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Using Nextera XT DNA Sample Preparation Kits and Enabling Dual Indexing

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

In addition, Illumina® now supports up to 96 Nextera XT indices per lane on all Illumina sequencing systems. If sequencing a Nextera XT 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 XT libraries can be found below.

Table 1   Nextera XT DNA Sample Preparation Kits

Kit Name	Catalog #
Nextera XT DNA Sample Preparation Kit (96 Samples)	FC-131-1096
Nextera XT DNA Sample Preparation Kit (24 Samples)	FC-131-1024
Nextera XT Index Kit (96 Indices, 384 Samples)*	FC-131-1002
Nextera XT Index Kit (24 Indices, 96 Samples)*	FC-131-1001

* Please note that these index kits are required to complete the sample preparation even if not pooling and the corresponding XT Index Kit is required for use with the XT Sample Preparation Kit.

Table 2   Sequencing Primer Kits for Sequencing Nextera XT 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, use the 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 system, and is available for download on MyIllumina. IEM indicates whether the indices chosen provide appropriate sequence diversity to avoid registration failures during sequencing. For the MiSeq system the PCR Amplicon workflow requires a Manifest file to be used with MiSeq Reporter and can be created with IEM. See the IEM User Guide for more information.

Pooling Guidelines

When using less than the full complement of indices in the kit, refer to the Nextera XT 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 XT 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 XT on other Illumina Sequencers

When using the Nextera XT Sample Prep kit for sequencing on HiSeq, HiScanSQ, or GAIIx, there are workflow changes in clustering, sequencing and analysis that require ordering additional kits. Please order the TruSeq Dual Index Sequencing Primer Boxes (Table 2); these kits contain the new primers for both clustering and sequencing. CASAVA is not compatible with Manifest files and therefore is unable to analyze data generated on these instruments.

cBot™ or Cluster Station Workflow

▸ **Primers**: If preparing Nextera XT libraries for cluster generation on HiSeq, HiScanSQ, or GAIIx system, 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. 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. Download the installer from MyIllumina.

Sequencing Workflow

- **Primers**: If sequencing Nextera XT libraries with HiSeq, HiScanSQ, or GAIIx system, 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 XT 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 XT 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

A new PCR Amplicon analysis workflow is available in MiSeq Reporter v1.3 (MSR) for the MiSeq system. The PCR Amplicon workflow requires specifying a manifest - a list of all the targeted regions and their chromosome start and end positions. The manifest specifies regions of interest (ROIs) for the aligner and variant caller, which results in faster analysis times and visualization of results specific for only the ROIs. CASAVA is not compatible with Manifest files. Refer to the IEM User Guide.

Software Requirements

The following software versions are required to run Nextera XT prepared libraries. To download the software, go to the following locations on https://my.illumina.com/Download/Listing/Software:

- **MCS software for MiSeq**: MCS 1.2 Software and Reference Genomes 1.1 or higher
- **Illumina Experiment Manager**: IEM 1.2 or higher
- **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 or higher
- **SCS software for Genome Analyzer**: Genome Analyzer SCS 2.10/RTA 1.13 Software or higher

Supporting Documentation

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

Title	Part #
Nextera XT DNA Sample Preparation Guide	15031942
Nextera XT DNA Sample Preparation Experienced User Card	15031943
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
Illumina Experiment Manager User Guide	15031335

Technical Assistance

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

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