



## User Guide

# Guidelines for uploading Illumina<sup>®</sup> instrument run data to BaseSpace<sup>™</sup> Sequence Hub for analysis in the UST TELL-Seq<sup>™</sup> app

For Research Use Only. Not for use in diagnostic procedures.

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## **Background**

TELL-Seq™ libraries from Universal Sequencing Technology Corporation (UST) are compatible with Illumina® sequencing instruments and run data can be uploaded to BaseSpace™ Sequence Hub (BSSH) for use with the TELL-Seq app for secondary analysis. This document provides guidelines for how to upload instrument data to BSSH for use as app input and for correct demultiplexing.

## **Sample sheet and FASTQ generation considerations**

TELL-Seq libraries contain an 18 base unique molecular identifier (UMI) in index 1 and an 8 base barcode used for demultiplexing in index 2. To correctly process the UMI data in index 1 and demultiplex the libraries based on index 2, BCL Convert is required; bcl2fastq or on-instrument solutions such as Local Run Manager (LRM) do not have the necessary UMI handling functions. The BCL Convert app in BSSH requires the use of a v2 sample sheet. Examples of v2 sample sheets that will process the TELL-Seq UMI are provided (See Appendix) and the relevant sections explained below.

## **Sample sheet index 2 orientation**

When selecting the correct sample sheet template, use either the Forward Strand template or the Reverse Complement template depending on the instrument type:

- Forward Strand template for MiSeq™, HiSeq™ 2000/2500, NovaSeq™ 6000 v1 reagents, NextSeq™ 1000/2000)
  - Note: The NextSeq 1000/2000 uses the Reverse Complement indexing workflow, however index 2 is automatically reverse complemented by BCL Convert for this platform.
- Reverse Complement template for iSeq™ 100, MiniSeq™, NextSeq™ 500/550, HiSeq 3000/4000, NovaSeq 6000 with v1.5 reagents
- See [Indexed Sequencing on Illumina Systems](#) for more details.

## **V2 Sample sheet information**

[Header]	
FileFormatVersion	2
RunName	UST_TellSeq_Forward
InstrumentPlatform	NextSeq1k2k
InstrumentType	NextSeq2000

The Header section contains information about the run. The RunName should contain letters, numbers, dashes, underscores, and spaces, but no other special characters. The RunName value is used to name the run in BSSH and will also be used to name the resulting project after FASTQ generation if a project name is not provided in the Cloud\_Data section.

[Reads]	
Read1Cycles	146
Read2Cycles	146
Index1Cycles	18
Index2Cycles	8

The Reads section is required to have the cycle numbers performed listed for each read, including the index cycles.

[BCLConvert_Settings]	
SoftwareVersion	3.8.4
AdapterRead1	CTGTCTCTTATACACATCT
AdapterRead2	CTGTCTCTTATACACATCT
OverrideCycles	Y146;U18;I8;Y146

The BCLConvert\_Settings section lists the specific version of the BCLConvert pipeline being used; Adapter trimming information, and the OverrideCycles information. OverrideCycles is used to inform BCL Convert how to process each read, either as a data read, UMI, or index. The cycle number values for each read in OverrideCycles must match the cycle number values in the Reads section. For TELL-Seq libraries the index 1 cycles are designated as UMI by the U18 value in OverrideCycles.

[BCLConvert_Data]	
Sample_ID	Index2
UST-TellSeq-1	TGAACCTT
UST-TellSeq-2	TGCTAAGT
UST-TellSeq-3	TGTTCTCT
UST-TellSeq-4	TAAGACAC
UST-TellSeq-5	CTAATCGA
UST-TellSeq-6	CTAGAACA
UST-TellSeq-7	TAAGTTCC
UST-TellSeq-8	TAGACCTA

The BCLConvert\_Data section contains the Sample\_ID and index values. The Sample\_ID values can contain only letters, numbers, dashes, and underscores, and no spaces or special characters. The default first index column (Index) should be deleted as it is not used for analyzing TELL-Seq libraries. The second index column (Index2) contains the barcode sequence used for demultiplexing and needs to have the sequence in the correct orientation, as described above. No other columns can be added to this section, including Sample\_Name or Description; adding any other columns to BCLConvert\_Data will result in an analysis error.

[Cloud_Settings]	
BsshApp	illumina-inc.bcl-convert.1.2.1

The Cloud\_Settings section must contain the BsshApp and illumina-inc.bcl-convert.### values to automatically launch BCL Convert for FASTQ generation instead of bcl2fastq after the run upload completes. If this section is omitted bcl2fastq will be used for FASTQ generation by default, and analysis

will fail. Note that the numerical values specify the BCL Convert app release number (different than the pipeline version). At the time of writing this guide the current app version is 1.2.1, but the current value may change with future releases. Please confirm the current app version number in BSSH before uploading data.

[Cloud_Data]
Sample_ID
UST-TellSeq-1
UST-TellSeq-2
UST-TellSeq-3
UST-TellSeq-4
UST-TellSeq-5
UST-TellSeq-6
UST-TellSeq-7
UST-TellSeq-8

The Cloud\_Data section must minimally contain a Sample\_ID column, and the values in that column must match the values used in the Sample\_ID column in the BCLConvert\_Data section. Omitting this section or having mismatching Sample\_ID values will result in an analysis error. Sample\_ID values should be unique for BSSH use to avoid unintended data aggregation.

### **Instrument run uploads to BSSH**

For instruments without local analysis options, see the system guides for information on uploading run data to BSSH.

- [HiSeq 2500](#): Select Sequence, New Run, then select BaseSpace for storage and analysis and select the sample sheet to upload.
- [HiSeq 3000/4000](#): Select Sequence, Connect to BaseSpace for storage and analysis and select the sample sheet to upload.
- [NovaSeq 6000](#): Configure BaseSpace Sequence Hub from the Settings menu for run monitoring and storage, and select the sample sheet during run setup.

For most instruments with local analysis options, the run should be performed in manual mode, connecting to BSSH. Instructions specific to MiSeq users are below.

- [iSeq 100](#): Configure the instrument to upload to BSSH and run in Manual mode in the Settings menu and select the sample sheet during run setup.
- [MiniSeq](#) (control software version 2 only): Select Sequence, Manual mode, and Use BaseSpace Sequence Hub for run monitoring and storage and select the sample sheet for upload. For MiniSeq control software v1 users, see the instructions for run data uploads with BaseSpace CLI below.
- [NextSeq 500/550](#) (control software version 4 only): Select Sequence, Manual mode, and Use BaseSpace Sequence Hub for run monitoring and storage and select the sample sheet for

upload. For NextSeq control software v2 users, see the instructions for run data uploads with BaseSpace CLI below.

- [NextSeq 1000/2000](#): Configure the run mode to standalone, and select “Proactive, Run Monitoring and Storage” after starting the run setup. Select the sample sheet for upload.

### **MiSeq run uploads to BSSH**

The MiSeq does not have a manual/standalone mode that allows you to upload data to BSSH. A run must be configured using either a placeholder v1 sample sheet or LRM run setup, depending on the system version. After the run is uploaded, the data can be manually analyzed using the BCL Convert app. The run data can also be saved locally and then uploaded to BSSH using BaseSpace CLI with a v2 sample sheet as described in the next section.

- [MiSeq Control Software v2/MiSeq Reporter](#): Create a temporary sample sheet with [Illumina Experiment Manager](#) (MiSeq, Other, FASTQ Only) that is dual indexed and configured for the appropriate data read cycles with one sample; any indexes can be selected at this step. After saving the file, edit the first index to have 18 Ns, and the second index to have 8 Ns. Save the CSV file and use it to configure the run setup on the instrument.
- [MiSeq Control Software v3/Local Run Manager v2 \(LRM\)](#):
  - Option 1: Create a run in the Generate FASTQ module in LRM and select “Custom” as the kit type. Enter in the appropriate data read cycles and enter one sample in the data section. Enter 18Ns for the first index 1 and 8 Ns for the second index. Start the run in LRM mode.
  - Option 2: Create a sample sheet as described in the MiSeq Reporter section above and start the run in Sample Sheet mode.
- [MiSeq Control Software v4/LRM v3](#):
  - Option 1: Create a run in the Generate FASTQ module in LRM. Select “Custom” as the Library Prep Kit type, and the Index Kit will automatically be set as Custom. Enter in the appropriate data read cycles and enter one sample in the data section. Enter 18Ns for the first index 1 and 8 Ns for the second index. Start the run in LRM mode.
  - Option 2: Download a sample sheet template from the [LRM Generate FASTQ v3.0 support site](#). Use one sample in the Data section with 18 Ns for the first index and 8 Ns for the second index. Start the run in Sample Sheet mode.

### **Run data uploads with BaseSpace CLI**

Run data not streamed to BSSH during run setup can be uploaded after the run is complete using [BaseSpace CLI](#); note that using BaseSpace CLI requires familiarity with working in a command line environment, and builds are available for Linux, Windows, and Mac OS X.

- Use the v2 sample sheet template appropriate for the instrument type from which the data were generated. Save the sample sheet as SampleSheet.csv and copy it to the top level of the run folder.
- Instructions for how to use BaseSpace CLI to upload run data are available in a [support bulletin](#), along with a [video walkthrough](#).

- FASTQ generation will start automatically once the upload is complete as long as the SampleSheet.csv file is present in the run folder.

### **Manually analyzing run data with the BCL Convert app**

In order to manually analyze a run with a v2 sample sheet, or to analyze a MiSeq run uploaded with a v1 sample sheet, launch the [BCL Convert](#) app in BSSH. To perform analysis with sample sheet different than the file uploaded with the run, upload the new sample sheet to a project first using the [Import](#) option and the Misc/Other file type. Launch the BCL Convert app and select the run folder along with the uploaded sample sheet as inputs and launch the app.

### **Analyzing TELL-Seq data in BSSH**

Launch the [TELL-Seq Data Analysis](#) app from the BSSH app page:

- Select the output project for the TELL-Seq analysis.
- Select the pipeline configuration, and optionally a reference FASTA.
- Select the uploaded run folder for input. Enter the UST TELL-Seq sample index names as a comma separated list and list the genome if a reference FASTA is used.
  - Starting analysis from Biosample FASTQs is not currently supported but is planned for a future update.
- Select Launch Application to begin analysis.

# Appendix

## Example V2 Sample Sheet for TELL-Seq Run

### 1. Forward Strain Index 2

[Header]	
FileFormatVersion	2
RunName	UST_TellSeq_Forward
InstrumentPlatform	NextSeq1k2k
InstrumentType	NextSeq2000
[Reads]	
Read1Cycles	146
Read2Cycles	146
Index1Cycles	18
Index2Cycles	8
[BCLConvert_Settings]	
SoftwareVersion	3.8.4
AdapterRead1	CTGTCTCTTATACACATCT
AdapterRead2	CTGTCTCTTATACACATCT
OverrideCycles	Y146;U18;I8;Y146
[BCLConvert_Data]	
Sample_ID	Index2
UST-TellSeq-1	TGAACCTT
UST-TellSeq-2	TGCTAAGT
UST-TellSeq-3	TGTTCTCT
UST-TellSeq-4	TAAGACAC
UST-TellSeq-5	CTAATCGA
UST-TellSeq-6	CTAGAACA
UST-TellSeq-7	TAAGTTCC
UST-TellSeq-8	TAGACCTA
[Cloud_Settings]	
BsshApp	illumina-inc.bcl-convert.1.2.1

[Cloud\_Data]  
Sample\_ID  
UST-TellSeq-1  
UST-TellSeq-2  
UST-TellSeq-3  
UST-TellSeq-4  
UST-TellSeq-5  
UST-TellSeq-6  
UST-TellSeq-7  
UST-TellSeq-8

## 2. Reverse Complement Strain Index 2

[Header]  
FileFormatVersion                    2  
RunName                                UST\_TellSeq\_RC  
InstrumentPlatform                    MiniSeq  
InstrumentType                        MiniSeq

[Reads]  
Read1Cycles                            146  
Read2Cycles                            146  
Index1Cycles                           18  
Index2Cycles                           8

[BCLConvert\_Settings]  
SoftwareVersion                       3.8.4  
AdapterRead1                         CTGTCTCTTATACACATCT  
AdapterRead2                         CTGTCTCTTATACACATCT  
OverrideCycles                        Y146;U18;I8;Y146

[BCLConvert\_Data]  
Sample\_ID                              Index2  
UST-TellSeq-1                         AAGGTTCA  
UST-TellSeq-2                         ACTTAGCA  
UST-TellSeq-3                         AGAGAACA  
UST-TellSeq-4                         GTGTCTTA  
UST-TellSeq-5                         TCGATTAG

UST-TellSeq-6  
UST-TellSeq-7  
UST-TellSeq-8

TGTTCTAG  
GGAACTTA  
TAGGTCTA

[Cloud\_Settings]

BsshApp

illumina-inc.bcl-  
convert.1.2.1

[Cloud\_Data]

Sample\_ID  
UST-TellSeq-1  
UST-TellSeq-2  
UST-TellSeq-3  
UST-TellSeq-4  
UST-TellSeq-5  
UST-TellSeq-6  
UST-TellSeq-7  
UST-TellSeq-8

This document is proprietary to Universal Sequencing Technology Corporation and is intended solely for the use of its customers in connection with the use of the products described herein and for no other purposes.

The instructions in this document must be followed precisely by properly trained personnel to ensure the proper and safe use of the TELL-Seq kit.

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**Revision History**

Document #	Version	DCR Reference and Comment
100026-USG	1.0	DCR-210082 Initial Release