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Sequencing and Analysis with TruSeq Synthetic Long-Read DNA Library Prep

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The Illumina® TruSeq® Synthetic Long-Read DNA Library Prep Kit provides a library prep workflow for Human Genome Phasing to identifying co-inherited alleles and phasing of de novo mutations, as well as synthetic long-reads for genome finishing, metagenomics, and de novo sequencing. The informatics for these two applications is exclusively enabled by two BaseSpace® apps: TruSeq Long-Read Assembly and TruSeq Phasing Analysis. Illumina supports sequencing of TruSeq Synthetic Long-Read DNA libraries on HiSeq® 1000, 2000, 1500, and 2500. The sequencing run must be streamed to BaseSpace to utilize the corresponding app for data analysis.

Sample Sheet Preparation

A custom sample sheet is required before starting a sequencing run with libraries prepared using the TruSeq Synthetic Long-Read DNA Library Prep Kit. This custom sample sheet enables correct identification of the sequenced library using the TruSeq Synthetic Long-Read apps in BaseSpace. This custom sample sheet can be created using Illumina Experiment Manager (IEM) v1.8 or later by selecting the **HiSeq FASTQ Only** and **TruSeq Synthetic Long-Read DNA** sample prep kit options.

cBot™ Workflow

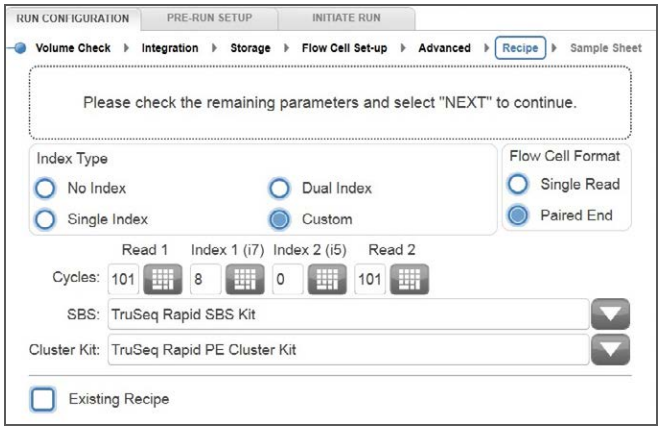
The TruSeq Synthetic Long-Read DNA workflow on the HiSeq is single indexing. However, TruSeq Synthetic Long-Read DNA Library Prep is Nextera® based, therefore use the TruSeq Dual Index Sequencing Primer Box, Paired-End (Catalog # PE-121-1003) when performing cluster generation with the TruSeq PE Cluster Kit v3. Use the TruSeq Dual Index Sequencing Primer (HP10) in place of the existing Read 1 sequencing primer (HP6). If you are using TruSeq PE Rapid Cluster kit or HiSeq PE Cluster Kit v4, no additional primers are required. For required software version and recipes for each cluster kit, see the *cBot User Guide (part # 15006165)*.

Sequencing Workflow

- **Primers**: If sequencing TruSeq Synthetic Long-Read DNA libraries with the TruSeq PE Cluster Kit v3, use the sequencing primers HP11 and HP12 within the TruSeq Dual Index Sequencing Primer Box, Paired-End (catalog # PE-121-1003). If using TruSeq PE Rapid Cluster kit or TruSeq PE Cluster Kit v4, no additional primers are required.
- **HiSeq Software**: Sequencing TruSeq Synthetic Long-Read DNA libraries on HiSeq requires HCS 2.0 or later and a connection to BaseSpace. Software is available for download from MyIllumina.
- **Workflow**: Sequencing TruSeq Synthetic Long-Read DNA libraries requires paired-end reads of 100–126 (depending on the SBS kit) and a single eight-cycle Index 1 (i7) Read. A different sequencing workflow is recommended for each SBS kit to accommodate reagent usage during run.

Kit	Catalog #	Recipe
TruSeq SBS Kit v3 – 200 cycles	FC-401-3001	100 + 8 (Index 1) + 100
TruSeq Rapid SBS Kit – 200 cycles	FC-401-4001	101 + 8 (Index 1) + 101
HiSeq SBS Kit v4 – 250 cycles	FC-401-4001	101 + 8 (Index 1) + 101 or 126 + 8 (Index 1) + 126

- **HiSeq Run Parameters**: Select **Use BaseSpace** for storage and analysis of TruSeq Synthetic Long-Read DNA libraries. On the recipe screen, select **Custom**, then select **Flow Cell Format: Paired End**, and then select the number of cycles for each read.



Analysis Workflow

TruSeq Synthetic Long-Read apps on BaseSpace are required to analyze TruSeq Synthetic Long-Read DNA library data generated on the HiSeq system. A sample sheet generated by IEM v1.8 or higher is required for analysis in BaseSpace. For more information, see the *TruSeq Long-Read Assembly User Guide (part # 15055850)* or *TruSeq Phasing Analysis User Guide (part # 15055852)*.

Software Requirements

Software upgrades might be required to run TruSeq Synthetic Long-Read DNA libraries. To download the software version required for your Illumina instrument, go to support.illumina.com/sequencing/downloads.ilmn:

- **IEM**: IEM 1.8 or later
- **cBot software (if needed)**: cBot 1.4 software or later (v3) or cBot 2.0.1 or later (v4); the recipe installer can also be found under this listing.
- **HCS software for HiSeq**: HCS 2.0/RTA 1.17 or later

Supporting Documentation and Training

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

Document Title	Part #
BaseSpace User Guide	15044182
cBot User Guide	15006165
HiSeq 1000 User Guide	15023355
HiSeq 1500 User Guide	15035788
HiSeq 2000 User Guide	15011190
HiSeq 2500 User Guide	15035786
IEM TruSeq Synthetic Long-Read DNA Quick Reference Card	15056316
Illumina Experiment Manager User Guide	15031335
TruSeq Long-Read Assembly User Guide	15055850
TruSeq Phasing Analysis User Guide	15055852
TruSeq Synthetic Long-Read DNA Library Prep Guide	15047264
TruSeq Synthetic Long-Read DNA Library Prep Experienced User Card and Lab Tracking Form	15047265

View TruSeq Synthetic Long-Read DNA Library Prep online training courses on the Illumina website:

1. Go to support.illumina.com.
2. Select **Sequencing**.
3. Select **Kits & Reagents**.
4. Select **TruSeq Synthetic Long-Read DNA Library Prep Kit** to open the support page.
5. Select **Training**.

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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