

Using Nextera DNA Sample Preparation Kits and Enabling Dual Indexing

FOR RESEARCH USE ONLY

The Nextera® DNA Sample Preparation Kits (Table 1) provide a rapid and streamlined workflow enabling library preparation in less than 90 minutes from only 50 ng of input DNA.

In addition, Illumina® now supports up to 96 Nextera indices per lane on all Illumina sequencing systems. If sequencing a Nextera prepared library on HiSeq®, HiScanSQ™, or GAIIX an accessory sequencing primer kit is required to enable dual indexing. The MiSeq® System reagent kits already include the appropriate primers to enable dual indexing and these accessory sequencing primer kits are not required. Additional details on completing a successful dual indexing sequencing run with Nextera libraries can be found below.

Table 1 Nextera DNA Sample Preparation Kits

Kit Name	Catalog #
Nextera DNA Sample Preparation Kit (96 Samples)	FC-121-1031
Nextera DNA Sample Preparation Kit (24 Samples)	FC-121-1030
Nextera Index Kit (96 Indices, 384 Samples)*	FC-121-1012
Nextera Index Kit (24 Indices, 96 Samples)*	FC-121-1011

* Please note that these index kits are required to complete the sample preparation even if not pooling.

Table 2 Sequencing Primer Kits for Sequencing Nextera Libraries on HiSeq, HiScanSQ, and GAIIX

Kit Name	Catalog #
TruSeq Dual Index Sequencing Primer Kit, Single Read (single use kit)	FC-121-1003
TruSeq Dual Index Sequencing Primer Kit, Paired End (single use kit)	PE-121-1003



NOTE

Order the appropriate sequencing primer box based on the flow cell type. For example, a single-read dual-indexed run on a paired-end flowcell, requires the Paired-End Dual Index Sequencing Primer box.

Sample Sheet Preparation

To ensure proper index combinations for pooled libraries with dual-indexed experiments, please use the new Illumina Experiment Manager (IEM) prior to performing

sample preparation. IEM is a tool that helps create the required sample sheet for use with HiSeq, HiScanSQ, GAIIX or MiSeq, and is available for download on MyIllumina. IEM also indicates whether the indices chosen provide appropriate sequence diversity to avoid registration failures during sequencing.

Pooling Guidelines

When using less than the full complement of indices in the kit, refer to the Nextera DNA Sample Preparation Guide for a compatibility chart for pooling guidelines. To assist in correctly arranging index primers during the PCR Amplification steps, a TruSeq Index Plate Fixture Kit is available to order (Catalog # FC-130-1005).

Using Nextera on MiSeq

If preparing libraries to run on MiSeq, the appropriate primers are included in the reagent cartridge and the on-instrument analysis software (MiSeq Reporter) automatically performs dual index processing. When using IEM for sample sheet generation, select **Adapter Trimming** when you create your sample sheet. Shorter inserts may lead to sequencing into the adapter, and this feature helps filter out adapter sequence from the final sequence data.

Using Nextera on other Illumina Sequencers

When using the Nextera Sample Prep kit for sequencing on HiSeq, HiScanSQ, or GAIIX, there are workflow changes in clustering, sequencing and analysis that require ordering additional kits.



NOTE

Please note that new primers are required for cluster generation and sequencing when using a Nextera Sample Prep kit, whether performing indexing or not.

Please order the TruSeq Dual Index Sequencing Primer Boxes (Table 2); these kits contain the new primers for both clustering and sequencing.

cBot™ or Cluster Station Workflow

- ▶ **Primers:** If preparing Nextera libraries for cluster generation on HiSeq, HiScanSQ, or GAIIX, you must use the TruSeq Dual Index Sequencing Primer Boxes for all sequencing run types: non-indexed, single-

indexed, and dual-indexed. The TruSeq Dual Index Sequencing primers will be used in place of the existing sequencing primers (HP6, HP7, and HP8). Please see table 2 for catalog numbers.

- ▶ **Workflow Updates:** Dual indexing now requires aliquotting the new primer mixes HP10 into strip tubes on cBot. Because of this, the appropriate single read or paired end “Amp_Lin_Block_TubeStripHyb” recipe is required. Please refer to the updated cBot documentation for additional information. For HiSeq users Illumina recommends upgrading the cBot recipe installer to version 1.0.13 or later. This installer can be found on MyIllumina.

Sequencing Workflow

- ▶ **Primers:** If sequencing Nextera libraries with HiSeq, HiScanSQ, or GAIIX, you must be sure to use the TruSeq Dual Index Sequencing Primer Boxes (Table 2) for all sequencing run types: non-indexed, single-indexed, and dual-indexed. These add on kits are not required if sequencing a Nextera prepared library with the MiSeq System.
- ▶ **Workflow Updates:** Dual indexing now requires 23 additional cycles of sequencing on paired-end flow cells or 16 additional cycles of sequencing on single-read flow cells – eight cycles for the Index 1 (i7) Read, eight cycles for the Index 2 (i5) Read, plus for paired-end flow cells, seven non-imaging, chemistry-only cycles at the beginning of the Index 2 (i5) Read. Due to these additional cycles, there are new recommendations for SBS reagent preparation.



NOTE

A 200-cycle kit will not contain sufficient overfill for a 2x101-cycle dual-indexed run on HiSeq (now 225 cycles). Instead, it is recommended to combine four 50-cycle kits.

- ▶ **Software:** Sequencing Nextera prepared libraries on HiSeq/HiScanSQ requires an upgrade to HCS 1.5/RTA 1.13 to enable the dual indexing workflow. GAIIX requires an upgrade to SCS 2.10/RTA 1.13 as it contains new v10 recipes for dual-indexed sequencing. Software is available for download from MyIllumina.

Analysis Workflow

CASAVA 1.8.2 is required to analyze dual-indexed data generated on the HiSeq, HiScanSQ, or GAIIX instruments. A sample sheet in the new dual-indexed format is also required, and can be generated using IEM. Please refer to the updated *CASAVA User Guide* and Release Notes for more information.

Software Requirements

Software upgrades are required to run Nextera prepared libraries. To download the updated software, go to the following locations on

<https://my.illumina.com/Download/Listing/Software>:

- ▶ **Illumina Experiment Manager sample sheet creator**
- ▶ **cBot software (if needed):** cBot 1.4 Software; the recipe installer can also be found under this listing.
- ▶ **HCS software for HiSeq/HiScanSQ:** HCS 1.5/RTA 1.13 Software
- ▶ **SCS software for Genome Analyzer:** Genome Analyzer SCS 2.10/RTA 1.13 Software
- ▶ **CASAVA:** CASAVA 1.8.2 Software

Supporting Documentation

To download the latest version of the documentation, go to <http://www.illumina.com/support>.

Title	Part #
Nextera DNA Sample Preparation Guide	15027987
Nextera DNA Sample Preparation Experienced User Card	15028285
MiSeq System User Guide	15027617
cBot User Guide	15006165
HiSeq 2000 User Guide	15011190
HiSeq 1000 User Guide	15023355
HiScanSQ User Guide	15015392
GAIIX User Guide	15030966
CASAVA 1.8 User Guide	15011196
Illumina Experiment Manager User Guide	15031335

Technical Assistance

For questions, go to the Support tab on illumina.com. If you do not find the information you need there, contact Illumina Technical Support by email or phone.

Copyright and Trademarks

© 2012 Illumina, Inc. All rights reserved.

Illumina, illuminaDx, BaseSpace, BeadArray, BeadXpress, cBot, CSPro, DASL, DesignStudio, Eco, GAIIX, Genetic Energy, Genome Analyzer, GenomeStudio, GoldenGate, HiScan, HiSeq, Infinium, iSelect, MiSeq, Nextera, Sentrix, SeqMonitor, Solexa, TruSeq, VeraCode, the pumpkin orange color, and the Genetic Energy streaming bases design are trademarks or registered trademarks of Illumina, Inc. All other brands and names contained herein are the property of their respective owners.