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Nextera® DNA Library Prep Reference Guide

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Document # 15027987 v01
January 2016

Customize a short end-to-end workflow guide with the Custom Protocol Selector
support.illumina.com/custom-protocol-selector.html

Revision History

Document	Date	Description of Change
Document # 15027987 v01	January 2016	• Changed title of this document to Reference Guide • Updated to new library prep style • Updated design of workflow diagram • 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 references to Custom Protocol selector and new protocol guide and checklist
Part # 15027987 Rev. B	July 2012	• Added a table showing different applications for Nextera Kits to the Introduction • Revised the Input DNA Quantitation section with additional details on quantitation methods • Modifications were added in PCR Clean-Up for 2x250 runs on the MiSeq • Modifications were added in Validate Library for 2x250 runs on the MiSeq • Changed the normalization concentration from 4nM to 2nM in the Pool Libraries section
Part # 15027987 Rev. A	May 2012	• Initial Release

Table of Contents

Overview

Chapter 1

Introduction

This protocol explains how to prepare up to 96 pooled indexed paired-end libraries from genomic DNA (gDNA) for subsequent cluster generation and DNA sequencing using the reagents provided in the Illumina Nextera® DNA Library Preparation Kit. The goal of this protocol is to fragment and add adapter sequences onto template DNA with a single tube Nextera reaction to generate multiplexed single read or paired-end sequencing libraries.

The Nextera DNA Library Prep protocol offers:

- Rapid and streamlined workflow
  - Complete protocol in less than 90 minutes
  - Single-well enzymatic reaction both fragments and adds adapter, no mechanical fragmentation/shearing required
  - Master mix reagents to reduce reagent containers, pipetting and hands-on time
- Lowest DNA input—Only 50 ng input DNA needed
- Higher throughput
  - Optimized for plate-based processing for simultaneous preparation of 96 samples
  - Master-mixed reagents and automation-friendly configurations
  - Volumes optimized for standard 96-well plate workflow
- Higher indexing—96 indexes available and supported on all Illumina sequencing systems

Example of Applications for Different Nextera Kits

Nextera	Nextera XT
Large / complex genomes	Small genomes, amplicons, plasmids
Human genomes	PCR Amplicons (> 300 bp)*
Nonhuman mammalian genomes (eg mouse, rat, bovine)	Plasmids
Plant genomes (eg Arabidopsis, maize, rice)	Microbial Genomes (eg prokaryotes, archea)
Invertebrates genomes (eg Drosophila)	Concatenated Amplicons
	double-stranded cDNA

* Use > 300 bp to ensure even coverage across the length of the DNA fragment. There is an expected drop off in sequencing coverage ~50 bp from each distal end of a fragment. The drop off occurs because the tagmentation reaction cannot add an adapter right at the distal end of a fragment. To avoid drop off for PCR amplicon sequencing, design your amplicons to be to be ~100 bases larger than the desired insert.

DNA Input Recommendations

The Nextera DNA Library Prep Kit protocol is optimized for 50 ng of genomic DNA total. Quantifying the starting genomic material is highly recommended.

Input DNA Quantification

The Nextera DNA Library Prep protocol uses an enzymatic DNA fragmentation step and can be more sensitive to DNA input compared to mechanical fragmentation methods. The success of library prep strongly depends on using an accurately quantified amount of input DNA library. Therefore, the correct quantification of the DNA library is essential.

To obtain an accurate quantification of the DNA library, quantify the starting DNA library using a fluorometric based method specific for duplex DNA such as the Qubit dsDNA BR Assay system. Illumina recommends using 2 µl of each DNA sample with 198 µl of the Qubit working solution for sample quantification. Avoid using methods that measure total nucleic acid content (eg NanoDrop or other UV absorbance methods) because common contaminants such as ssDNA, RNA, and oligos are not substrates for the Nextera DNA Library Prep assay.

Assessing DNA Quality

Absorbance measurements at 260 nm are commonly used to quantify DNA. 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.

Additional Resources

Visit the Nextera 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	http://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 DNA Library Prep Protocol Guide (document # 1000000006836)	Provides only protocol instructions. The protocol guide is intended for experienced users.
Nextera DNA Checklist (document # 1000000006828)	Provides a checklist of the protocol steps. The checklist is intended for experienced users.
Nextera Low Plex Pooling Guidelines Tech Note	Provides pooling guidelines and dual indexing strategies for Nextera library preparation.

Protocol

Chapter 2

Introduction

This section describes the Nextera DNA Library Prep protocol.

- Follow the protocol in the order described using the specified parameters.
- Review Best Practices from the Nextera DNA Library Prep support page on the Illumina website.
- Before proceeding, confirm kit contents and make sure that you have the required equipment and consumables. See *Consumables and Equipment* on page 26.
- Include a common index in each column. A common index facilitates pipetting operations when dispensing index adapters and pooling indexed libraries. For more information, see the *Nextera Low Plex Pooling Guidelines Tech Note*.

Prepare for Pooling

If you plan to pool libraries, record information about your samples before beginning library prep. Different methods are available depending on the sequencing instrument you are using. See the Nextera DNA Library Prep support page for more information.

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.
  - To pellet beads, centrifuge at 280 × g for 1 minute.

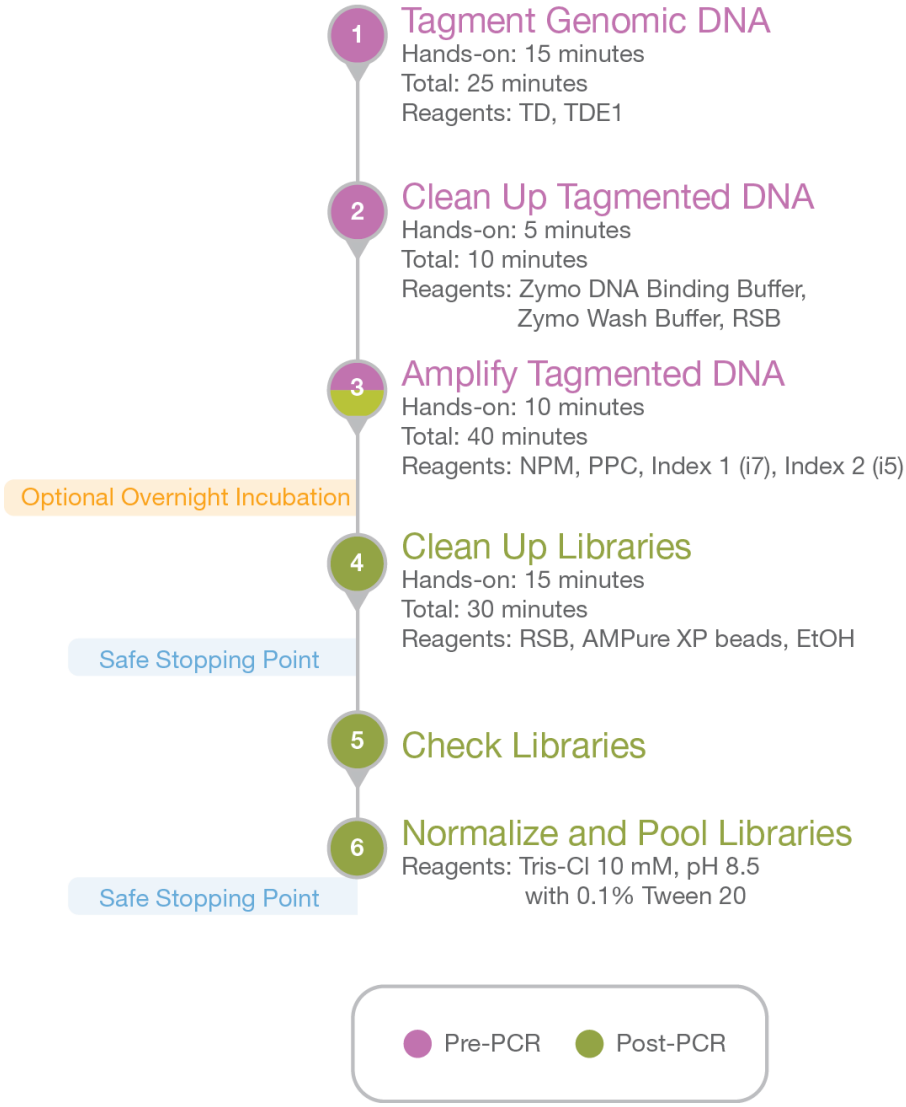
Handling Beads

- 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 DNA Library Prep kit. Safe stopping points are marked between steps.

Figure 1 Nextera DNA Library Prep Workflow



Tagment Genomic DNA

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

Consumables

- TD (Tagment DNA Buffer)
- TDE1 (Tagment DNA Enzyme)
- Genomic DNA (2.5 ng/µl)
- 96-well Hard-Shell TCY plate
- Microseal 'B' adhesive seal

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
TD	-25°C to -15°C	Thaw on ice. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.
TDE1	-25°C to -15°C	Thaw on ice. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.
Genomic DNA	-25°C to -15°C	Thaw on ice. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.

2 Label a new 96-well TCY plate NSP1 with a marker.

3 Save the following program as TAG NSP1 on a thermal cycler with a heated lid.
- Choose the preheat lid option and set to 55°C
- 55°C for 5 minutes
- Hold at 10°C

Procedure

1 Add 20 µl of genomic DNA at 2.5 ng/µl (50 ng total) to each sample well of the NSP1 plate.

2 Add 25 µl of TD Buffer to the wells containing genomic DNA.

3 Add 5 µl of TDE1 to the wells containing genomic DNA and TD Buffer.

4 Pipette up and down 10 times to mix.

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

6 Place on the preprogrammed thermal cycler and run the TAG NSP1 program. While the program is running, perform steps 2–4 from the Clean Up Tagmented DNA Preparation section.

Clean Up Tagmented DNA

The tagmented DNA is purified from the Nextera transposome. This step is critical because the Nextera transposome can bind tightly to DNA ends and interfere with downstream processes if not removed.

Consumables

- RSB (Resuspension Buffer)
- Zymo DNA binding buffer
- Zymo wash buffer (EtOH added)
- Zymo-Spin I-96 Plate
- Zymo Collection plate
- 96-well Hard-Shell TCY plate
- 96-well midi plate

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.

2 Label a new midi plate NSP2 with a marker.

3 Label a new TCY plate NSP3 with a marker.

4 Add 180 µl Zymo DNA binding buffer to each well of the NSP2 plate with a sample in the corresponding well of the NSP1 plate.

Procedure

1 Transfer 50 µl from each well of NSP1 to the corresponding well of NSP2. Pipette up and down 10 times to mix.

2 Place the Zymo-Spin I-96 Plate on the Collection Plate.

3 Transfer sample mixture from NSP2 to the Zymo-Spin I-96 Plate.

4 Centrifuge at 1300 × g at 20°C for 2 minutes.

5 Discard the flow-through.

NOTE
Use open-top plate holders for the centrifugation step to accommodate the plate stack.

6 Wash 2 times as follows.

a Add 300 µl of Zymo wash buffer from a trough to each sample well. Change tips between columns.
b Centrifuge at 1300 × g at 20°C for 2 minutes.
c Discard the flow-through.

7 Centrifuge at 1300 × g for 2 minutes to make sure that there is no residual wash buffer.

8 Place the Zymo-Spin I-96 Plate on NSP3.

9 Add 25 µl of RSB directly to the column matrix in each well.

10 Incubate for 2 minutes at room temperature.

11 Centrifuge at 1300 × g at 20°C for 2 minutes.

NOTE
[Optional] Check the products of the tagmentation reaction by loading 1 µl of undiluted Zymo eluate on an HS Bioanalyzer chip. This step produces a broad distribution of DNA fragments with a size range from ~150 bp – < 1 kb.

Amplify Tagmented DNA

This step amplifies purified tagmented DNA and adds index adapters using a 5-cycle PCR program. The PCR step adds Index 1 (i7) and Index 2 (i5), sequencing, and common adapters (P5 and P7) required for cluster generation and sequencing. Use the full amount of recommended input DNA and do not add extra PCR cycles.

Consumables

- Index 1 (i7) adapters and orange tube caps
- Index 2 (i5) adapters and white tube caps
- NPM (Nextera PCR Master Mix)
- PPC (PCR Primer Cocktail)
- TruSeq Index Plate Fixture
- Microseal 'A' adhesive film
- 96-well Hard-Shell TCY plate

About Reagents

- If you plan to pool less than a full set of libraries for sequencing, see the *Nextera Low Plex Pooling Guidelines* tech note for the correct Index 1 and Index 2 adapters. Then use the Illumina Experiment Manager to make sure that the correct index primers have been selected.

Preparation

1. Prepare the following consumables.

Item	Storage	Instructions
Index adapters (i7 and i5)	-25°C to -15°C	Thaw on ice for ~20 minutes. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.
NPM	-25°C to -15°C	Thaw on ice for ~20 minutes. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.
PPC	-25°C to -15°C	Thaw on ice for ~20 minutes. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.

   Use 1.7 ml Eppendorf tubes as adapters for the microcentrifuge.

2. Label a new 96-well microplate NAP1 with a marker.
3. Save the following program as PCR AMP on a thermal cycler with a heated lid.
   - Choose the preheat lid option and set to 100°C
   - 72°C for 3 minutes
   - 98°C for 30 seconds
   - 5 cycles of:
     - 98°C for 10 seconds
     - 63°C for 30 seconds
     - 72°C for 3 minutes
   - Hold at 10°C

Procedure

1. Arrange Index 1 (i7) adapters in the TruSeq Index Plate Fixture as follows:
   - 24 libraries—Columns 1–6

- 96 libraries—Columns 1–12

2 Arrange Index 2 (i5) adapters in the TruSeq Index Plate Fixture as follows:
   - 24 libraries—Rows A–D
   - 96 libraries—Rows A–H

3 Place the plate on the TruSeq Index Plate Fixture.

Figure 2 TruSeq Index Plate Fixture Arrangement (24 libraries)

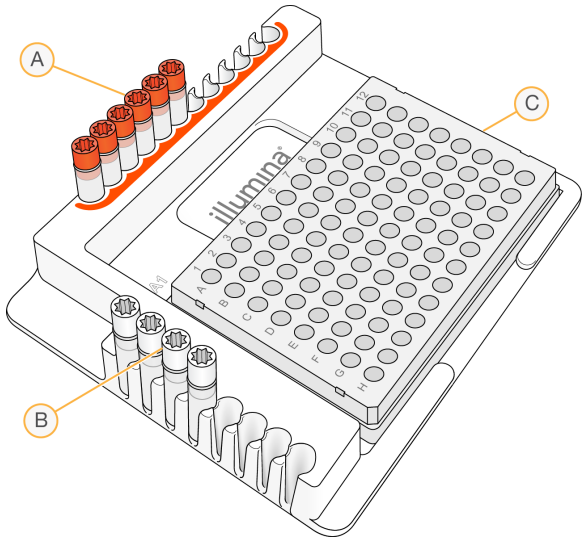
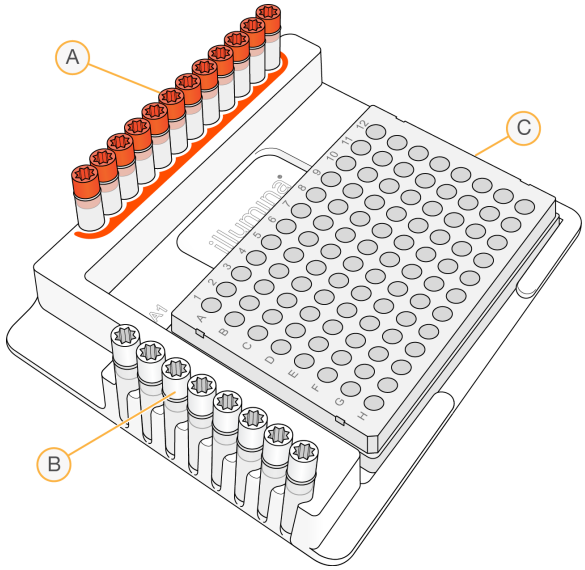


Figure 3 TruSeq Index Plate Fixture (96 libraries)



A Columns 1–12: Index 1 (i7) adapters (orange caps)
B Rows A–H: Index 2 (i5) adapters (white caps)
C NAP1 plate

4 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.

5 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.

6 Add 15 µl NPM to each well.

7 Add 5 µl PPC to each well containing index primers and NPM.

8 Transfer 20 µl from each well of NSP3 to NAP1. Pipette up and down 3–5 times to mix.

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

10 Transfer the NAP1 plate to the post-amplification area.

11 Place on the preprogrammed thermal cycler and run the PCR AMP program.

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 provides a size selection step that removes short library fragments.

Consumables

- RSB (Resuspension Buffer)
- AMPure XP beads
- Freshly prepared 80% ethanol (EtOH)
- 96-well midi plate
- 96-well TCY plate

About Reagents

- 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. Bring the AMPure XP beads to room temperature
2. Prepare fresh 80% ethanol from absolute ethanol.
3. Label a new midi plate NAP2 with a marker.
4. Label a new TCY plate NLP with a marker.

Procedure

1. Centrifuge NAP1 at 280 × g at 20˚C for 1 minute to collect condensation.
2. Transfer the contents of each well of NAP1 to NAP2.
3. Vortex AMPure XP beads for 30 seconds. Add an appropriate volume of beads to a trough.
4. Add 30 µl AMPure XP beads to NAP2.
   For 2 × 250 runs on MiSeq, add 25 µl AMPure XP beads to NAP2.
5. Add 30 µl AMPure XP beads to NAP2.
6. Pipette up and down 10 times to mix.
   Alternatively, shake at 1800 rpm for 2 minutes.
7. Incubate at room temperature without shaking for 5 minutes.
8. Place on a magnetic stand and wait until the liquid is clear (~2 minutes). Keep on magnetic stand until step 13.
9. Remove and discard supernatant.

10 Wash 2 times as follows.

   a Add 200 µl freshly prepared 80% EtOH. Avoid disturbing the beads.
   b Incubate on the magnetic stand for 30 seconds or until the supernatant appears clear.
   c Remove and discard all supernatant from each well.

11 Remove residual EtOH from each well.

12 Air-dry the beads for 15 minutes.

13 Remove from the magnetic stand.

14 Add 32.5 µl RSB.

15 Pipette up and down 10 times to mix.
Alternatively, shake at 1800 rpm for 2 minutes.

16 Incubate at room temperature for 2 minutes.

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

18 Transfer 30 µl supernatant from NAP2 to NLP.

SAFE STOPPING POINT

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

Check Libraries

Perform the following procedures for quality control analysis on your sample library and quantification of the DNA library templates.

Quantify Libraries

To achieve the highest-quality data on Illumina sequencing platforms, create optimum cluster densities across every lane of the flow cell. This step requires accurate quantification of DNA library templates. Quantify your libraries using a dsDNA-specific fluorescent dye method, such as Qubit or PicoGreen. Based on an average library size of 500 bp, convert the library concentration using the formula 1 ng/ul = 3 nM.

Quality Control

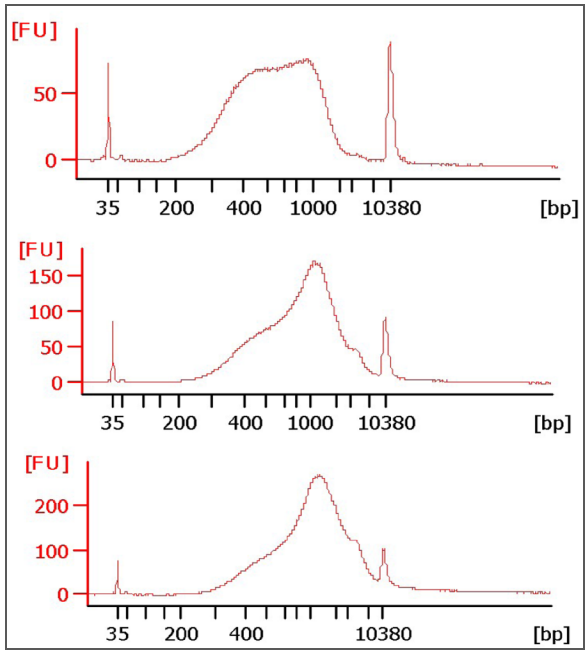
Run 1 µl of 1:3 diluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip to check the size distribution of your libraries.

Figure 4 shows example traces of successfully sequenced libraries. Typical libraries show a broad size distribution from ~250 bp to 1000 bp, as in the top panel. Fragment sizes as small as 250 bp to as large as 1000–1500 bp can be sequenced. For larger libraries, as in the bottom panel, adjust the DNA concentration used for clustering. Libraries with average size greater than 1000 bp can require clustering at several concentrations to achieve optimal density.

The modified *PCR Clean-Up* step for 2 × 250 runs on the MiSeq System results in libraries with larger average size. Adjust the DNA concentration conversion formula to 1ng/µl = 1.5 nM. Clustering at several different DNA concentrations can be required to achieve optimal cluster density.

Library Size from Bioanalyzer in bp	Conversion Factor for ng/µl > nM	DNA Concentration for Cluster Generation
250	1 ng/µl = 6 nM	6–12 pM
500	1 ng/µl = 3 nM	6–12 pM
1000–1500	1 ng/µl = 1.5 nM	12–20 pM

Figure 4 Successful Human Genomic DNA Library Size Distributions Sequenced on HiSeq



Normalize and Pool Libraries

This process describes how to pool libraries before sequencing. Normalize indexed libraries to 2 nM in the Nextera Dilution Plate and then pool in equal volumes in the Nextera Pooled Plate. Normalize nonindexed libraries to 2 nM in the Nextera Dilution Plate without pooling.

Consumables

- NDP (Nextera Dilution Plate) barcode label
- NPP (Nextera Pooled Plate) barcode label (for multiplexing only)
- Microseal 'B' adhesive film
- Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20
- 96-well midi plate

Preparation

1. Apply the NDP barcode label to a new 96-well midi plate.
2. Apply the NPP barcode label to a new 96-well midi plate (for indexed libraries).
3. If NLP was stored frozen, thaw at room temperature and then centrifuge at 280 × g for 1 minute.

Make NDP

1. Transfer 10 µl library from each well of NLP to the corresponding well of NDP.
2. Normalize the concentration in each well to 2 nM using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.

   NOTE
   Depending on the yield quantification data of each library, the final volume in the NDP plate can vary from 10 µl to 100 µl.

3. Shake at 1000 rpm for 2 minutes.
4. Centrifuge at 280 × g for 1 minute.

Make NPP [For Indexed Libraries]

1. Transfer 5 µl from each well in column 1 of NDP to column 1 of NPP.
2. Repeat step 1 for the remaining columns of NDP until samples are pooled in column 1 of NPP.

   NOTE
   Track which sample goes into which well to avoid pooling 2 samples with the same index.

3. Combine the contents of each well of column 1 into well A2 of NPP.
4. Shake at 1800 rpm for 2 minutes.
5. Denature and dilute pooled libraries to the loading concentration for the instrument you are using. See the denature and dilute libraries guide for your instrument.

SAFE STOPPING POINT

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

Supporting Information

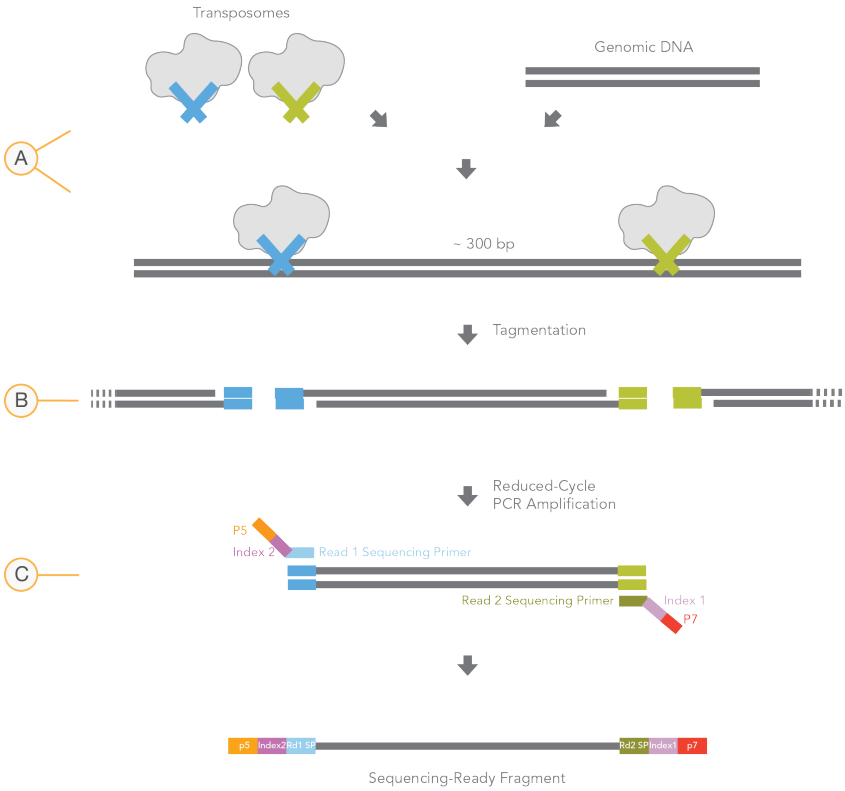
Appendix A

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 does the Nextera DNA Assay Work?

The Nextera 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 a single step. A limited-cycle PCR step uses the adapters to amplify the insert DNA. The PCR step adds index adapter sequences on both ends of the DNA, which enables dual-indexed sequencing of pooled libraries on Illumina sequencing platforms.



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

Acronyms

Acronym	Definition
NAP1	Nextera Amplification Plate 1
NAP2	Nextera Amplification Plate 2
NDP	Nextera Dilution Plate
NLP	Nextera Library Plate
NPM	Nextera PCR Master Mix
NSP1	Nextera Sample Plate 1
NSP2	Nextera Sample Plate 2
NSP3	Nextera Sample Plate 3
NPP	Nextera Pooled Plate
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
TD	Tagment DNA Buffer
TDE1	Tagment DNA Enzyme

Kit Contents

The Nextera DNA Library Prep Kit is packaged in 96 or 24 sample boxes and shipped on dry ice unless specified otherwise. Each kit has a corresponding Index Kit that contains 96 or 24 indexes. When you receive your kit, store the kit components at the specified temperature.

If sequencing with HiSeq 1000/2000, HiSeq 1500/2500 in High Output mode, Genome Analyzer IIx, or HiScan, use 1 of the following sequencing primer boxes for all sequencing run types:

- TruSeq Dual Index Sequencing Primer Box, Single read
- TruSeq Dual Index Sequencing Primer Box, Paired end

Use the TruSeq Index Plate Fixture Kit to help with correctly arranging the index primers during the Amplify Tagmented DNA. Each kit contains 2 fixtures and can be used for both the 24-sample kit and 96-sample kit.

Kit Name	Catalog #	Number of Samples
Nextera DNA Library Prep Kit (24 Samples)	FC-121-1031	24
Nextera Index Kit (24 Indexes, 96 Samples)	FC-121-1011	24
Nextera DNA Library Prep (96 Samples)	FC-141-1007	96
Nextera Index Kit (96 Indexes, 384 Samples)	FC-121-1012	96
TruSeq Dual Index Sequencing Primer Box, Single-Read	FC-121-1003	24/96
TruSeq Dual Index Sequencing Primer Box, Paired-End	PE-121-1003	24/96
TruSeq Index Plate Fixture Kit	FC-130-1005	24/96

Nextera DNA Library Prep Kit, Store at -25°C to -15°C

Quantity		Acronym	Reagent Name
24 samples	96 samples		
1	2	TD	Tagment DNA Buffer
1	1	TDE1	Tagment DNA Enzyme
1	1	NPM	Nextera PCR Master Mix
1	2	PPC	PCR Primer Cocktail
1	4	RSB	Resuspension Buffer

Nextera Index Kit, Store at -25°C to -15°C

Quantity		Reagent Name
24 samples	96 samples	
6, N701–N706	12, N701–N712	Index 1 (i7) Adapters
4, N501–N504	8, N501–N508	Index 2 (i5) Adapters

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 and Equipment

Consumable	Supplier
10 µl barrier pipette tips	General lab supplier
10 µl multichannel pipettes	General lab supplier
10 µl single channel pipettes	General lab supplier
1000 µl barrier pipette tips	General lab supplier
1000 µl multichannel pipettes	General lab supplier
1000 µl single channel pipettes	General lab supplier
200 µl barrier 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, part # AB-0859
Agencourt AMPure XP 60 ml kit	Beckman Coulter Genomics, part # A63881
Distilled water	General lab supplier
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
Microseal 'A' film	Bio-Rad, part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
PCR grade water (for gel-free method)	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
Tris-Cl 10 mM, pH8.5 with 0.1% Tween 20	General lab supplier
Tween 20	Sigma, part # P7949
Ultrapure water	General lab supplier

Consumable	Supplier
Microseal 96-well PCR plates (TCY plate)	Bio-Rad, part # HSP-9601
Zymo Purification Kit (ZR-96 DNA Clean & Concentrator™-5)	Catalog # D4023 or D4024

Equipment

Equipment	Supplier
96-well thermal cycler (with heated lid)	See table in *Thermal Cycler* section.
Magnetic stand-96	Ambion, part # AM10027
Microplate centrifuge	General lab supplier
Vortexer	General lab supplier
High-Speed Microplate Shaker	VWR, catalog # 13500-890 (110 V/120 V) VWR, catalog # 14216-214 (230 V)

Thermal Cyclers

The following table lists the recommended settings for the recommended thermal cycler, and other comparable models. If your lab has a thermal cycler that is not listed, validate the thermal cycler before performing the protocol.

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

Index Sequences

The Illumina dual-index strategy adds 2 8-base indexes, Index 1 (i7) and Index 2 (i5), to each sample.

There are 12 different Index 1 (i7) adapters (eg, N705) and up to 8 different Index 2 (i5) adapters (eg, N505), depending on the kit you are using. In the Index adapter name:

- N refers to Nextera
- 7 refers to Index 1 (i7)
- 5 refers to Index 2 (i5)
- 01–12 refers to the index number

Use the following bases for entry on your sample sheet.

Table 1 Index Adapter Sequences

Index 1 (i7)	Sequence		Index 2 (i5)	Sequence
N701	TAAGGCGA		N502	CTCTCTAT
N702	CGTACTAG		N503	TATCCTCT
N703	AGGCAGAA		N504	AGAGTAGA
N704	TCCTGAGC		N505	GTAAGGAG
N705	GGACTCCT		N506	ACTGCATA
N706	TAGGCATG		N507	AAGGAGTA
N707	CTCTCTAC		N508	CTAAGCCT
N708	CAGAGAGG		N517	GCGTAAGA
N709	GCTACGCT			
N710	CGAGGCTG			
N711	AAGAGGCA			
N712	GTAGAGGA			

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 2 Illumina General Contact Information

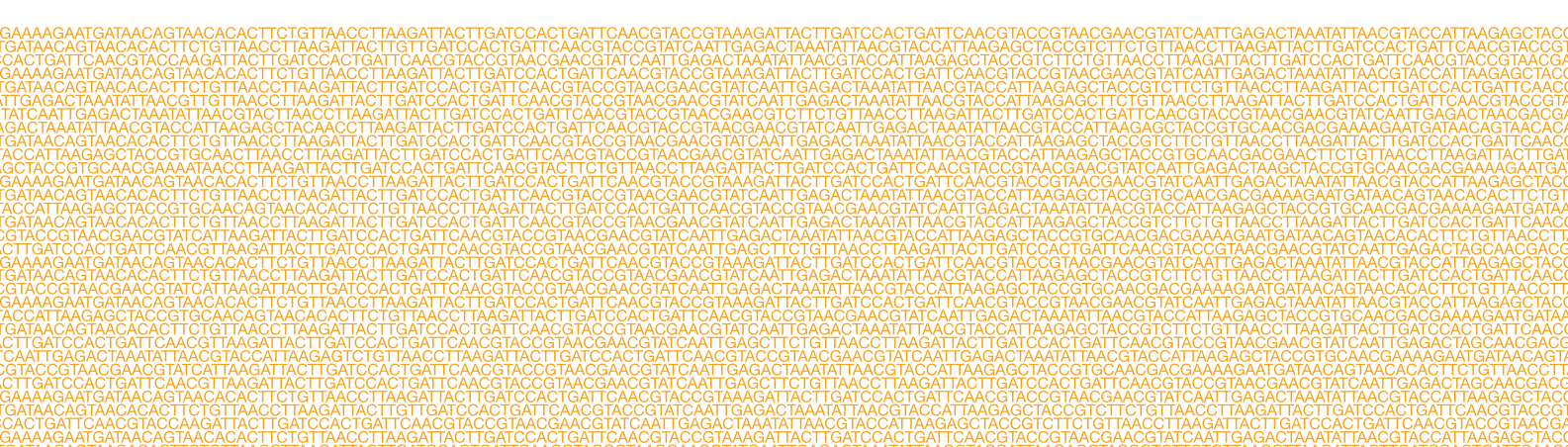
Website	www.illumina.com
Email	techsupport@illumina.com

Table 3 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

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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