

Sequencing with Nextera Rapid Capture Enrichment Kits

FOR RESEARCH USE ONLY

Nextera® Rapid Capture Enrichment kits are available in various configurations:

Samples	Enrichment Reactions	Plexity
8	8	1
24	8	3
48	8	6
72	8	9
24	2	12
48	4	12
96	8	12
288	24	12

Running On MiSeq®

If preparing Nextera Rapid Capture Enrichment Custom libraries to run on MiSeq, the primers are included in the reagent cartridge. The on-instrument MiSeq Reporter analysis software automatically performs dual index processing.

Running On NextSeq®

If preparing libraries to run on NextSeq, the primers are included in the reagent cartridge. Demultiplexing of sequencing runs is performed with BaseSpace®.

Running On HiSeq® or GAIIx

When clustering libraries on the cBot™ and sequencing on the HiSeq 2000, GAIIx, and in High Output mode on the HiSeq 2500, new primers are required for a non-indexed, single-indexed, or dual-indexed run. Use the TruSeq® Dual Index Sequencing Primer Kit for Paired End runs (catalog # PE-121-1003). The primer kit is good for a single run and contains the required primers for sequencing (HP10, HP11, HP12). These primers are included with HiSeq 2500 rapid run reagent kits.

cBot or Cluster Station Workflow

- ▶ **Primers:** If preparing libraries for cluster generation for HiSeq, HiScanSQ, or GAIIx, use the TruSeq Dual Index Sequencing Primer Box. The Read 1 sequencing primer (HP10) is used in place of the existing sequencing primer (HP6).
- ▶ **Workflow Updates:** Aliquot the new primer mix HP10 into 8-tube strips on the cBot. Because of this, the "PE_Amp_Lin_Block_TubeStripHyb" recipe is required for

paired-end flow cells. 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.

Sequencing Workflow

- ▶ **Primers:** If sequencing libraries with the HiSeq, HiScanSQ™, or GAIIx systems use the TruSeq Dual Index Sequencing Primers HP11 and HP12 to replace HP7 and HP8. These primers are included in the MiSeq System and HiSeq 2500 rapid run reagent kits.
- ▶ **Workflow Updates:** Dual indexing requires 23 additional cycles of sequencing – 8 cycles for the Index 1 (i7) Read, 8 cycles for the Index 2 (i5) Read, plus 7 nonimaging, 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. See the HiSeq 2000, HiSeq 2500, HiScanSQ, or GAIIx guides for reagent kit configurations.
- ▶ **Software:** Sequencing prepared libraries on HiSeq/HiScanSQ requires an upgrade to HCS 1.5/RTA 1.13 or later to enable the dual indexing workflow. GAIIx requires an upgrade to SCS 2.10/RTA 1.13 or later as it contains new v10 recipes for dual-indexed sequencing.
- ▶ **HiSeq Run Parameters:** Choose the appropriate Indexing Option for your run type. Select **Dual Index Sequencing Primer Box** as the Indexing Chemistry to run dual indexing or 8 cycle single indexing chemistry.

Analysis Workflow

BaseSpace can be used to analyze Nextera Rapid Capture Enrichment data generated on all sequencing systems and also enables you to organize samples, libraries, pools, and sequencing runs in a single environment. Refer to BaseSpace help (help.basespace.illumina.com) for more information.

HiSeq Analysis Software is required to analyze Nextera Rapid Capture Enrichment data generated on the HiSeq, HiScanSQ, or GAIIx sequencing systems. A sample sheet in the dual-indexed format is required and can be generated using IEM. Refer to the Illumina Experiment Manager Guide and release notes for more information.

Manifest Files

A manifest file is required to analyze Nextera Rapid Capture Enrichment data. Download manifest files as follows:

- ▶ For the Nextera Rapid Capture Exome and Expanded Exome Enrichment Kits, go to the Illumina website at www.support.illumina.com. Then, go to the Nextera Rapid Capture Exome and Expanded Exome Enrichment Kits support page, then click **Downloads**.
- ▶ For Nextera Rapid Capture Custom Enrichment Kits, after your order ships access all your Nextera Rapid Capture Custom Enrichment Kits manifest files through your MyIllumina account. Follow the links in the **Custom Products** tab to access the Product Files for Nextera Rapid Capture Enrichment.

Software Requirements

Software upgrades might be required to run Nextera Rapid Capture Enrichment prepared libraries. To download the software version required, go to support.illumina.com/sequencing/downloads.ilmn:

- ▶ **BaseSpace**: see basespace.illumina.com
- ▶ **IEM**: Illumina Experiment Manager 1.5 or later
- ▶ **cBot software (if needed)**: 1.4 or later; the recipe installer can also be found under this listing.
- ▶ **HCS software for HiSeq/HiScanSQ**: HCS 1.5/RTA 1.13 or later
- ▶ **SCS software for Genome Analyzer**: Genome Analyzer SCS 2.10/RTA 1.13 or later
- ▶ **HiSeq Analysis Software (HAS)**: any version
- ▶ **MiSeq Control Software (MCS)**: 2.2 or later
- ▶ **MiSeq Reporter Software**: 2.2 or later
- ▶ **NextSeq Control Software (NCS)**

Supporting Documentation

To download the latest version of the documentation, go to support.illumina.com/sequencing/documentation.ilmn.

Document Title	Part #
Illumina Experiment Manager User Guide	15031335
IEM Nextera Quick Reference Card	15037155
Nextera Rapid Capture Enrichment Guide	15037436
Nextera Rapid Capture Enrichment Experienced User Card and Lab Tracking Form	15037437
cBot User Guide	15006165
GAIIX User Guide	15030966

Document Title	Part #
HiScanSQ User Guide	15015392
HiSeq 1000 User Guide	15023355
HiSeq 1500 User Guide	15035788
HiSeq 2000 User Guide	15011190
HiSeq 2500 User Guide	15035786
Sequencing Dual-Indexed Libraries on the HiSeq System User Guide	15032071
HiSeq Analysis Software User Guide	15041353
MiSeq System User Guide	15027617
MiSeq Reporter User Guide	15042295
NextSeq System User Guide	15046563

To order a free printed copy of Nextera Rapid Capture Enrichment documentation, log in to www.illumina.com. Click **Quick Order**, then enter the catalog number:

Document Title	Catalog #
Nextera Rapid Capture Enrichment Reference Guide	FC-140-9001DOC

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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