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Dual Index Sequencing with TruSeq HT Library Prep

FOR RESEARCH USE ONLY

TruSeq® HT library prep kits provide a streamlined library preparation workflow using an adapter plate that contains 96 uniquely dual-indexed adapter combinations designed for manual or automated preparation of 96 uniquely dual-indexed samples. Illumina® supports up to 96 TruSeq HT indexes per lane on all Illumina sequencing systems.

Sample Sheet Preparation

To ensure proper index combinations for pooled libraries with dual-indexed experiments, use the Illumina Experiment Manager (IEM) or BaseSpace before performing library prep.

- **IEM**—A desktop application that helps you create a sample sheet for dual-indexed sequencing on the HiSeq®, HiScanSQ™, GAIIx, MiSeq®, or NextSeq® systems. You can download IEM from the Illumina website and install it on any Windows computer. IEM confirms whether selected indexes provide appropriate sequence diversity to avoid registration failures during sequencing. See the *Illumina Experiment Manager Guide* for more information.
- **BaseSpace**®—Provides tools to organize samples and sequencing runs, and to analyze TruSeq HT data generated on all sequencing systems. See the *BaseSpace User Guide* for more information.

Pooling Guidelines

The *TruSeq Library Prep Pooling Guide* contains complete details on the adapter plate index sequences, layout, and handling, as well as pooling guidelines when using less than the full complement of indexes.

Sequencing Primers and Reagents

Dual-indexed sequencing is supported on HiSeq 2500, 2000, and 1500, GAIIx, MiSeq, and NextSeq systems. Illumina kits contain sequencing primers and reagents required for dual-indexed sequencing, except for TruSeq v3 reagent kits on HiSeq systems and TruSeq v5 reagent kits on the GAIIx.

- **HiSeq 2500, 2000, 1500**—To perform dual-indexed sequencing on a single-read flow cell using TruSeq v3 chemistry, you need an additional sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box (Single Read), catalog # FC-121-1003. This kit contains HP9, which is the Index 2 Read sequencing primer that is compatible with the single-read flow cell. All other kits that are packaged with a single-read flow cell contain

HP9, including HiSeq v4 kits and any kits for rapid run sequencing. The TruSeq Dual Index Sequencing Primer Box is not required when performing dual-indexed sequencing of TruSeq HT libraries on a paired-end flow cell.

- **GAIIx**—To perform dual-indexed sequencing on a single-read flow cell using TruSeq v5 chemistry, you need an additional sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box (Single Read), catalog # FC-121-1003. This kit contains HP9, which is the Index 2 Read sequencing primer that is compatible with the single-read flow cell.
- **MiSeq and NextSeq**—All kits include sequencing primers and reagents for dual-indexed sequencing. No additional reagents are required.

cBot™ Workflow

Follow the standard cluster generation workflow on the cBot. No changes to the workflow are required for clustering TruSeq HT libraries.

Sequencing Workflow

Primers—If you are using the TruSeq v3 chemistry (HiSeq systems) or TruSeq v5 chemistry (GAIIx) on a single-read flow cell, you need HP9 from the TruSeq Dual Index Sequencing Primer Box (Single Read), catalog # FC-121-1003. All other reagent kits contain the required sequencing primers.

Workflow—Dual-indexed sequencing performs additional cycles for the index reads and follows a similar workflow for each instrument with the following exceptions:

- To perform the maximum number of cycles with 2 index reads using the TruSeq v3 chemistry (HiSeq systems) or TruSeq v5 chemistry (GAIIx), use multiple SBS kits to provide sufficient reagents.
- To perform dual-indexed sequencing on a single-read flow cell using TruSeq v3 chemistry (HiSeq systems) or TruSeq v5 chemistry (GAIIx), load HP9 from the TruSeq Dual Index Sequencing Primer Box (Single Read), catalog # FC-121-1003.

For more information about kit requirements, see the documentation for the kits that you are using. For an overview of the dual-indexing workflow, see the *Indexed Sequencing Overview Guide*.

Analysis Workflow

BaseSpace can be used to analyze TruSeq HT data generated on all sequencing systems and enables you to organize samples and sequencing runs in a single environment. See the *BaseSpace User Guide* for more information.

Software Requirements

The following software versions are required for your Illumina instrument to sequence TruSeq HT libraries. To download the software, go to support.illumina.com/sequencing/downloads.html:

- **IEM**—v1.2 or later
- **BaseSpace**—Any version
- **cBot software**—v1.4 or later. The recipe installer is also available on the Downloads tab on the cBot support page.
- **Sequencing Control Software for GAIIx**—v2.10 or later
- **HiSeq Control Software (HCS)**—v1.5 or later
- **MiSeq Control Software (MCS)**—v2.2 or later
- **MiSeq Reporter Software**—v2.2 or later
- **NextSeq Control Software (NCS)**—v1.2 or later

Supporting Documentation

The following documentation, as well as kit-specific documentation, are available for download from the Illumina website. To download documentation, go to support.illumina.com/documentation.html.

Document Title	Part #
Illumina Experiment Manager Guide	15031335
IEM TruSeq DNA, RNA, or ChIP Quick Reference Card	15037152
BaseSpace User Guide	15044182
TruSeq Library Prep Pooling Guide	15042173
cBot User Guide	15006165
GAIIx User Guide	15030966
HiScanSQ User Guide	15015392
HiSeq 1500 User Guide	15035788
HiSeq 2000 User Guide	15011190
HiSeq 2500 User Guide	15035786
MiSeq System User Guide	15027617
NextSeq 500 System User Guide	15046563
Indexed Sequencing Overview Guide	15057455

Training

To access TruSeq library prep online training courses, go to support.illumina.com/training/sequencing_training.html.

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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