

Signatures

Protocol

This section describes the Nextera XT DNA protocol.

- Review the planned complete sequencing workflow, fr
  ensure compatibility of products and experiment para
- Before proceeding, confirm kit contents and make sur
  components, equipment, and consumables. This protc
  reagents and index adapters. Index adapters are sold
  Supplied Consumables.
- Follow the protocols in the order shown, using the spe
  parameters.

Library Prep Workflow

Tips and Techniques

Safe Stopping Point

Avoiding Cross-Contamination

Sealing the Plate

IPB 100 ml Bottle Resuspension

Preparing IDT for Illumina DNA/RNA Unique

Tagment Genomic DNA

This step uses the Nextera XT transposome to tagment gl
tags DNA with adapter sequences.

Consumables

Preparation

Procedure

Amplify Libraries

This step amplifies the tagmented DNA using a limited-cy
Index 1 (i7) adapters, Index 2 (i5) adapters, and sequence
generation. To confirm indexes selected for low plexity po
refer to the *Index Adapters Pooling Guide*.

Index adapter tubes or plates are ordered separately from
compatible index adapters for use with this protocol, refer

Consumables

Preparation

Procedure

SAFE STOPPING POINT

If you are stopping, seal the plate, and store at 2°C to 8°C
thermal cycler overnight.

Clean Up Libraries

This step uses single-sided bead purification to purify am

Consumables

Preparation

Procedure

SAFE STOPPING POINT

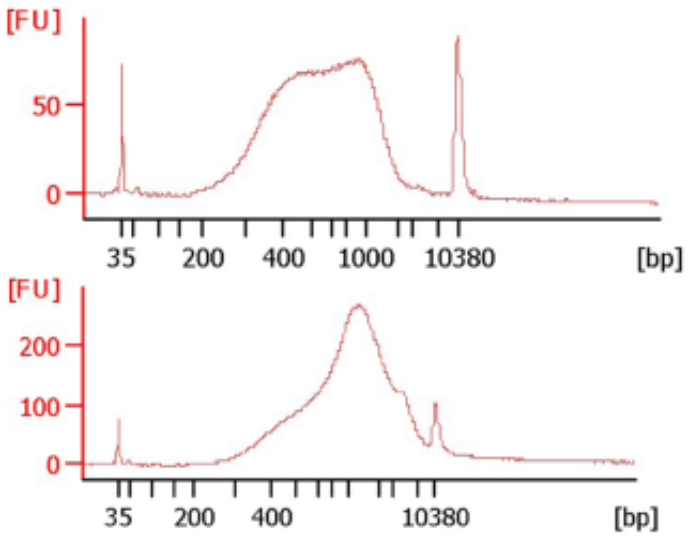
If you are stopping, seal the plate with Microseal 'B' adhes
at -25°C to -15°C for up to 7 days.

Check Library Quality

Run 1 µl undiluted library on an Agilent Technology 2100 B

Typical libraries show a broad size distribution of ~250–10
libraries can be sequenced with average fragment sizes a

Figure 1: Example Bioanalyzer Trace



Quality Metrics

Two factors can cause cluster density fluctuations in librai
Library Prep:

- An average sample size that is too large or too small a
- A final sample concentration that is too low due to a lc
  based normalization step.

To troubleshoot fluctuations in cluster density, consider cl
concentration. For more information, refer to *Nextera XT L
(Pub. No. 770-2015-015)*.

Check Library Size

Check Library Concentration

Normalize Libraries

This step normalizes the quantity of each library made wit
Kits to ensure more equal library representation in the poc

Do not follow the normalization protocol and instead use *L
Concentration* for manual normalization:

- If you are using IDT for Illumina Nextera UD Indexes.
- If the final library yield is < 10 nM.
- If your sequencing system uses onboard denaturation

Consumables

Preparation

Procedure

SAFE STOPPING POINT

If you are stopping, seal the plate with Microseal 'B' adhes
at -25°C to -15°C for up to 7 days.

Dilute Libraries to the Startin

This step dilutes libraries to the starting concentration for
step in a serial dilution. After diluting to the starting conce
denatured and diluted to the final loading concentration.

Use this procedure when the *Normalize Libraries* protocol

For sequencing, Illumina recommends the read lengths inc
compatible products support page. If you would like additi
adjusted IPB recommendations for ≥ 2 × 250 cycles, you c
it is not required.

IDT for Illumina DNA/RNA UD Indexes uses 10 base pair in
and Nextera XT v2 indexes, which use eight base pair inde
codes can require adjustments to your sequencing run set

Procedure

Resources & References

Additional Resources

The following resources provide instructions and guideline
the Illumina support page for additional information.

- Compatible products and requirements for recording s
  libraries, and analyzing data.
- Questions and answers about using the kit.
- Training videos about the kit and courses for related p
- The latest versions of the kit documentation.

Table 2: Additional Recommended Resources

Resource	Description
Custom Protocol Selector	A tool for generating end-to- library prep method, run para with options to refine the leve
Index Adapters Pooling Guide	Provides pooling guidelines a using the 10-base pair IDT fo or 8-base pair Nextera XT an the Nextera XT DNA Library F
Illumina Adapter Sequences	Provides the nucleotide sequ oligonucleotides used in Illum
IDT for Illumina DNA/RNA UD Indexes support page	Provides information about ID Dual (UD) Indexes.

Revision History

Document	Date	Description of Char
Document # 15031942 v07	April 2023	Clarified Nextera XT overview. Corrected normalize name LNB1. Clarified instructions Clarified instructions Removed statement TruSeq v3 primers c System. Updated kit options Updated list of addi Removed reference embedded (FFPE) a the workflow.

		Removed list of acro Added HTML forma
Document # 15031942 v06	August 2021	Add IPB bead resus techniques Replaced AMPure X Replaced Vortex ver Changed storage te Clean Up Libraries p IPB bead added to c sections
Document # 15031942 v05	May 2019	Added information c UD Indexes sets A, I contents, preparatic resources. Removed plate layo Removed the Pool L Check Library Qualit Libraries section. Revised Additional F clarity on the resour Revised language th consistency across preparation referenc Added protocol for c concentration. Removed obsolesce Indexes, 384 Sampl Contents.
Document # 15031942 v04	January 2019	Added information c workflows to ensure methods.
Document # 15031942 v03	February 2018	Updated the normal indicate that shakin elution is necessary resuspended. Reorganized kit con renaming some sect identify storage tem Corrected the diagr XT assay works to c has two of the same
Document # 15031942 v02	April 2017	Added the following • Supported genom • The ratio of absor contaminants. • Recommendation

		• AMPure XP bead ≥ 2 × 250 cycles. • Reagent and libra PCR plate after th amplification step • Beckman Coulter for Agencourt AM • Illumina catalog # 121-1003 for the Sequencing Prime Added the following additional resources • *Best Practices for Normalization in Preparation Kits (* • *Nextera XT Librar Troubleshooting (* Consolidated steps Identified the NaOH biology grade. Specified the use of mM Tris-HCl, pH 7.5 for DNA quality asse Specified proceedin tagmentation is com occurs while the tra Specified a thaw tim (Nextera PCR Maste Updated the normal to various sample nu Updated TCY plate plate, skirted. Updated magnetic s Scientific. Corrected the catalo provided in the intro Corrected the illustr assay works.
Document # 15031942 v01	January 2016	Updated design of v Renamed and comb needed to improve Simplified consumal beginning of each s Revised step-by-ste succinct. Removed reference Cards and added re Selector. Clarified AMPure XP nonamplicon applica

		Added information a libraries. See Norma Corrected index ada diagram.
15031942 Rev. E	January 2015	Corrected kit conter Preparation Index Ki include index N715.
15031942 Rev. D	September 2014	Added info for new i preparation of up to libraries. Updated DNA Input starting material and incomplete tagment Added new Nextera information on how cluster density. Removed Dual Index Pooling Guidelines s be found in the Next Guidelines Tech Not Library Prep suppor References to read l updated for v3 chen Added instructions f fewer than 24 samp beads in Library Nor Added NaOH 1N pH Equipment list as a u Removed Tween 20 Equipment list. Cons
15031942 Rev. C	October 2012	Modifications were a 2×300 runs on the M New section for clus HiScanSQ, and GAIIx HiSeq, HiScanSQ, ar The *Dual Indexing P* catalog numbers for correct catalog num Emphasized making Tagment Buffer) and Storage Buffer 1) rea before use in the pr Removed reference 0.1% Tween 20 from Consumables table library preparation.
15031942 Rev. B	July 2012	

		Emphasized making Tagment Buffer) and Storage Buffer 1) rea before use in the pr Removed reference 0.1% Tween 20 from Consumables table library preparation.
15031942 Rev. A	May 2012	Initial release.

Document # 15031942 v07