

Dual Index Sequencing with TruSeq Stranded Total RNA HT Sample Prep

FOR RESEARCH USE ONLY

The TruSeq® Stranded Total RNA HT Sample Preparation Kits provide a streamlined library preparation workflow using a RNA Adapter Plate (RAP) containing 96 uniquely dual-indexed adapter combinations designed for manual or automated preparation of 96 uniquely dual-indexed samples.

Illumina® supports up to 96 TruSeq Stranded Total RNA HT indices per lane on all Illumina sequencing systems. If sequencing a TruSeq Stranded Total RNA HT prepared library on a HiScanSQ™ or GAIIX system on a single-read flow cell, or HiSeq® system on a v3 single-read flow cell, the TruSeq Dual Index Sequencing Primer Box, Single Read (single use box) (catalog# FC-121-1003) is required to enable dual indexing. An accessory sequencing primer box is not required for dual-indexed sequencing of TruSeq Stranded Total RNA HT libraries on paired-end flow cells for any Illumina sequencing platform or for HiSeq 2500 Rapid run two lane single-read flow cells.

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 also indicates whether the indices chosen provide appropriate sequence diversity to avoid registration failures during sequencing.

Pooling Guidelines

The *TruSeq Sample Preparation Pooling Guide* contains complete details on the RAP index sequences, layout, and handling, as well as pooling guidelines when using less than the full complement of indices.

Using TruSeq on MiSeq

If preparing TruSeq Stranded Total RNA HT libraries to run on MiSeq, the primers are included in the reagent cartridge and the on-instrument analysis software (MiSeq Reporter) automatically performs dual index processing.

Using TruSeq on other Illumina Sequencers

When using a TruSeq Stranded Total RNA HT Sample Prep Kit for dual-indexed sequencing on single-read flow cells for HiSeq (except for Rapid run single-read flow cells), HiScanSQ, or GAIIX, there are workflow changes in clustering, sequencing

and analysis that require ordering the TruSeq Dual Index Sequencing Primer Box, Single Read. Sequencing libraries prepared with a TruSeq Stranded Total RNA HT Sample Prep Kit on paired-end flow cells for HiSeq, HiScanSQ or GAIIX do not require workflow changes or additional boxes.

cBot™ or Cluster Station Workflow

- ▶ **Primers:** If preparing TruSeq Stranded Total RNA HT libraries for cluster generation on single-read flow cells for HiSeq, HiScanSQ, or GAIIX, you must use the TruSeq Dual Index Sequencing Primer Box, Single Read for dual-indexed runs. The TruSeq Dual Index Sequencing primers (HP10, HP11, and HP12) will be used in place of the existing sequencing primers (HP6, HP7, and HP8).
- ▶ **Workflow Updates:** Dual indexing on single-read flow cells now requires aliquotting the new primer mixes HP10 into eight-tube strips on cBot. Because of this, the “SR_Amp_Lin_Block_TubeStripHyb” recipe is required for single read 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. Download the installer from MyIllumina.

Sequencing Workflow

- ▶ **Primers:** If sequencing TruSeq Stranded Total RNA HT libraries with the HiSeq, HiScanSQ, or GAIIX system on single-read flow cells, use the TruSeq Dual Index Sequencing Primer Box, Single Read for dual-indexed runs. This add-on box is not required if sequencing a TruSeq Stranded Total RNA HT prepared library with the MiSeq System, on paired-end flow cells for HiSeq, HiScanSQ, GAIIX, or on HiSeq Rapid run flow cells.
- ▶ **Workflow Updates:** Dual indexing on paired-end and single-read flow cells 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 TruSeq Stranded Total RNA HT 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. Software is available for download from MyIllumina.
- ▶ **HiSeq Run Parameters:** Choose the appropriate Indexing Option for your run type. Select **Dual Index Sequencing Primer Box** as the Indexing Chemistry in order to run dual indexing or eight cycle single indexing chemistry, even if the primers contained in the box are not being used.

Analysis Workflow

CASAVA 1.8.2 is required to analyze TruSeq Stranded Total RNA HT data generated on the HiSeq, HiScanSQ, or GAIIX systems. A sample sheet in the new dual-indexed format is required and can be generated using IEM. Refer to the CASAVA 1.8.2 User Guide and release notes for more information.

Software Requirements

Software upgrades might be required to run TruSeq Stranded Total RNA HT prepared libraries. To download the software version required for your Illumina platform, go to support.illumina.com/sequencing/downloads.ilmn:

- ▶ **IEM:** IEM 1.2 software or later
- ▶ **cBot software (if needed):** cBot 1.4 software or later; the recipe installer can also be found under this listing.
- ▶ **HCS software for HiSeq/HiScanSQ:** HCS 1.5/RTA 1.13 software or later
- ▶ **SCS software for Genome Analyzer:** Genome Analyzer SCS 2.10/RTA 1.13 software or later
- ▶ **HiSeq Analysis Software (HAS):** any version or **CASAVA:** CASAVA 1.8.2 software or later
- ▶ **MiSeq Control Software (MCS):** 2.2 software or later
- ▶ **MiSeq Reporter Software (MSR):** 2.2 software or later

Supporting Documentation and Training

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

Document Title	Part #
CASAVA 1.8 User Guide	15011196
cBot User Guide	15006165
GAIIX User Guide	15030966
HiScanSQ User Guide	15015392
HiSeq 1000 User Guide	15023355
HiSeq 1500 User Guide	15035788
HiSeq 2000 User Guide	15011190
HiSeq 2500 User Guide	15035786
Illumina Experiment Manager User Guide	15031335
IEM TruSeq DNA, RNA, or ChIP Quick Reference Card	15037152
MiSeq System User Guide	15027617
Sequencing Dual-Indexed Libraries on the HiSeq System User Guide	15032071
TruSeq Stranded Total RNA Sample Preparation Guide	15031048
TruSeq Stranded Total RNA Sample Preparation LS Experienced User Card and Lab Tracking Form	15031060
TruSeq Stranded Total RNA Sample Preparation HS Experienced User Card and Lab Tracking Form	15031059

To access TruSeq Stranded Total RNA Sample Preparation online training courses, go to www.illumina.com/support/training/sequencing_training.ilmn.

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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