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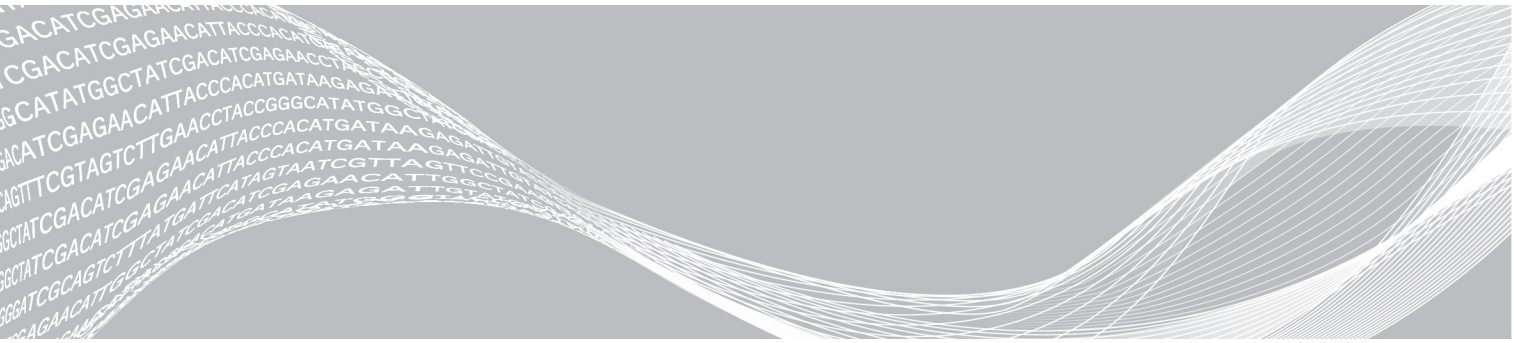
Nextera XT DNA Library Prep Kit

Reference Guide



Document # 15031942 v02

April 2017

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Revision History

Document	Date	Description of Change
Document # 15031942 v02	April 2017	Added the following information: • Supported genome size of < 5 Mb. • The ratio of absorbance that indicates contaminants. • Recommendations for PCR amplicons. • AMPure XP bead recommendations for runs ≥ 2 × 250 cycles. • Reagent and library volumes in the PCR plate after the tagmentation and amplification steps. • Beckman Coulter Genomics item # A63880 for Agencourt AMPure XP, 5 ml. • Illumina catalog # PE-121-1003 and # FC-121-1003 for the TruSeq Dual Index Sequencing Primer Box. Added the following technical notes to the list of additional resources: • *Best Practices for Standard and Bead-Based Normalization in Nextera XT DNA Library Preparation Kits (Pub. No. 470-2016-007)* • *Nextera XT Library Prep: Tips and Troubleshooting (Pub. No. 770-2015-015)* Consolidated steps in the pool libraries procedure. Identified the NaOH consumable as molecular biology grade. Specified the use of molecular-grade water or 10 mM Tris-HCl, pH 7.5–8.5 to dilute starting material for DNA quality assessment. Specified proceeding immediately when tagmentation is complete so that neutralization occurs while the transposome is active. Specified a thaw time of 20 minutes for NPM (Nextera PCR Master Mix). Updated the normalize libraries procedure to apply to various sample numbers, not only 96. Updated TCY plate to Hard-Shell 96-well PCR plate, skirted. Updated magnetic stand supplier to Thermo Fisher Scientific, Corrected the catalog numbers for Nextera kits provided in the introduction. Corrected the illustration showing how the Nextera assay works.
Document # 15031942 v01	January 2016	Updated design of workflow diagram. Renamed and combined some procedures as needed to improve continuity. Simplified consumables information at the beginning of each section. Revised step-by-step instructions to be more succinct. Removed reference to obsolete Experienced User Cards and added reference to the Custom Protocol Selector. Clarified AMPure XP bead recommendation for nonamplicon applications. See Clean Up Libraries. Added information about normalizing low yield libraries. See Normalize Libraries. Corrected index adapter labels on the assay diagram.
15031942 Rev. E	January 2015	Corrected kit contents for Nextera XT DNA Library Preparation Index Kit v2 Set A (FC-131-2001) to include index N715.

Document	Date	Description of Change
15031942 Rev. D	September 2014	Added info for new index kits that enable preparation of up to 384 indexed paired-end libraries. Updated DNA Input Recommendations for diluting starting material and the potential results of incomplete tagmentation. Added new Nextera XT Quality Metrics with new information on how to troubleshoot fluctuations in cluster density. Removed Dual Indexing Principle and Low Plexity Pooling Guidelines sections. This information can be found in the Nextera Low-Plex Pooling Guidelines Tech Note on the Nextera XT DNA Library Prep Kit support page. References to read lengths on the MiSeq were updated for v3 chemistry. Added instructions for alternate tip if processing fewer than 24 samples while transferring LNB1 beads in Library Normalization. Added NaOH 1N pH > 12.5 to the Consumables and Equipment list as a user-supplied consumable. Removed Tween 20 from Consumables and Equipment list. Consumable not used in protocol.
15031942 Rev. C	October 2012	Modifications were added in *PCR Clean-Up* for 2x300 runs on the MiSeq. New section for clustering samples on the HiSeq, HiScanSQ, and GAIIx. See Clustering Samples for HiSeq, HiScanSQ, and GAIIx. The *Dual Indexing Principle* section listed incorrect catalog numbers for the Nextera XT Index kits. The correct catalog numbers are now listed. Added emphasis on making sure the NT (Neutralize Tagment Buffer) and LNS1 (Library Normalization Storage Buffer 1) reagents are at room temperature before use in the protocol. Removed reference to Tris-Cl 10 mM, pH8.5 with 0.1% Tween 20 from the User-Supplied Consumables table because it is not used in this library preparation.
15031942 Rev. B	July 2012	Added emphasis on making sure the NT (Neutralize Tagment Buffer) and LNS1 (Library Normalization Storage Buffer 1) reagents are at room temperature before use in the protocol. Removed reference to Tris-Cl 10 mM, pH8.5 with 0.1% Tween 20 from the User-Supplied Consumables table because it is not used in this library preparation.
15031942 Rev. A	May 2012	Initial release.

Table of Contents

Chapter 1 Overview

Introduction

This protocol explains how to prepare up to 384 indexed paired-end libraries from DNA for subsequent sequencing on Illumina® sequencing systems. Reagents provided in the Nextera® XT Library Prep Kit and Nextera XT Index Kit are used to fragment DNA and add adapter sequences onto the DNA template.

The kit has the following features:

- Uses tagmentation, an enzymatic reaction, to fragment DNA and add partial adapter sequences in only 5 minutes.
- Master mixed reagents reduce reagent containers, pipetting, and hands-on time.
- Only 1 ng input DNA is needed.
- Nextera XT typically supports genomes that are < 5 Mb while Nextera DNA typically supports genomes > 5 Mb.

Table 1 Example Applications for Nextera Kits

Nextera XT (FC-131-1024, FC-131-1096)	Nextera DNA (FC-121-1030, FC-121-1031)
Small genomes, amplicons, plasmids	Large or complex genomes
PCR amplicons (> 300 bp)*	Human genomes
Plasmids	Nonhuman mammalian genomes (eg, mouse, rat, bovine)
Microbial genomes (eg, Prokaryotes, archaea)	Plant genomes (eg, Arabidopsis, maize, rice)
Concatenated amplicons	Invertebrate genomes (eg, Drosophila)
Double-stranded cDNA	
Single-cell RNA-Seq	

* Using a > 300 bp amplicon size ensures even coverage across the length of the DNA fragment. For more information, see *PCR Amplicons* on page 2.

DNA Input Recommendations

The Nextera XT protocol is optimized for 1 ng of input DNA.Quantify the starting material before preparing libraries. Dilute starting material in molecular-grade water or 10 mM Tris HCl, pH 7.5–8.5.

Input DNA Quantification

The enzymatic DNA fragmentation used for this protocol is more sensitive to DNA input compared to mechanical fragmentation. Success depends on accurate quantification of input DNA.

Use a fluorometric-based method to quantify input DNA. For example, if you use the Qubit dsDNA BR Assay system, use 2 µl of each DNA sample with 198 µl of the Qubit working solution. Avoid methods that measure total nucleic acid, such as NanoDrop or other UV absorbance methods.

Assess DNA Quality

UV absorbance is a common method for assessing the quality of a DNA sample. The ratio of absorbance at 260 nm to absorbance at 280 nm is used as an indication of sample purity. This protocol is optimized for DNA with absorbance ratio values of 1.8–2.0, which indicates a pure DNA sample. Target a 260/230 ratio of 2.0–2.2. Values outside this range indicate the presence of contaminants. For a complete list of contaminants, including sources, avoidance, and effects on the library, see *Nextera XT Library Prep: Tips and Troubleshooting (Pub. No. 770-2015-015)*.

Dilute the starting material in molecular-grade water or 10 mM Tris-HCl, pH 7.5–8.5. Incomplete tagmentation can cause library preparation failure, poor clustering, or an unexpectedly high scaffold number.

PCR Amplicons

The PCR amplicon must be > 300 bp. Shorter amplicons can be lost during the library cleanup step.

Tagmentation cannot add an adapter directly to the distal end of a fragment, so a drop in sequencing coverage of ~50 bp from each distal end is expected. To ensure sufficient coverage of the amplicon target region, design primers to extend beyond the target region by 50 bp per end.

Additional Resources

Visit the Nextera XT DNA Library Prep Kit support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

The following documentation is available for download from the Illumina website.

Resource	Description
Custom Protocol Selector	support.illumina.com/custom-protocol-selector.html A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
Nextera XT DNA Library Prep Kit Checklist (document # 1000000006566)	Provides a checklist of the protocol steps. The checklist is intended for experienced users.
Nextera Low-Plex Pooling Guidelines (Pub. No. 770-2011-044)	Provides pooling guidelines and dual indexing strategies for Nextera XT library prep.
Best Practices for Standard and Bead-Based Normalization in Nextera XT DNA Library Preparation Kits (Pub. No. 470-2016-007)	Provides best practices for bead-based normalization of Nextera XT libraries.
Nextera XT Library Prep: Tips and Troubleshooting (Pub. No. 770-2015-015)	Provides best practices for addressing undertagmentation, sample contaminants, and other problems that can occur when preparing Nextera XT libraries.

Chapter 2 Protocol

Introduction

This chapter describes the Nextera XT DNA Library Prep Kit protocol.

- Review Best Practices before proceeding. See *Additional Resources* on page 2 for information on accessing Best Practices on the Illumina website.
- Before proceeding, confirm kit contents and make sure that you have the required equipment and consumables. See *Supporting Information* on page 15.
- Follow the protocols in the order shown, using the specified volumes and incubation parameters.

Prepare for Pooling

If you plan to pool libraries, record information about your samples before beginning library prep. For more information, see the Nextera XT DNA Library Prep Kit support page.

Review *Nextera Low-Plex Pooling Guidelines (Pub. No. 770-2011-044)* when preparing libraries for Illumina sequencing systems that require balanced index combinations.

Tips and Techniques

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Avoiding Cross-Contamination

- When adding or transferring samples, change tips between ***each sample***.
- When adding adapters or primers, change tips between ***each row*** and ***each column***.
- Remove unused index adapter tubes from the working area.

Sealing the Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Vortexing steps
  - Centrifuge steps
  - Thermal cycling steps
- Apply the adhesive seal to cover the plate and seal with a rubber roller.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates. Use Microseal 'B' for shaking, centrifuging, and long-term storage.
- Microseal 'A' adhesive film is effective for thermal cycling and easy to cut when using fewer than 96 wells.

Plate Transfers

- When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.

Centrifugation

- Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.

Handling Beads

- Pipette bead suspension slowly.
- When mixing, mix thoroughly.
- If beads are aspirated into the pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).
- When washing beads:
  - Use the appropriate magnet for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Keep the plate on the magnet until the instructions specify to remove it.
  - Do not agitate the plate while on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

The following diagram illustrates the workflow using a Nextera XT DNA Library Prep Kit for eight samples. Safe stopping points are marked between steps.

Figure 1 Nextera XT Workflow

Tagment Genomic DNA

This step uses the Nextera transposome to tagment gDNA, which is a process that fragments DNA and then tags the DNA with adapter sequences in a single step.

Consumables

- ATM (Amplicon Tagment Mix)
- TD (Tagment DNA Buffer)
- NT (Neutralize Tagment Buffer)
- gDNA (0.2 ng/µl per sample)
- Hard-Shell 96-well PCR plate, skirted
- Microseal 'B' adhesive seals

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
gDNA	-25°C to -15°C	Thaw on ice. Invert the thawed tubes 3–5 times, and then centrifuge briefly.
ATM	-25°C to -15°C	Thaw on ice. Invert the thawed tubes 3–5 times, and then centrifuge briefly.
TD	-25°C to -15°C	Thaw on ice. Invert the thawed tubes 3–5 times, and then centrifuge briefly.
NT	15°C to 30°C	Check for precipitates. If present, vortex until all particulates are resuspended.

2 Save the following tagmentation program on the thermal cycler:
- Choose the preheat lid option
- 55°C for 5 minutes
- Hold at 10°C

Procedure

1 Add the following volumes in the order listed to each well of a new Hard-Shell skirted PCR plate. Pipette to mix.
- TD (10 µl)
- Normalized gDNA (5 µl)

2 Add 5 µl ATM to each well. Pipette to mix.

3 Centrifuge at 280 × g at 20°C for 1 minute.

4 Place on the preprogrammed thermal cycler and run the tagmentation program. When the sample reaches 10°C, ***immediately*** proceed to step 5 because the transposome is still active.

5 Add 5 µl NT to each well. Pipette to mix.

6 Centrifuge at 280 × g at 20°C for 1 minute.

7 Incubate at room temperature for 5 minutes.
The PCR plate contains 25 µl tagmented and neutralized gDNA, all of which is used in the next step.

Amplify Libraries

This step amplifies the tagmented DNA using a limited-cycle PCR program. PCR adds the Index 1 (i7), Index 2 (i5), and full adapter sequences to the tagmented DNA from the previous step. The index primers and Nextera PCR Master Mix are added directly to the 25 µl of tagmented gDNA from the previous step.

The adapters and sequences are required for cluster formation. Use the full amount of recommended input DNA and the specified number of PCR cycles, which helps ensure high-quality sequencing results.

When planning the index scheme for libraries, use the same index Index 1 (i7) index in each column of the PCR plate. This scheme allows use of a multichannel pipette to transfer indexes from the tubes to the plate. See *Additional Resources* on page 2 for information on accessing the tech note on low-plex pooling.

Consumables

- NPM (Nextera PCR Master Mix)
- Index 1 primers (N7XX)
- Index 2 primers (S5XX)
- TruSeq® Index Plate Fixture
- Microseal 'A' film

Preparation

1 Prepare the following consumables:

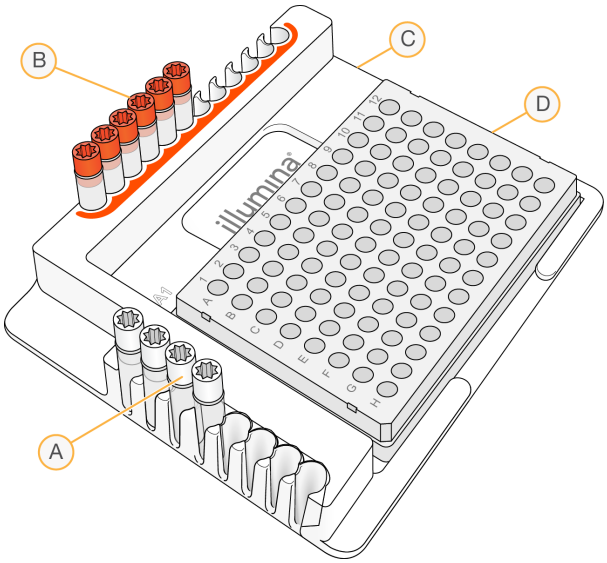
Item	Storage	Instructions
Index adapters (i5 and i7)	-25°C to -15°C	Only prepare adapters being used. Thaw at room temperature for 20 minutes. Invert each tube to mix. Centrifuge briefly.
NPM	-25°C to -15°C	Thaw on ice for 20 minutes.

2 Save the following program on the thermal cycler:
- Choose the preheat lid option.
- 72°C for 3 minutes
- 95°C for 30 seconds
- 12 cycles of:
  - 95°C for 10 seconds
  - 55°C for 30 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 10°C

Procedure

1 [24 libraries] Arrange the index primers in the TruSeq Index Plate Fixture as follows.
- Arrange Index 1 (i7) adapters in columns 1–6 of the TruSeq Index Plate Fixture.
- Arrange Index 2 (i5) adapter in rows A–D of the TruSeq Index Plate Fixture.

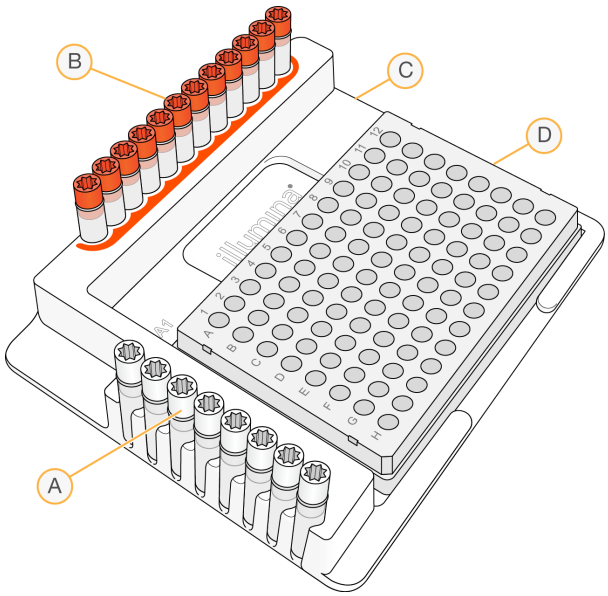
Figure 2 Setup of TruSeq Index Plate Fixture for 24 Libraries



A Rows A–D: Index 2 (i5) adapters (white caps)
B Columns 1–6: Index 1 (i7) adapters (orange caps)
C TruSeq Index Plate Fixture
D Hard-Shell PCR plate

2 [96 libraries] Arrange the index primers in the TruSeq Index Plate Fixture as follows.
- Arrange Index 1 (i7) adapters in columns 1–12 of the TruSeq Index Plate Fixture.
- Arrange Index 2 (i5) adapter in rows A–H of the TruSeq Index Plate Fixture.

Figure 3 Setup of TruSeq Index Plate Fixture for 96 Libraries



A Rows A–H: Index 2 (i5) adapters (white caps)
B Columns 1–12: Index 1 (i7) adapters (orange caps)

C TruSeq Index Plate Fixture
D Hard-Shell PCR plate

3 Using a multichannel pipette, add 5 µl of each Index 1 (i7) adapter down each column. Replace the cap on each i7 adapter tube with a new orange cap.

4 Using a multichannel pipette, add 5 µl of each Index 2 (i5) adapter across each row. Replace the cap on each i5 adapter tube with a new white cap.

5 Add 15 µl NPM to each well containing index adapters. Pipette to mix.

6 Centrifuge at 280 × g at 20°C for 1 minute.

7 Place on the preprogrammed thermal cycler and run the PCR program.
The volume is 50 µl.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Libraries

This step uses AMPure XP beads to purify the library DNA and remove short library fragments.

Consumables

- RSB (Resuspension Buffer)
- AMPure XP beads
- Freshly prepared 80% ethanol (EtOH)
- 96-well midi plate
- Hard-Shell 96-well PCR plate, skirted

About Reagents

- The AMPure XP beads are a user-supplied consumable.
- Vortex AMPure XP beads before each use.
- Vortex AMPure XP beads frequently to make sure that beads are evenly distributed.
- Always prepare fresh 80% ethanol for wash steps. Ethanol can absorb water from the air, impacting your results.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
RSB	-25°C to -15°C	Thaw at room temperature. RSB can be stored at 2°C to 8°C after the initial thaw.
AMPure XP Beads	2°C to 8°C	Let stand on the benchtop for 30 minutes to bring to room temperature.

2 Prepare fresh 80% ethanol from absolute ethanol.

Procedure

1 Centrifuge at 280 × g at 20°C for 1 minute.

2 Transfer 50 µl PCR product from each well of the PCR plate to corresponding wells of a new midi plate.

NOTE

The ratio of PCR product to volume of beads is 3:2. For example, 50 µl PCR product to 30 µl AMPure. If you pull less than 50 µl of PCR product, adjust your ratio of AMPure beads accordingly.

3 Add 30 µl AMPure XP beads to each well.
Smaller amplicons in Nextera XT library preps typically yield smaller insert size ranges. To maximize recovery of smaller fragments from the bead cleanup step, use the following conditions.

Input Size (bp)	AMPure XP Recommendation	AMPure XP Volume (µl)
300–500	1.8x AMPure XP	90
> 500	0.6x AMPure XP (0.5x AMPure XP for ≥ 2 x 250 cycles)*	30 (25 µl for ≥ 2 x 250 cycles)*
gDNA or other genomic input	0.6x AMPure XP	30

*Applicable only to the MiSeq® or HiSeq® 2500 using HiSeq Rapid v2 reagents.

4 Shake at 1800 rpm for 2 minutes.

5 Incubate at room temperature for 5 minutes.

6 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

7 Remove and discard all supernatant from each well.

8 Wash two times as follows.

a Add 200 µl fresh 80% EtOH to each well.

b Incubate on the magnetic stand for 30 seconds.

c Remove and discard all supernatant from each well.

9 Using a 20 µl pipette, remove residual 80% EtOH from each well.

10 Air-dry on the magnetic stand for 15 minutes.

11 Remove from the magnetic stand.

12 Add 52.5 µl RSB to each well.

13 Shake at 1800 rpm for 2 minutes.

14 Incubate at room temperature for 2 minutes.

15 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

16 Transfer 50 µl supernatant from the midi plate to a new Hard-Shell PCR plate.

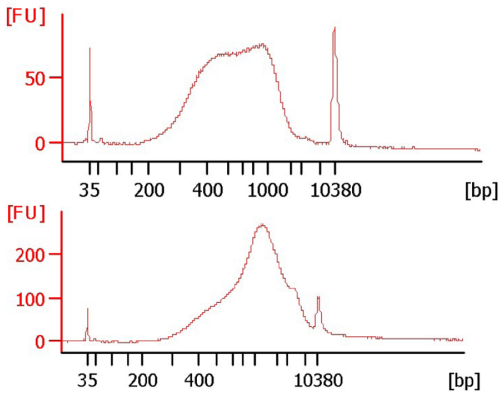
SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Check Libraries

1 Run 1 µl of undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip. The following figure shows example traces of libraries successfully sequenced on a HiSeq 2500 system. Typical libraries show a broad size distribution of ~250–1000 bp, as shown in the top panel. Various libraries can be sequenced with average fragment sizes as small as 250 bp or as large as 1500 bp.

Figure 4 Library Size Distributions of Control gDNA



Normalize Libraries

This process normalizes the quantity of each library to ensure more equal library representation in the pooled library.

NOTE

Manually normalize libraries when the final library yield is less than 10–15 nM. Bead-based normalization on low yield libraries can result in overly diluted samples and low sequencing yields. For more information, see *Best Practices for Standard and Bead-Based Normalization in Nextera XT DNA Library Preparation Kits (Pub. No. 470-2016-007)*.

Consumables

- LNA1 (Library Normalization Additives 1)
- LNB1 (Library Normalization Beads 1)
- LNW1 (Library Normalization Wash 1)
- LNS1 (Library Normalization Storage Buffer 1)
- 0.1 N NaOH (fewer than 7 days old) (3 ml per 96 samples)
- 96-well midi plate
- Hard-Shell 96-well PCR plate, skirted
- 15 ml conical tube
- Microseal 'B' adhesive seals

About Reagents

- Vortex LNA1 vigorously to make sure that all precipitates have dissolved. Inspect in front of a light.

- Vortex LNB1 vigorously, with intermittent inversion (at least 1 minute). Repeat until all beads are resuspended and no beads are present at the bottom of the tube when it is inverted.
- Always use a wide-bore pipette tip for LNA1.
- Mix only the required amounts of LNA1 and LNB1 for the current experiment. Store the remaining LNA1 and LNB1 separately at the recommended temperatures.
- Aspirate and dispense beads slowly due to the viscosity of the solution.

WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
LNA1	-25°C to -15°C	Prepare under a fume hood. Bring to room temperature. Use a 20°C to 25°C water bath as needed.
LNB1	2°C to 8°C	Bring to room temperature. Use a 20°C to 25°C water bath as needed.
LNW1	2°C to 8°C	Bring to room temperature. Use a 20°C to 25°C water bath as needed.
LNS1	Room temperature	Bring to room temperature.

Procedure

1 Transfer 20 µl supernatant from the Hard-Shell PCR plate to a new midi plate.

2 Add 44 µl LNA1 per sample to a new 15 ml conical tube. Calculate about 5% extra sample to account for sample loss due to pipetting.
For example: for 96 samples, add 4.4 ml LNA1 to the tube (100 samples × 44 µl = 4.4 ml).

3 Thoroughly resuspend LNB1. Pipette to mix.

4 Transfer 8 µl LNB1 per sample (including the 5% extra) to the 15 ml conical tube containing LNA1. Invert to mix.
For example: for 96 samples, transfer 800 µl LNB1 to the tube of LNA1 (100 samples × 8 µl = 800 µl).

5 Pour the bead mixture into a trough.

6 Add 45 µl combined LNA1 and LNB1 to each well containing libraries.

7 Shake at 1800 rpm for 30 minutes.

8 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

9 Remove and discard all supernatant from each well.

10 Wash two times as follows.

   a Add 45 µl LNW1 to each well.

   b Shake at 1800 rpm for 5 minutes.

c Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

d Remove and discard all supernatant from each well.

11 Add 30 µl 0.1 N NaOH to each well.

12 Shake at 1800 rpm for 5 minutes.

13 During the 5 minute elution, label a new 96-well PCR plate SGP for storage plate.

14 Add 30 µl LNS1 to each well of the SGP plate. Set aside.

15 After the 5 minute elution, make sure that all samples in the midi plate are resuspended. Pipette to mix or lightly tap the plate on the bench.

16 Shake at 1800 rpm for 5 minutes.

17 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

18 Transfer the supernatant from the midi plate to the SGP plate.

19 Centrifuge at 1000 × g for 1 minute.

NOTE

After denaturation, the libraries are single-stranded DNA, which resolves poorly on an agarose gel or Bioanalyzer chip. For quality control, use the double-stranded DNA saved from step 16 of the cleanup procedure.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Pool Libraries

Pooling libraries combines equal volumes of normalized libraries in a single tube. After pooling, dilute and heat-denature the library pool before loading libraries for the sequencing run.

Consumables

- Adhesive PCR foil seal
- Eppendorf LoBind microcentrifuge tubes
- PCR eight-tube strip

Preparation

1 To prepare for the sequencing run, begin thawing reagents according to the instructions for your instrument.

2 If the SGP plate was stored frozen at -25°C to -15°C, thaw at room temperature. Pipette to mix.

Procedure

1 Centrifuge at 1000 × g at 20°C for 1 minute.

2 Label a new Eppendorf tube PAL.

3 Transfer 5 µl of each library from the SGP plate to the PAL tube. Invert to mix.

4 Dilute pooled libraries to the loading concentration for your sequencing system. For instructions, see the denature and dilute libraries guide for your system.

5 Store unused pooled libraries in the PAL tube and SGP plate at -25°C to -15°C for up to 7 days.

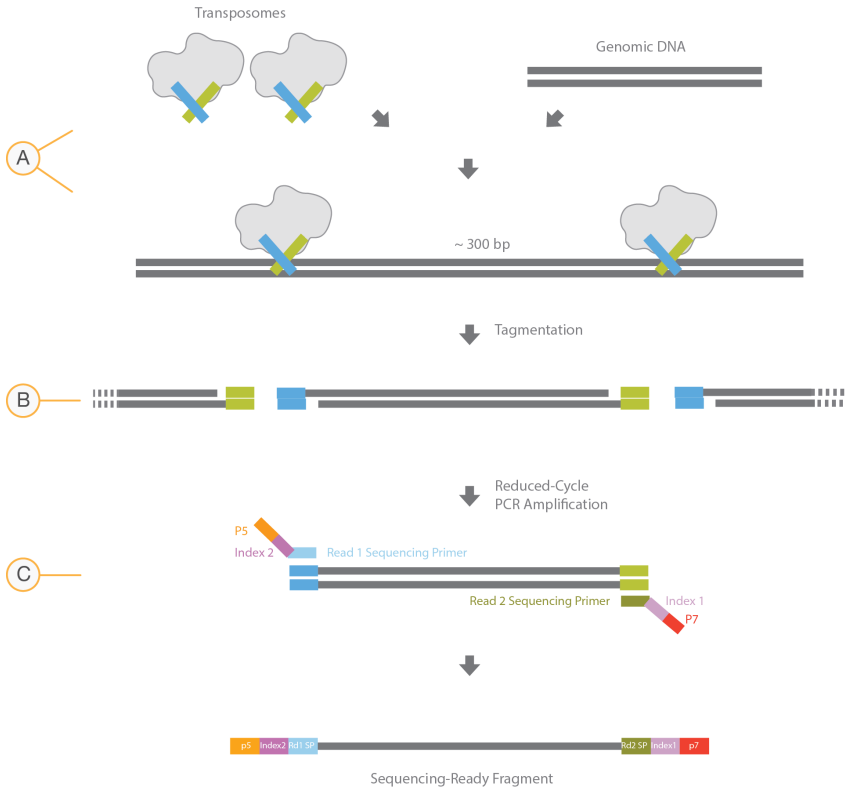
Supporting Information

Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all the required consumables and equipment.

How the Nextera XT Assay Works

The Nextera XT DNA Library Prep Kit uses an engineered transposome to tagment genomic DNA, which is a process that fragments DNA and then tags the DNA with adapter sequences in one step. Limited-cycle PCR uses the adapters to amplify the insert DNA. The PCR step also adds index adapter sequences on both ends of the DNA, which enables dual-indexed sequencing of pooled libraries on Illumina sequencing platforms.



A Nextera XT transposome with adapters combined with template DNA
B Tagmentation to fragment and add adapters
C Limited-cycle PCR to add index adapter sequences

Nextera XT Quality Metrics

Two factors can cause cluster density fluctuations in libraries prepared with the Nextera XT DNA Library Prep Kit:

- An average sample size that is too large or too small after tagmentation.
- A final sample concentration that is too low due to a low yield when starting the bead-based normalization step.

To troubleshoot fluctuations in cluster density, consider checking library size and library concentration. For more information, see *Nextera XT Library Prep: Tips and Troubleshooting (Pub. No. 770-2015-015)*.

Check Library Size

Larger molecules cluster less efficiently than smaller molecules. If the fragment size after tagmentation is larger than expected, low cluster numbers are possible. The inverse is also true. The average expected library size after tagmentation is between 400 bp and 1.2 kb.

Check the library size with a high sensitivity Bioanalyzer trace after the PCR cleanup step. Look for a long low plateau. Alternatively, PCR-amplify the library with qPCR primers and run the product on an agarose gel. The sequence for these primers is available in the *Sequencing Library qPCR Quantification Guide (document # 11322363)*.

- **Short libraries indicate too little input DNA**—Requantify the input DNA with a fluorometric method. Start with 10%–25% more input DNA. If the library peak is below 400 bp and you want to continue with this library, dilute the library further.
- **Long libraries indicate too much input DNA or the presence of inhibitors**—Start with less input DNA, make sure that the input DNA is free from inhibitors, and repeat the quantification step.

For more information on library dilution, see the denature and dilute libraries guide for your sequencing system.

Check Library Concentration

Bead-based normalization is most efficient when the library yield after amplification is 10–15 nM, or higher. Measure library concentration using high sensitivity dsDNA Qubit after library cleanup, and measure library size with a Bioanalyzer to calculate molarity.

If you are starting with high-quality DNA and see low yield after library cleanup, there are possible issues with AMPure cleanup or the amplification step. If results show either condition, confirm proper storage of the PCR master mix at -25°C to -15°C in a no-frost freezer. Confirm minimal freeze-thaw cycles.

The following resources are available on the Illumina website:

- **Best practices for bead handling**—From the Nextera XT DNA Library Prep Kit support page, select the Best Practices tab and review Handling Magnetic Beads.
- **Online training module**—Review section 2.4 of the *TruSeq: Sample Purification Bead Size Selection and Best Practices*, which is a short training with guidance on bead handling. To access this training, select the Training tab on the Nextera XT DNA Library Prep Kit support page.

Acronyms

Acronym	Definition
ATM	Amplicon Tagment Mix
CAA	Clean Amplified Plate
CAN	Clean Amplified NTA Plate
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNS1	Library Normalization Storage Buffer 1
LNW1	Library Normalization Wash 1
LNP	Library Normalization Plate
NT	Neutralize Tagment Buffer
NPM	Nextera PCR Master Mix
NTA	Nextera XT Tagment Amplicon Plate
PAL	Pooled Amplicon Library
RSB	Resuspension Buffer
SGP	Storage Plate
TD	Tagment DNA Buffer

Nextera XT DNA Library Prep Kit

The Nextera XT DNA Library Prep Kit is packaged in either 96-index (384 samples) or 24-index (96 samples) boxes, and shipped on dry ice unless otherwise specified. Certain kit components are stored at a temperature that differs from the shipping temperature. Make sure that you store kit components at the specified storage temperatures.

Each kit has a corresponding index kit that contains 96 indexes or 24 indexes. Combining Nextera XT Index Kit v2 Sets A–D achieves 384 index combinations.

Sequencing primers provided in TruSeq v3 reagent kits are not compatible with Nextera XT libraries. Thus, sequencing Nextera XT libraries on a HiSeq 2500 using TruSeq v3 reagents requires the sequencing primers provided in the Illumina TruSeq Dual Index Sequencing Primer Box. This box is provided in a paired-end (PE-121-1003) and single-read (FC-121-1003) version. One box is needed for each run.

Nextera XT DNA Library Prep Kit - 96 Indexes

Consumable	Catalog #
Nextera XT DNA Library Prep Kit	FC-131-1096
Nextera XT Index Kit (96 Indexes, 384 Samples)	FC-131-1002
Nextera XT Index Kit v2 Set A (96 Indexes, 384 Samples)*	FC-131-2001
Nextera XT Index Kit v2 Set B (96 Indexes, 384 Samples)*	FC-131-2002
Nextera XT Index Kit v2 Set C (96 Indexes, 384 Samples)*	FC-131-2003
Nextera XT Index Kit v2 Set D (96 Indexes, 384 Samples)*	FC-131-2004
TruSeq Index Plate Fixture Kit	FC-130-1005

* Combining Sets A–D achieves 384 index combinations.

Nextera XT DNA Library Prep Kit, 96 Indexes (FC-131-1096)

96-Index Kit Contents, Box 1

Quantity	Acronym	Reagent Name	Storage Temperature
1	ATM	Amplicon Tagment Mix, 96 rxn	-25°C to -15°C
2	TD	Tagment DNA Buffer	-25°C to -15°C
1	NPM	Nextera PCR Master Mix	-25°C to -15°C
4	RSB	Resuspension Buffer	-25°C to -15°C
1	LNA1	Library Normalization Additives 1	-25°C to -15°C
2	LNW1	Library Normalization Wash 1	2°C to 8°C
1	HT1	Hybridization Buffer	-25°C to -15°C

96-Index Kit Contents, Box 2

Quantity	Acronym	Reagent Name	Storage Temperature
1	NT	Neutralize Tagment Buffer	Room temperature
1	LNB1	Library Normalization Beads 1	2°C to 8°C
1	LNS1	Library Normalization Storage Buffer 1	Room temperature

Index Kit (96 Indexes) (FC-131-1002)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S502 to S508, and S517	-25°C to -15°C
12 tubes	Index Primers, N701 to N712	-25°C to -15°C

Index Kit v2 Set A (FC-131-2001)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S502, S503, S505 to S508, S510, and S511	-25°C to -15°C
12 tubes	Index Primers, N701 to N707, N710 to N712, N714, and N715	-25°C to -15°C

Index Kit v2 Set B (FC-131-2002)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S502, S503, S505 to S508, S510, and S511	-25°C to -15°C
12 tubes	Index Primers, N716, N718 to N724, and N726 to N729	-25°C to -15°C

Index Kit v2 Set C (FC-131-2003)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S513, S515 to S518, and S520 to S522	-25°C to -15°C
12 tubes	Index Primers, N701 to N707, N710 to N712, N714, and N715	-25°C to -15°C

Index Kit v2 Set D (FC-131-2004)

Quantity	Reagent Name	Storage Temperature
8 tubes	Index Primers, S513, S515 to S518, and S520 to S522	-25°C to -15°C
12 tubes	Index Primers, N716, N718 to N724, and N726 to N729	-25°C to -15°C

Nextera XT DNA Library Prep Kit - 24 Indexes

Consumable	Catalog #
Nextera XT DNA Library Prep Kit	FC-131-1024
Nextera XT Index Kit (24 Indexes, 96 Samples)	FC-131-1001
TruSeq Index Plate Fixture Kit	FC-130-1005

Nextera XT DNA Library Prep Kit, 24 Indexes (FC-131-1024)

24-Index Kit Contents, Box 1

Quantity	Acronym	Reagent Name	Storage Temperature
1	ATM	Amplicon Tagment Mix, 24 rxn	-25°C to -15°C
1	TD	Tagment DNA Buffer	-25°C to -15°C
1	NPM	Nextera PCR Master Mix	-25°C to -15°C
1	RSB	Resuspension Buffer	-25°C to -15°C
1	LNA1	Library Normalization Additives 1	-25°C to -15°C
1	LNW1	Library Normalization Wash 1	2°C to 8°C
1	HT1	Hybridization Buffer	-25°C to -15°C

24-Index Kit Contents, Box 2

Quantity	Acronym	Reagent Name	Storage Temperature
1	NT	Neutralize Tagment Buffer	Room temperature
1	LNB1	Library Normalization Beads 1	2°C to 8°C
1	LNS1	Library Normalization Storage Buffer 1	Room temperature

Index Kit (24 Indexes) (FC-131-1001)

Quantity	Reagent Name	Storage Temperature
4 tubes	Index Primers, S502 to S504, and S517	-25°C to -15°C
6 tubes	Index Primers, N701 to N706	-25°C to -15°C

TruSeq Index Plate Fixture Kit

Each kit contains two index plate fixtures to help arrange index primers before dispensing to the 96-well plate during library amplification. The Index Plate Fixture can be used with both the 24-sample kit and 96-sample kit.

Consumable	Catalog #
TruSeq Index Plate Fixture Kit	FC-130-1005

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol.

The protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Consumables

Consumable	Supplier
10 µl pipette tips	General lab supplier
10 µl multichannel pipettes	General lab supplier
10 µl single channel pipettes	General lab supplier
1000 µl pipette tips	General lab supplier
1000 µl multichannel pipettes	General lab supplier
1000 µl single channel pipettes	General lab supplier
200 µl pipette tips	General lab supplier
200 µl multichannel pipettes	General lab supplier
200 µl single channel pipettes	General lab supplier
96-well storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, catalog # AB-0859
Agencourt AMPure XP, 60 ml kit or 5 ml kit	Beckman Coulter Genomics, item # A63881 (60 ml) Beckman Coulter Genomics, item # A63880 (5 ml)
Distilled water	General lab supplier
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, product # E7023
Microseal 'A' film	Bio-Rad, catalog # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, catalog # MSB-1001
NaOH 1 N, pH > 12.5, molecular biology grade	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, catalog # 89094-658
Ultrapure water	General lab supplier
Hard-Shell 96-well PCR plates	Bio-Rad, catalog # HSP-9601

Equipment

Equipment	Supplier
High-Speed microplate shaker	VWR, catalog # 13500-890 (110 V/120 V) VWR, catalog # 14216-214 (230 V)
Magnetic stand-96	Thermo Fisher Scientific, catalog # AM10027
Microplate centrifuge	General lab supplier
Vortexer	General lab supplier

Thermal Cyclers

Use the following recommended settings for selected thermal cycler models. Before performing library prep, validate any thermal cyclers not listed.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, Constant at 100°C	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

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Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
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Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

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