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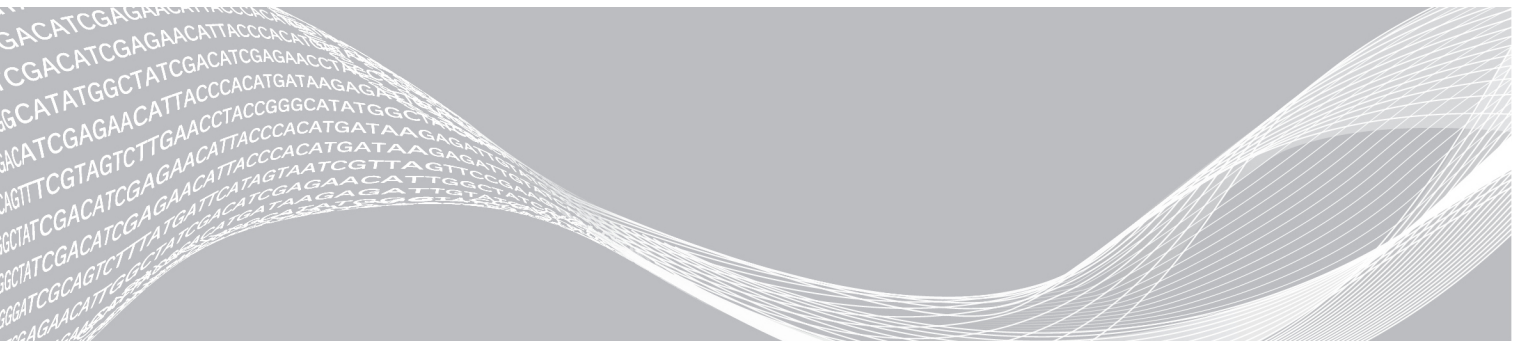
Nextera™ DNA Flex Library Prep

Reference Guide



Document # 1000000025416 v01

ILLUMINA PROPRIETARY

April 2018

Revision History

Document	Date	Description of Change
Document # 1000000025416 v01	April 2018	Replaced references to the *Nextera DNA Flex Pooling Guide (document # 1000000031471)* with the *Index Adapters Pooling Guide (document # 1000000041074)*. Pooling information is consolidated into the *Index Adapters Pooling Guide*.
Document # 1000000025416 v00	October 2017	Initial release.

Table of Contents

Chapter 1 Overview

Introduction

This protocol explains how to prepare up to 96 indexed paired-end libraries from genomic DNA for subsequent sequencing on an Illumina sequencing system.

The Nextera™ DNA Flex Library Prep protocol:

- Uses an enzymatic reaction, called tagmentation, to fragment DNA and add adapter sequences in only 15 minutes
- Innovative sample normalization at inputs > 100 ng
- Streamlines sample pooling and sequencing
- Reduces excessive pipetting and overall hands-on time, while optimizing use of consumables by using master mix reagents
- Generates libraries from as little as 1 ng input
- Prepares libraries directly from blood or saliva samples

Genomic DNA Input Recommendations

The Nextera DNA Flex Library Prep protocol is compatible with DNA inputs ranging from 1–500 ng, or higher. For human DNA samples and other large complex genomes, the recommended DNA input is between 100–500 ng. For small genomes, the DNA input amount can be reduced to as low as 1 ng (modifying the PCR cycling conditions accordingly).

For DNA inputs between 100–500 ng, accurate quantification of the initial DNA sample is not required, and normalization of the final yield is expected.

If you are using less than 100 ng DNA input, we recommend quantification of the initial DNA sample to determine the number of PCR cycles required. In this case, because final libraries yields from low inputs are not normalized by this library prep method, quantification and normalization of libraries before sequencing is recommended.

Table 1 DNA Input Recommendations

Total DNA Input (ng)	Quantification of Input DNA Recommended	Recommended # of PCR Cycles	Normalized Library Yield
1–9	Yes	12	No
10–24		8	
25–49		6	
50–99		5	
100–500	No	5	Yes
Blood/Saliva	No	5	Yes

NOTE

Protocols specific to blood and saliva are included in the *Supporting Information* section of this guide. The blood protocol requires the Flex Lysis Reagent Kit, which is not provided with the Nextera DNA Flex Library Prep Kit. This kit is sold separately—refer to Illumina catalog #20015884.

Input DNA Quantification (less than 100 ng)

When input is less than 100 ng, use a fluorometric-based method to quantify input DNA. Avoid methods that measure total nucleic acid, such as NanoDrop or other UV absorbance methods.

If you use the Qubit dsDNA BR Assay Kit and/or HS Kit, use 2 µl of each DNA sample with 198 µl of the Qubit Working Solution.

Assess DNA Quality

UV absorbance is a common method used for assessing the quality of a DNA sample. The ratio of absorbance at 260 nm to absorbance at 280 nm provides 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 260/280 ratio of 2.0–2.2. Values outside this range indicate the presence of contaminants that may cause incomplete tagmentation and adversely impact the final library yield. For a complete list of contaminants, including sources, avoidance, and effects on the library, see the *Nextera XT Troubleshooting Technical Note*.

NOTE

Incomplete tagmentation caused by contaminants may result in library preparation failure, poor clustering, or an unexpectedly high scaffold number.

Additional Resources

The Nextera DNA Flex Library Prep support pages on the Illumina website provide additional resources. These resources include software, training, compatible products, best practices, and the following documentation. Always check the support pages for the latest versions.

Resource	Description
Custom Protocol Selector	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.
Index Adapter Pooling Guide (document # 1000000041074)	Provides pooling guidelines and dual indexing strategies for using the Nextera DNA Flex Library Prep kit.
Nextera™ DNA Flex Library Prep Checklist (document # 1000000033561)	Provides a checklist of the protocol steps. The checklist is intended for experienced users.
Nextera™ DNA Flex Library Prep Consumables and Equipment List (document # 1000000033564)	Provides an interactive checklist of user-provided consumables and equipment.

Chapter 2 Protocol

Introduction

This chapter describes the Nextera DNA Flex Library Prep protocol.

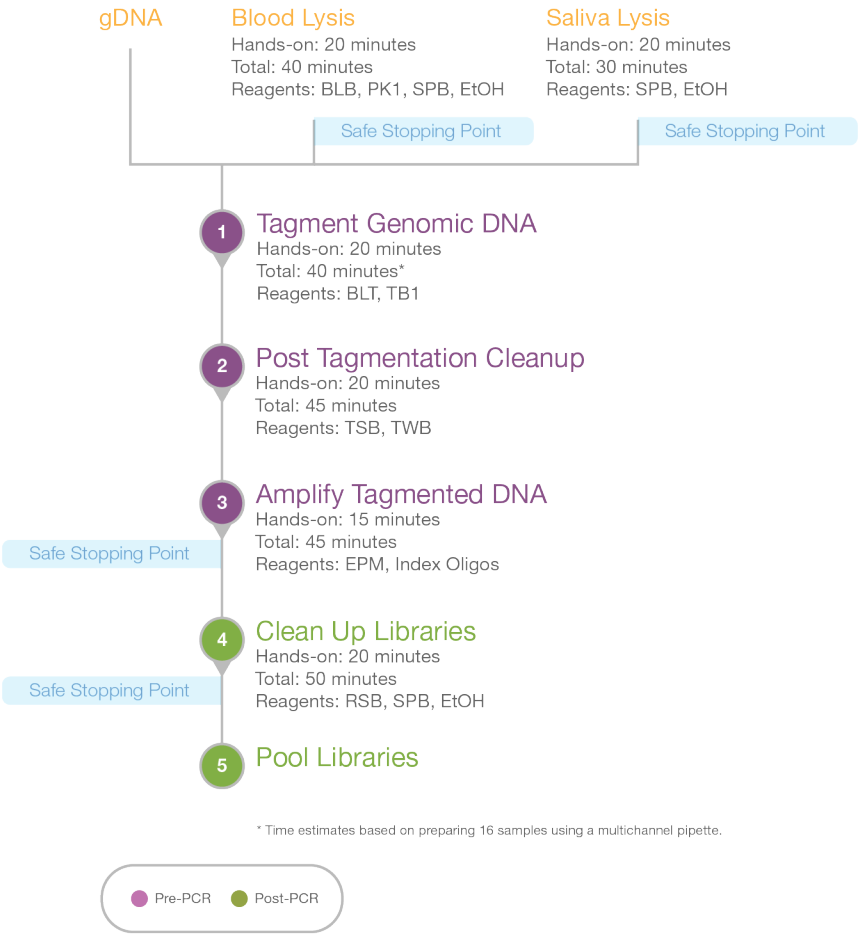
- Review Best Practices before proceeding. See *Additional Resources* on page 2 for information on how to access Nextera DNA Flex Library Prep Best Practices on the Illumina website.
- Before proceeding, confirm kit contents and make sure that you have the required equipment and consumables. See *Supporting 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 DNA Flex Library Prep support page.

Nextera DNA Flex Library Prep Workflow

Figure 1 Nextera DNA Flex Library Prep Workflow



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 or reagent master mixes, change tips between ***each sample***.
- When adding index adapters, change tips between ***each row*** and ***each column***.
- Remove unused index adapter tubes from the working area.
- Open only one index adapter tube at a time, to prevent misplacing caps.

Sealing the Plate

- Always seal the 96-well plate before any thermal cycling steps in the protocol.
- Apply the adhesive seal to cover the plate and seal with a rubber roller.

- Microseal ‘F’ adhesive foils are effective at temperatures down to -70°C and are recommended for long-term storage of the 96-well plates containing the final libraries.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates. Microseal 'B' seals can be for thermal cycling or short-term storage.
- Microseal 'A' adhesive film is effective for thermal cycling.

Handling Bead-Linked Transposomes (BLT)

- Store the BLT stock tube upright in the refrigerator to make sure that the beads are always submerged in the buffer.
- Vortex the BLT stock tube thoroughly to make sure that the beads remain in suspension while in aliquot. Centrifugation before pipetting is not recommended.
- If beads are adhered to the side or top of a 96-well plate, centrifugation is acceptable. Fully resuspend the bead pellet by pipetting until thoroughly mixed.
- When performing wash steps:
  - Use an appropriate magnet for the plate. See *Consumables and Equipment* on page 19.
  - Keep the plate on the magnet, unless instructed otherwise.
  - Avoid agitating the plate, or disturbing the bead pellet, while the plate is on the magnet.
  - If beads are accidentally aspirated into the pipette tip, dispense all liquid back into the well and leave until clear to allow the beads to settle.
  - Dispense tagment wash buffer directly onto the beads.
  - If liquid becomes adhered to the side or top of the tube or well, pulse centrifuge briefly to pull volume into solution.

Handling Tagment Wash Buffer (TWB)

- Minimize the potential of TWB foaming during tagmentation with a deliberately gentle pipetting technique.

Tagment Genomic DNA

This step uses the Bead-Linked Transposomes BLT to tagment DNA. This process fragments and tags the DNA with adapter sequences.

Consumables

- BLT (Bead-Linked Transposomes)
- TB1 (Tagmentation Buffer)
- Nuclease-free water
- 96-well PCR plate
- Microseal 'B' adhesive seal
- 1.7 ml microcentrifuge tubes

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
BLT	2°C to 8°C NOTE: Storing at temperatures below 2°C renders the BLT unusable.	Bring to room temperature. Vortex to mix.
TB1	-25°C to -15°C	Bring to room temperature. Vortex to mix.

2 Save the TAG program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 55°C for 15 minutes
- 10°C hold
- Each well or tube contains 50 µl

See *Genomic DNA Input Recommendations* on page 1 for recommended DNA input amounts and sample types.

Procedure

1 Transfer between 2 µl and 30 µl of DNA into the wells of a 96-well PCR plate, so that the total input amount (ng) is within the desired range.

2 Add nuclease-free water to the DNA samples to bring the total volume to 30 µl.

3 Vortex the BLT vigorously for 10 seconds, then visually check the beads for complete resuspension. Repeat as necessary.

4 Prepare tagmentation master mix. For each reaction use:

Reagent	Volume per reaction (µl)
BLT	11 µl
TB1	11 µl

5 Vortex the tagmentation master mix thoroughly to make sure the BLT beads are evenly resuspended BLT in the buffer.

NOTE

Make sure that the tagmentation master mix is vortexed well and the beads remain evenly resuspended in the mix while it is being aliquoted into the 96-well plate.

6 Using fresh tips, transfer 20 µl of tagmentation master mix to each well containing a sample.

7 Pipette mix the 50 µl reaction mix to resuspend.

8 Seal the plate with Microseal 'B', place on the preprogrammed thermal cycler and run the TAG program.

Post Tagmentation Cleanup

This step washes the adapter-tagged DNA on the BLT before PCR amplification.

Consumables

- TSB (Tagment Stop Buffer)
- TWB (Tagment Wash Buffer)
- 96-well plate magnet
- Microseal 'B' adhesive seal

Preparation

Prepare the following consumables:

Item	Storage	Instructions
ST2	15°C to 30°C	Check for any precipitates. If present, heat the buffer at 37°C for 10 minutes, and vortex until they dissolve. Use at room temperature.
TWB	15°C to 30°C	Use at room temperature.

Procedure

1 Add 10 µl of TSBto the tagmentation reaction.

2 Gently pipette mix the entire volume to resuspend the beads.

3 Seal the plate and incubate at 37°C for 15 minutes on a thermal cycler with heated lid set at 100° C and volume reaction of 60 µl. Then hold at 10° C.

4 Place the plate on the magnet for 3 minutes or until solution is clear.

5 Using a multichannel pipette, remove supernatant and discard.

NOTE

If beads became disturbed during aspiration, redisperse solution into the wells. Keep plate on the magnet to let beads settle.

6 Remove the plate from the magnet and add 100 µl TWB. Gently pipette mix until beads are fully resuspended.

7 Place the plate on the magnet for 3 minutes or until solution is clear.

8 Remove supernatant with a multichannel pipette and discard.

9 Repeat steps 6 through 8 for a total of 2 washes.

10 Remove the plate from the magnet and add 100 µl TWB. Gently pipette mix until beads are fully resuspended.

11 Seal the plate and place the plate with TWB on the magnet and allow it to incubate until step 3 in the *Amplify Tagmented DNA*. The plate should incubate for at least 3 minutes or until clear.

NOTE

Keep the pellet in TWB to help prevent any chance of over drying the beads.

12 While the samples are incubating, continue with the protocol.

Amplify Tagmented DNA

This step amplifies the tagmented DNA using a limited-cycle PCR program. The PCR step adds Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for sequencing cluster formation.

Consumables

- EPM (Enhanced PCR Mix)
- Nextera DNA Flex index adapters
- Nuclease-free water

- Microseal 'A' adhesive seal
- Microseal 'B' adhesive seal
- 1.7 ml microcentrifuge tubes
- P20 multichannel pipette
- P200 multichannel pipette

Preparation

Prepare the following consumables:

Item	Storage	Instructions
EPM	-25°C to -15°C	Thaw on ice. Invert to mix, then briefly centrifuge.
Nextera DNA Flex Indexes	-25°C to -15°C	Thaw at room temperature. For index tubes: Vortex to mix, then centrifuge briefly. For plates: Spin briefly before use.

To yield robust libraries, use this number of PCR cycles, depending on expected initial DNA input amount:

DNA Input (ng)	Number of PCR Cycles
1–9	12
10–24	8
25–49	6
50–100	5
>100	5
Blood/Saliva	5

Procedure

1 Prepare the PCR master mix.

Reagent	Volume per reaction
EPM	22 µl
Nuclease-free water	22 µl

2 Vortex and spin down the PCR master mix.

3 Remove the third TWB wash from the samples while on the magnet. Use a P20 multichannel pipette to remove any excess liquid from the plate.
Any remaining foam on the well walls does not adversely affect the library.

4 Remove the plate from the magnet. Proceed *immediately* to the next step to prevent excessive drying of the beads.

5 Add 40 µl of the PCR master mix to each sample well. Pipette mix to make sure that the beads are thoroughly resuspended.

6 Add index adapters to each sample. Check volumes in the following table. For low-plexity conditions, refer to the *Index Adapters Pooling Guide (document # 1000000041074)*.

Index Kit Type	Kit Configuration	Volume of Index Adapter per Sample
24 plex (dual index)	Individual tubes	5 µl i5 adapter 5 µl i7 adapter
96 plex (dual index)	96-well plate	10 µl of primer mix

NOTE

For tubes, open only one index adapter tube at a time to prevent misplacing caps; alternatively, use fresh caps after opening each tube.
For plates, each well of the index plate is for a single use only.

7 Using a pipette set to 40 µl, pipette mix a minimum of 10 times to mix the entire reaction volume.

8 Seal the plate, place in the thermal cycler and run the program.
- 68°C, 3 min
- 98°C, 3 min
- Repeat cycling conditions below for the total number of cycles listed in *DNA Volume-Based Recommendations*:
  - 98°C, 45 sec
  - 62°C, 30 sec
  - 68°C, 2 min
- 68°C, 1 min
- 10°C hold

9 Remove the plate from the thermal cycler when the PCR program completes.

10 Centrifuge for 1 minute at 280 × g to make sure all the liquid is at the bottom of the well.

SAFE STOPPING POINT

If you are stopping, seal the plate with a Microseal 'B' adhesive seal, and store at 2°C to 8°C for up to 3 days.

Clean up Libraries

This step purifies the amplified libraries through a double-sided bead purification procedure.

Consumables

- Purification Beads
- Freshly prepared 80% ethanol (EtOH)
- RSB (Resuspension Buffer)
- Midi plates (2)
- 96-well PCR plate
- Microseal 'B' adhesive seal
- Microseal 'F' foil seals
- 1.7 ml microcentrifuge tubes
- 96-well plate magnet

- Nuclease-free water

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
PB	2°C to 8°C	Let stand at room temperature for 30 minutes. Vortex and invert to mix.
RSB	-25°C to -15°C	Thaw and bring to room temperature. Vortex to mix.

Procedure

1 Place the plate on the magnet for 5 minutes or until the supernatant is clear.

2 Transfer 45 µl of the PCR supernatant into a fresh midi plate.

3 Vortex and invert PB multiple times to ensure full resuspension.

4 Prepare a master mix of diluted SPB:

Reagent	Volume per reaction (µl)
PB	45 µl
Nuclease-free water	40 µl

5 Vortex the diluted PB master mix thoroughly and add 85 µl mix to each PCR product.

6 Pipette mix a minimum of 10 times or until thoroughly mixed.

WARNING

Complete mixing is critical to proper size distribution of libraries.

7 Seal the plate and incubate at room temperature for 5 minutes.

8 Place the midi plate on a plate magnet for 5 minutes or until supernatant is clear.

9 During incubation, vortex the PB (*undiluted* stock tube) thoroughly, and then add 15 µl to each well in a *new* midi plate.

10 Transfer 125 µl of supernatant from the first midi plate into the second midi plate (containing the 15 µl of PB).

11 Pipette mix 10 times.

NOTE

Mixing is critical for proper size distribution of libraries.

12 Seal the second midi plate and incubate at room temperature for 5 minutes.

13 Place midi plate on a magnet for 5 minutes or until clear.

14 Remove and discard supernatant without disrupting the beads.

15 With the plate on the magnet, add 200 µl of fresh 80% ethanol without mixing and incubate for 30 seconds.

16 Pipette to remove the ethanol.

17 Repeat steps 15 and 16 for a total of 2 washes.

18 Use a P20 pipette to remove any excess liquid from the midi plate.

19 Air-dry on the magnetic stand until dry (~5 minutes).

20 Remove the midi plate from the magnet and add 32 µl of RSB to the beads.

21 Pipette mix until thoroughly resuspended.

22 Incubate at room temperature on the bench for 2 minutes.

23 Place the midi plate back on the magnet for 2 minutes or until clear.

24 Transfer 30 µl of the supernatant into a new 96-well PCR plate.

SAFE STOPPING POINT

If you are stopping, seal the plate with a Microseal 'F' foil seal and store at -25°C to -15°C for up to 30 days.

Pooling Libraries

When using DNA inputs of 100–500 ng, the normalization features of this library protocol mean that quantification and normalization of individual libraries, generated within a single experiment, is not necessary. There may, however, be slight variations in final yields between different library preparation events, so to achieve optimal cluster density we advise you to pool your libraries with equal volume and quantify the pool prior to sequencing.

HiSeqX and HiSeq 3000/4000 users

The wider range in acceptable clustering concentrations on these instruments means that if the Qubit/PicoGreen quantification for the library pool (as described in the section below) is between 9.5 ng/µl and 12.5 ng/µl, a 1:11 dilution (10 µl pooled library + 100 µl RSB) can be made to take the pool to the concentration required for sequencing (ie, 2–3 nM).

For DNA inputs of 100–500 ng

1 Pool 5 µl per sample of up to 96 libraries into a single 1.5 ml microcentrifuge tube. Mix by vortexing then spin down in a microfuge.

2 Quantify the single pooled library using a dsDNA specific fluorescent dye method such as Qubit or PicoGreen.

For DNA inputs of <100 ng

1 Quantify each library individually using Qubit or PicoGreen.

For Libraries generated from blood or saliva

The volumes of blood and saliva used in this protocol were developed to provide normalization for most samples; however, it should be noted that blood and saliva are heterogeneous sample types. The ability of Nextera DNA Flex to generate normalized libraries depends on the total amount of DNA obtained from the lysed sample, and this can be adversely affected by numerous factors independent of kit performance. These factors include, but are not limited to:

- viscosity of the saliva samples
- blood sample age
- storage conditions
- underlying medical conditions affecting white blood cell counts

Assuming you start with adequate DNA input (>100 ng), expect normalization of the libraries equal to that observed in 100–500 ng gDNA input. For improved confidence, you can quantify each library individually prior to pooling using Qubit or PicoGreen.

Check Library Quality (Optional)

Run 1 µl of either the pooled library, or the individual libraries, on an Advanced Analytical Fragment Analyzer with the HS-NGS High Sensitivity 474 kit or an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA kit. Typical library size profiles are shown below with the average fragment size expected to be around 600 bp (when analyzed using a size range of 150–1500 bp).

Figure 2 Final Library Run on Fragment Analyzer Using High Sensitivity NGS Kit

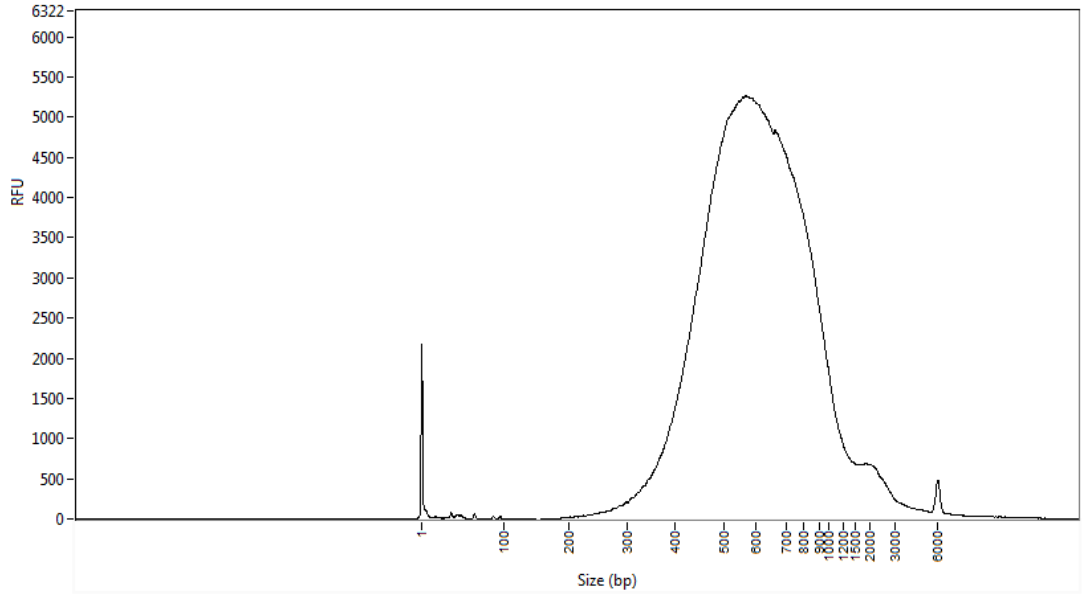
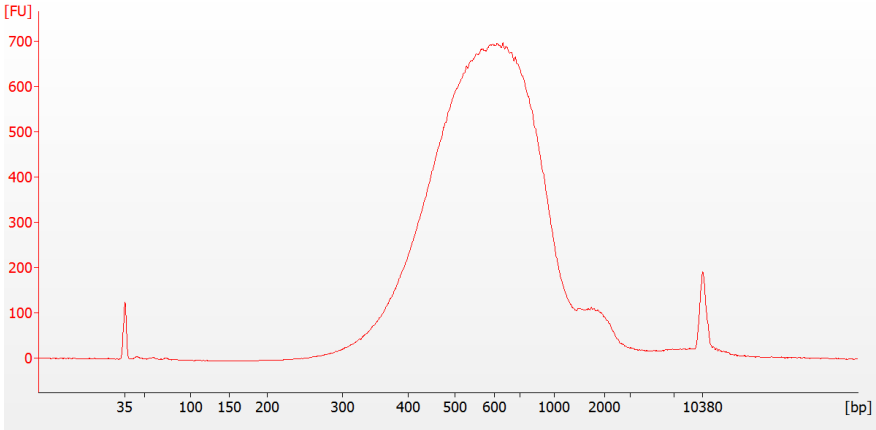


Figure 3 Final Library Run on Bioanalyzer Using High Sensitivity DNA Kit



Calculate Molarity

1 Calculate the molarity of the individual, or pooled library, using the following formula:

$$\frac{ng/\mu l x 10^6}{660\frac{g}{mol}\, x\ average\ library\ size\ (bp)} = Molarity$$

NOTE

If the individual, or pooled libraries, were run on a Bioanalyzer or Fragment Analyzer, use the average size obtained for the sample, otherwise use 600 bp as the average fragment length in the calculation.

2 After the molarity has been calculated:

a For libraries that were pooled prior to quantification
- Dilute the pool with RSB to .

b For libraries that have been quantified individually
- Dilute the individual samples with RSB to , then pool 10 µl of each diluted sample in a single tube.

Table 2 Recommended Loading Concentrations

Sequencer	Dilute To	Template Loading Concentration	Denaturation Instructions Document Number
NovaSeq	See document # 1000000019358	See document # 1000000019358	1000000019358
HiSeqX	2–3 nM	200–300 pM	15006165
HiSeq 3000/ 4000	2–3 nM	200–300 pM	15006165
HiSeq 2000/ 2500 High Output Mode	2 nM	12 pM	15006165
HiSeq 2500 Rapid Run Mode	2 nM	8.5 pM	15050107
NextSeq	2 nM	1.2–1.3 pM	15048776
MiSeq	4 nM	12 pM	15039740
MiniSeq	2 nM	1.2–1.3 pM	1000000002697

NOTE

Use these recommended loading concentrations only as a general guideline.

Optimize the loading concentrations for your workflow and quantification method.

Chapter 3 Sequencing

Nextera DNA Flex supports read lengths up to 2 x 151 cycles..

Table 3 Recommended Read Length on Illumina Sequencers

Sequencer	Read Length
NovaSeq, HiSeq X, HiSeq 3000/4000, NextSeq, MiSeq, MiniSeq, iSeq	2 x 151
HiSeq 2000, HiSeq 2500 High Output	2 x 126
HiSeq Rapid Run	2 x 101

Supporting Information

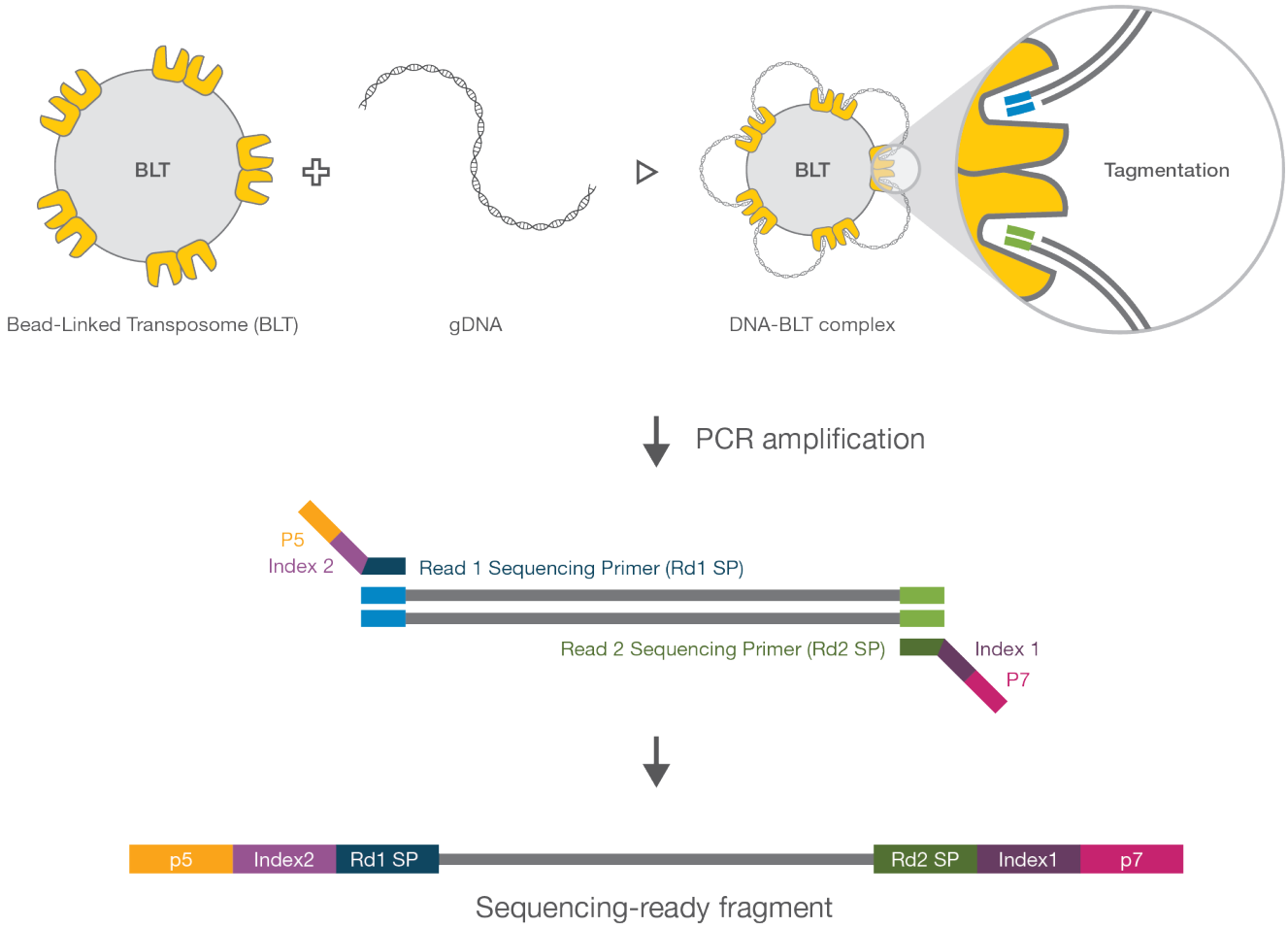
Introduction

The protocol described in this guide assumes that you have reviewed the contents of this section, confirmed workflow contents, and obtained all required consumables and equipment.

How the Nextera DNA Flex Assay Works

The Nextera DNA Flex library prep kit uses an innovative, bead-based transposome complex to tagment genomic DNA by fragmenting and adding adapter tag sequences in a single reaction step. Once saturated with input DNA, the bead-based transposome complex fragments a set number of DNA molecules, providing flexibility to use a wide DNA input range, consistent tight fragment size distribution, and normalized libraries Following the tagmentation step, a limited-cycle PCR step adds Nextera DNA Flex-specific index adapter sequences to the ends of a DNA fragment, enabling capability across all Illumina sequencing platforms. A subsequent Sample Purification Bead (SPB) cleanup step then purifies libraries for use on an Illumina sequencer.

Figure 4 Nextera DNA Flex Workflow



Acronyms

Acronym	Definition
BLB	Blood Lysis Buffer
BLT	Bead Linked Transposome
EPM	Enhanced PCR Mix
EtOH	Ethanol
PK1	Proteinase K
RSB	Resuspension Buffer
SPB	Sample Purification Beads
TB1	Tagmentation Buffer 1
TSB	Tagment Stop Buffer
TWB	Tagment Wash Buffer

Product Contents

Nextera DNA Flex Library Prep Kit Configurations

NOTE

Certain components of the kit are stored at a temperature that differs from the shipping temperature. Store kit components at the temperature specified.

Nextera DNA Flex Library Prep - 24 Samples

Box 1 of 3

Quantity	Acronym	Description	Storage Temperature
1	SPB	Sample Purification Beads	2°C to 8°C
1	TSB	Tagment Stop Buffer	Room temperature
1	TWB	Tagment Wash Buffer	Room temperature

Box 2 of 3

Quantity	Acronym	Description	Storage Temperature
1	RSB	Resuspension Buffer	-25°C to -15°C
1	TB1	Tagmentation Buffer 1	-25°C to -15°C
1	EPM	Enhanced PCR Mix	-25°C to -15°C

Box 3 of 3

Quantity	Acronym	Description	Storage Temperature
1	BLT	Bead-Linked Transposomes	2°C to 8°C

Nextera DNA Flex Library Prep - 96 Samples

Box 1 of 3

Quantity	Acronym	Description	Storage Temperature
1	SPB	Sample Purification Beads	2°C to 8°C
4	TSB	Tagment Stop Buffer	Room temperature
1	TWB	Tagment Wash Buffer	Room temperature

Box 2 of 3

Quantity	Acronym	Description	Storage Temperature
1	RSB	Resuspension Buffer	-25°C to -15°C
4	TB1	Tagmentation Buffer 1	-25°C to -15°C
4	EPM	Enhanced PCR Mix	-25°C to -15°C

Box 3 of 3

Quantity	Acronym	Description	Storage Temperature
4	BLT	Bead-Linked Transposomes	2°C to 8°C

Index Kit Contents

Make sure that you have all the reagents identified in this section before proceeding to the library preparation procedures. Kits are available in the following configurations.

Consumable	Catalog #
Nextera™ DNA CD Indexes (24 Indexes, 24 Samples)	20018707
Nextera™ DNA CD Indexes (96 Indexes, 96 Samples)	20018708

NOTE

Certain components of the kit are stored at a temperature that differs from the shipping temperature. Store kit components at the temperature specified in this protocol.

24 Dual Index (Tube Format) - 24 Samples

Quantity	Index Name	Description	Storage Temperature
1	H503	DNA Adapter	-25°C to -15°C
1	H505	DNA Adapter	-25°C to -15°C
1	H506	DNA Adapter	-25°C to -15°C
1	H517	DNA Adapter	-25°C to -15°C
1	H710	DNA Adapter	-25°C to -15°C
1	H705	DNA Adapter	-25°C to -15°C
1	H706	DNA Adapter	-25°C to -15°C
1	H707	DNA Adapter	-25°C to -15°C
1	H711	DNA Adapter	-25°C to -15°C
1	H714	DNA Adapter	-25°C to -15°C

96 Dual Index (Plate Format) - 96 Samples

Quantity	Description	Storage Temperature
1	96 Dual Adapter Index Plate	-25°C to -15°C

Blood Lysis Kit Contents

Confirm that all reagents identified in this section are available before proceeding to the library preparation procedures.

Consumable	Catalog #
Nextera DNA Flex Library Prep—Flex Lysis Reagent Kit	20018706

Flex Lysis Reagent Kit

Quantity	Acronym	Description	Storage Temperature
4	BLB	Blood Lysis Buffer	Room temperature
4	PK1	Proteinase K	-25°C to -15°C

NOTE

Purification Beads are not included in this kit, however sufficient SPB to run the blood lysis workflow are included in the 24-plex and 96-plex library kits.

Consumables and Equipment

Confirm that all required user-supplied consumables and equipment are present and available 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
20 µl pipette tips	General lab supplier
20 µl multichannel pipettes	General lab supplier
20 µl single channel pipettes	General lab supplier
200 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
200 µl single channel pipettes	General lab supplier
1000 µl pipette tips	General lab supplier
1000 µl single channel pipettes	General lab supplier
96-well storage plates, round well, 0.8 ml (midi plate) xs	Fisher Scientific, catalog # AB-0859
Hard-Shell 96-well PCR plates	Bio-Rad, catalog # HSP-9601
Microseal 'A' film	Bio-Rad, catalog # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, catalog # MSB-1001
Microseal 'F' foil seals	Bio-Rad, catalog # MSF-1001
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, catalog # 89094-658
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, product # E7023
Nuclease-free water	General lab supplier
[Optional] Agilent High Sensitivity DNA Kit	Agilent, catalog # 5067-4626
Qubit dsDNA HS Assay Kit	ThermoFisher Scientific, catalog # Q32851 or Q32854
Quant-iT™ PicoGreen® dsDNA Assay Kit	ThermoFisher Scientific, catalog # P11496
[Optional] High Sensitivity NGS Fragment Analysis Kit	Advanced Analytical, catalog # DNF-474
Flex Lysis Reagent Kit	Illumina, catalog # 20015884
EDTA Blood Collection tubes	Becton Dickinson
Oragene DNA Collection Kit for Saliva	DNA Genotek, catalog # OGR-500 or OGD-510

Equipment

Equipment	Supplier
Magnetic Stand-96	Thermo Fisher Scientific, catalog # AM10027
Microplate centrifuge	General lab supplier
Microcentrifuge	General lab supplier
Vortexer	General lab supplier
[Optional] 2100 Bioanalyzer System	Agilent, catalog # G2940CA
Qubit® Fluorometer 3.0	ThermoFisher Scientific, catalog # Q33216, Q33217 or Q33218
[Optional] Fragment Analyzer™ Analytical	Advanced Analytical

Thermal Cyclers

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

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad C-1000 Touch thermal cycler	Calculated	Heated	Plate
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate

Blood Lysis (Optional)

This protocol has been validated using fresh whole blood collected in EDTA collection tubes. Following collection, store the blood at 4°C and process it within 3 days.

NOTE

The use of frozen blood has not been validated, therefore cannot be recommended.

CAUTION

Blood is a potential source of infectious diseases. Follow site specific procedures to ensure the safe handling of blood samples. During the lysis protocol, ensure that the entire blood sample is fully lysed (ie, brown in color following the heat incubation step) before proceeding to subsequent steps.

Consumables

- Blood samples collected into EDTA collection tubes
- SPB (Sample Purification Beads)
- BLB (Blood Lysis Buffer)
- PK1 (Proteinase K)
- Nuclease-free water
- Freshly prepared 80% ethanol (EtOH)
- 96-well PCR plate
- 96-well plate magnet

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
BLB	15°C to 30°C*	BLB must be at room temperature for optimal use. Check for any precipitates. If present, heat at 37°C for 10 minutes and vortex until resuspended.
SPB	2°C to 8°C**	Let stand for 30 minutes to bring to room temperature. Keep at room temperature for later use in the protocol.
PK1	-25°C to -15°C	Place on ice until needed.

*BLB is shipped frozen but should be stored at room temperature.

**SPB is included in the Nextera DNA Flex Library Prep Kit.

Procedure

1 Create a lysis master mix containing the following volumes for each prep:

Reagent	Volume per reaction (µl)
BLB	7 µl
PK1	2 µl
Nuclease-free water	31 µl

2 Make sure that the EDTA blood tube is thoroughly mixed by inverting the tube.

3 Transfer 10 µl of blood into the well of a 96-well PCR plate.

4 Vortex and spin down the lysis master mix.

5 Add 40 µl of the master mix to each sample.

6 Vortex and invert SPB multiple times to ensure full resuspension.

7 Add 20 µl of SPB to the sample well.

8 Use a pipette set to 50 µl and gently mix 10 times to ensure that the beads are thoroughly mixed with the sample.

9 Seal the plate and incubate at 56°C for 10 minutes at on a thermal cycler with heated lid to 100°C.

10 Place the plate on a magnet for 5 minutes.

NOTE

The beads are not visible at this point due to the darker brown color of the blood from the lysis reaction. Allow the sample to sit on the magnet for 5 minutes to make sure that the beads fully migrate to the magnet.

11 Carefully pipette off supernatant without disturbing the beads. Check for the presence of the bead pellet within the sample well before discarding the supernatant.

NOTE

If the beads are accidentally aspirated, the sample can be dispensed back in the well and allowed to settle again before the supernatant is removed.

12 Add 150 µl of 80% ethanol (EtOH) and incubate for 30 seconds on the magnet.

13 Remove and discard all of the EtOH.

14 Use a P20 pipette to remove any residual EtOH.

15 Remove the plate from magnet.

16 Resuspend the beads in 30 µl of water and pipette mix until resuspended.

17 Proceed directly to step 3to add the tagmentation master mix directly to the sample well containing 30 µl of water and the beads.

SAFE STOPPING POINT

If you are stopping, seal the plate with a Microseal 'B' adhesive seal, and store at 2°C to 8°C for up to 3 days.

Saliva Lysis (Optional)

This protocol is validated for saliva collected only in Oragene DNA Saliva collection tubes. Following collection, the saliva is mixed with the Oragene DX Solution contained in the collection tube, making it stable at room temperature.

NOTE

Prior to performing the saliva lysis, the Oragene tubes containing the samples need to be incubated for at least 1 hour at 50°C in water or an air incubator (as recommended by DNA Genotek) to lyse the cells. This incubation step can be performed at any time after the sample is collected. Once heat treated, the samples can be stored at room temperature.

This protocol is expected to generate >100 ng of DNA output at the end of the saliva lysis step.

CAUTION

Saliva is a potential source of infectious diseases. Follow site specific procedures to ensure the safe handling of saliva samples.

Consumables

- Saliva samples (collected in Oragene DNA collection tubes and heat treated)
- SPB (Sample Purification Beads)
- Nuclease-free water
- Freshly prepared 80% ethanol (EtOH)
- 96-well PCR plate
- 96-well plate magnet

Preparation

1 Prepare the following consumable:

Item	Storage	Instructions
SPB	2°C to 8°C*	Let stand for 30 minutes to bring to room temperature. Keep at room temperature for later use in the protocol.

*SPB is included in the Nextera DNA Flex Library Prep Kit.

Procedure

1 Transfer 20 µl of water into a 96-well PCR plate (1 well per sample).

2 Vortex the heat treated Oragene DNA collection tube to make sure that sample is thoroughly mixed.

3 Transfer 30 µl of the sample into a 96-well plate containing water and slowly pipette mix.

NOTE

Some saliva samples can be viscous so using wide-bored pipette tips can allow more accurate pipetting.

4 Vortex and invert SPB multiple times to ensure full resuspension.

5 Add 20 µl of SPB to the sample well.

6 Use a pipette set to 50 µl and gently mix 10 times to ensure the beads are thoroughly mixed with the sample.

7 Leave the plate at room temperature for 5 minutes.

8 Place the plate on the magnet for 5 minutes.

9 Carefully pipette off supernatant without disturbing the beads. Check for the presence of the bead pellet within the sample well before discarding the supernatant.

NOTE

If the beads are accidentally aspirated, the sample can be dispensed back in the well and allowed to settle again before the supernatant is removed.

10 Add 150 µl of 80% ethanol (EtOH) to the SPB pellet and leave for 30 seconds on the magnet.

11 Remove and discard all the EtOH.

12 Use a P20 pipette to remove any residual EtOH.

13 Remove the plate from the magnet.

14 Resuspend the beads in 30 µl water and pipette mix until fully resuspended.

15 Proceed directly to step 3 of the *Tagment Genomic DNA Procedure* on page 6 to add the tagmentation master mix directly to the sample well containing 30 µl of water and the beads.

SAFE STOPPING POINT

If you are stopping, seal the plate with a Microseal 'B' adhesive seal, and store at 2°C to 8°C for up to 3 days.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
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**.

Document # 1000000025416 v01

Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

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