TELL-Seq™ Library Sequencing User Guide

For all Illumina® sequencing systems except iSeq 100

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

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1. Introduction

This protocol explains how to run any indexed paired-end TELL-Seq™ libraries on an Illumina® sequencing system.

A TELL-Seq library† requires custom sequencing primers for any Illumina sequencing systems and contains an 18-base index 1 sequence used as the molecular barcode for linked reads, which must be sequenced completely.

2. Kit Contents

TELL-Seq™ Illumina® Sequencing Primer Kit **(PN 100004)**

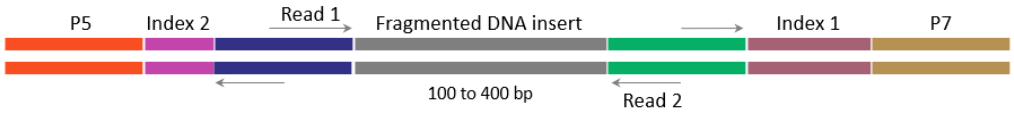
Component Name	Cap Color	Concentration	Volume (µL)	Storage Temperature
Read 1 Primer	CAP Black	100µM	50	-25°C to -15°C
Read 2 Primer	CAP White	100µM	50	-25°C to -15°C
Index 1 Primer	CAP Red	100µM	50	-25°C to -15°C
Index 2 Primer	CAP Yellow	100µM	50	-25°C to -15°C

PRO TIP: The minimum number of sequencing runs that can be performed using the amount of sequencing primers provided vary based on the sequencing system (see below).

Sequencing System	Number of runs	Is custom Index 2 Primer required?
NovaSeq	4	v1 reagent: No; v1.5 reagent: Yes
HiSeq 3000/4000	2	Yes
HiSeq 2000/2500	5	No
NextSeq	8	Yes
MiSeq	16	No
MiniSeq	8	Yes

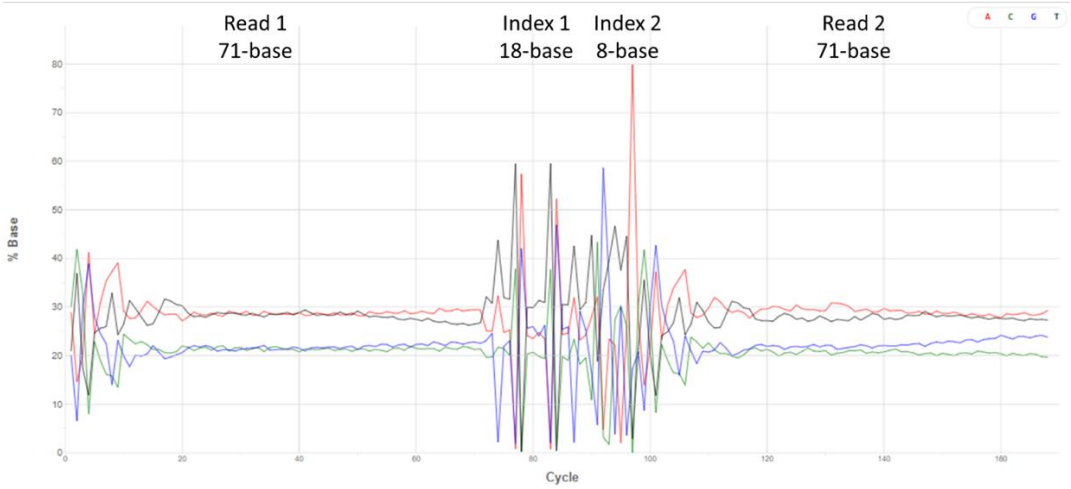
† Patent pending.

3. TELL-Seq™ Library Structure and Sequencing Scheme



Index 1 (i.e., I7 index) contains **18-base** TELL Bead sequences, which MUST be sequenced completely. Index 2 (i.e., I5 index) contains 8-base sample index primer sequences used in library amplification. Paired end sequencing is preferred. Minimal read length requirement is 2x96; Maximum read length requirement is 2x150.

Example of Illumina® Sequencing % Base by Cycle Chart



4. Illumina® Sequencing Guideline

1. Dilute TELL-Seq library according to Illumina® sequencing platform specific concentration and volume.
2. Libraries may be pooled together for sequencing when different multiplex primers are used in the library amplification step.
3. Custom sequencing primers are required to sequence TELL-Seq libraries and provided in the TELL-Seq Illumina Sequencing Primer Kit.
4. These custom sequencing primers can be loaded into the specified wells for custom primers. Alternatively, they can also be loaded into corresponding standard Illumina® sequencing primer wells when an Illumina® PhiX control library is spiked in a sequencing run.

5. Custom Index 2 primer is only needed when multiple TELL-Seq libraries with different multiplex primers are pooled for sequencing and when a sequencer requires an i5 index sequencing primer. **For MiSeq, HiSeq 2000/2500 and NovaSeq v1 reagent, custom Index 2 Primer is not required.**
6. The minimum number of sequencing runs can be performed using the amount of sequencing primers provided are varied based on the sequencing system.

Sequencing System	Number of runs	Is custom Index 2 Primer required?
NovaSeq	4	v1 reagent: No; v1.5 reagent: Yes
HiSeq 3000/4000	2	Yes
HiSeq 2000/2500	5	No
NextSeq	8	Yes
MiSeq	16	No
MiniSeq	8	Yes

5. Example Sample Sheet for MiSeq System

Below is an example sample sheet for a 2x146 PE run with 18-cycle index 1 (i.e., I7 index) and 8-cycle index 2 (i.e., I5 index) sequencing using custom sequencing primers for Read 1, Index 1 and Read 2 which will be **loaded into custom sequencing primer wells**. Because MiSeq uses a P5 primer on the flow cell surface as the sequencing primer for Index 2 reads, TELL-Seq custom Index 2 primer is not required for an MiSeq system. The demultiplexing of any pooled libraries will base on the sample index (i.e., index 2). It will be processed by TELL-read analysis pipeline separately after the sequencing run completes.

[Header]										
IEMFileVersion	5									
Experiment Name	UST209_11.14.19									
Date	1/1/2020									
Workflow	GenerateFASTQ									
Application	FASTQ Only									
Instrument Type	MiSeq									
Assay	AmpliSeq Library PLUS for Illumina									
Index Adapters	AmpliSeq CD Indexes (384)									
Chemistry	Amplicon									
[Reads]										
146										
146										
[Settings]										
CustomRead1PrimerMix	C1									
CustomIndexPrimerMix	C2									
CustomRead2PrimerMix	C3									
ReverseComplement	0									
Adapter	CTGTCTCTTATACACATCT									
[Data]										
Sample_ID	Sample_Plate	Sample_Well	Index_Plate	Index_Plate_Well	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project	Description
Sample1			B	A01	7027	NNNNNNNNNNNNNNNNNN	5001	NNNNNNNN		

Total 18 Ns

Total 8 Ns

6. Illumina® Sequencing Read Length Recommendation

1. Paired end sequencing is recommended.
2. TELL-Seq library Index 1 is 18-base, Index 2 is 8-base. There are total 26-base for both indexes compared to total 16-base for standard Illumina dual index. The extra 10-cycle required for sequencing TELL-Seq library index need to be deducted from read 1 and read 2 sequencing cycles evenly. Since Illumina sequencing reagent guarantee 2 extra cycles, 4-cycle for read 1 and 4-cycle for read 2 need to be deducted respectively. Recommended sequencing length are 2×96 PE to 2×146 PE for dual index run; 2x100 PE to 2x150 PE for a single sample run without need for Index 2 read.

7. Sequencing Depth Consideration

1. Adequate sequencing depth is required to get enough TELL Bead coverage. The more TELL Beads used in library amplification to generate a TELL-Seq library, the more sequencing reads will be required to get the desired sequencing depth. However, the fewer TELL Beads used for library amplification, the lower the library complexity will be, which may lead to a higher duplication rate of sequencing reads. The balance between TELL Beads used and TELL-Seq library complexity required may depend on the genome size and application.
2. For *de novo* assembly application, at least 50x genome coverage of the sample is recommended in general. However, lower sequencing coverage may also be enough depending on the amount of TELL Beads used for library amplification and TELL-Seq library complexity.

8. Library Multiplex Primer Index Sequences (i.e. Index 2 Read) for sample sheet of NovaSeq v1, MiSeq and HiSeq2000/2500

Library Multiplex Primer	Index Sequence
T501	TGAACCTT
T502	TGCTAAGT
T503	TGTTCTCT
T504	TAAGACAC
T505	CTAATCGA
T506	CTAGAACA
T507	TAAGTTCC
T508	TAGACCTA
T509	CATCCGAA
T510	TTATGAGT
T511	AGAGGCGC
T512	TAGCCGCG
T513	ACGAATAA
T514	TTCGTAGG
T515	GATCTGCT
T516	CGCTCCGC
T517	AGGCTATA
T518	GCCTCTAT
T519	AGGATAGG
T520	TCAGAGCC
T521	CTTCGCCT
T522	TAAGATTA
T523	AGTAAGTA
T524	GACTTCCT

9. Appendix

I. Spiking TELL-Seq Custom Primers into Illumina® Sequencing Primers

TELL-Seq™ libraries require custom sequencing primers for Illumina sequencing platforms. Spiking (or combining) custom TELL-Seq Illumina sequencing primers into the standard Illumina sequencing primers is necessary when including PhiX control library or other standard Illumina libraries with TELL-Seq libraries in a sequencing run.

Note: TELL-Seq Index 1 read has **18**-base and need **18**-cycle sequencing; Index 2 read has 8-base.

Procedure for spiking custom primers into Illumina primers

- The cartridge position, total volumes, and final concentration of custom primers for each platform are provided in the tables below.
- Calculate the volume of the custom primer to add to the Illumina primer cartridge position based on the final concentration of the custom primer in the cartridge.*
- After spiking in the custom primer, adjust the pipette to half of the total volume and gently pipette up and down five times to mix thoroughly.
- For MiSeq, MiniSeq, NextSeq and NovaSeq platforms, do not check the "custom primer" box position in the sample sheet or during the run setup.

* To calculate how much custom primer to spike into the well, use the (C1)*(V1) = (C2)*(V2) equation where:

C1 = 100µM (when using 100µM high concentration primer stock, the small additional volume to the final volume is negligible).

V1 = solve for the volume of the custom primer to be spiked in

C2 = the recommended custom primer final concentration from the chart below

V2 = total volume of Illumina primer in the charts below

Example for MiSeq platform:

100µM * **V1** = 0.5µM * 680µL
V1 = 3.4 µl

Important Note: The guidelines below are based on the current primer volumes. To ensure accuracy, measure total primer volumes in the cartridge with a pipette before proceeding with setup.

iSeq100
Due to the construction of the iSeq100 cartridge, it is not possible to load and use custom primers.

MiniSeq

Kit version	Illumina Primer (name)	Cartridge Position	Total Volume (µL)	Custom primer final concentration (µM)
High Output 75 cycles	Read 1 (BP10)	24	550	0.3
	Read 2 (BP11)	25	610	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	28	820	0.3
High Output 150 cycles	Read 1 (BP10)	24	550	0.3
	Read 2 (BP11)	25	610	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	28	857	0.3
High Output 300 cycles	Read 1 (BP10)	24	550	0.3
	Read 2 (BP11)	25	610	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	28	820	0.3
Mid Output 300 cycles	Read 1 (BP10)	24	550	0.3
	Read 2 (BP11)	25	610	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	28	820	0.3

NextSeq

Kit version	Illumina Primer (name)	Cartridge Position	Total Volume (mL)	Custom primer final concentration (µM)
High Output 75 cycles - V2 and V2.5	Read 1 (BP10)	20	1.72	0.3
	Read 2 (BP11)	21	1.98	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	22	2.83	0.3
High Output 150 cycles - V2 and V2.5	Read 1 (BP10)	20	1.73	0.3
	Read 2 (BP11)	21	1.98	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	22	2.83	0.3
High Output 300 cycles - V2 and V2.5	Read 1 (BP10)	20	1.73	0.3
	Read 2 (BP11)	21	1.98	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	22	2.83	0.3
Mid Output 150 cycles - V2 and V2.5	Read 1 (BP10)	20	1.34	0.3
	Read 2 (BP11)	21	1.51	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	22	2.09	0.3
Mid Output 300 cycles - V2 and V2.5	Read 1 (BP10)	20	1.33	0.3
	Read 2 (BP11)	21	1.52	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	22	2.09	0.3

MiSeq

Kit version	Illumina Primer (name)	Cartridge Position	Total Volume (µL)	Custom primer final concentration (µM)
V2 and V3	Read 1 (HP10)	12	680	0.5
	Index 1 (i7) (HP12)*	13	680	0.5
	Read 2 (HP11)	14	680	0.5

* There is no option for a custom Index 2 (i5) primer since the template uses the grafted P5 primer on the surface of the flow cell.

HiSeq 1000/2000 - 1500/2300

Kit version	Illumina Primer (name)	Position	Total Volume (mL)	Custom primer final concentration (µM)
High Output V4	Read 1 (HP10)	cBot plate - Row 2	0.3 per tube	0.5
	Index 1 (i7) (HP12)	PE rack - 17	2.5	0.5
	Index 2 (i5) (HP9)	PE rack - 16	2.5	0.5
	on a single read run*			
	Read 2 (HP11)	PE rack - 16	2.5	0.5
High Output V3	Read 1 (HP6)	cBot plate – Row 11	0.3 per tube	0.5
	Index 1 (i7) (HP8 or HP12)	PE rack - 17	3.15	0.5
	Index 2 (i5) (HP9)	PE rack - 16	3.15	0.5
	on a single read run*			
	Read 2 (HP7 or HP11)	PE rack - 16	3.15	0.5
Rapid Run V2	Read 1 (HP10)	PE rack -18	1.93	0.5
	Index 1 (i7) (HP12)	PE rack - 17	1.93	0.5
	Index 2 (i5) (HP9)	PE rack - 16	1.93	0.5
	on a single read run*			
	Read 2 (HP11)	PE rack - 16	1.93	0.5

* There is no option for a custom Index 2 (i5) primer since the template uses the grafted P5 primer on the surface of the flow cell.

HiSeq 3000/4000

Kit version	Illumina Primer (name)	Position	Total Volume (mL)	Custom primer final concentration (µM)
High Output	Read 1 (HP10)	cBot plate - Row 11	0.39 per tube	0.5
	Index 1 (i7) and Index 2 (i5) (HP14)	PE rack - 17	4.31	0.5
	Read 2 (HP11)	PE rack - 16	2.64	0.5

NovaSeq v1.0 Consumables

Kit version	Illumina Primer (name)	Cartridge Position	Total Volume (mL)	Custom primer final concentration (µM)
SP 100/200/300/500 cycles	Read 1 (BP10)	24	4	0.3
	Index 1 (i7) (BP14)*	23	5	0.3
	Read 2 (BP11)	13	2	0.3
S1 and S2 100/200/300 cycles	Read 1 (BP10)	24	4	0.3
	Index 1 (i7) (BP14)*	23	5	0.3
	Read 2 (BP11)	13	2	0.3
S4 200/300 cycles	Read 1 (BP10)	24	7.3	0.3
	Index 1 (i7) (BP14)*	23	5	0.3
	Read 2 (BP11)	13	3.5	0.3

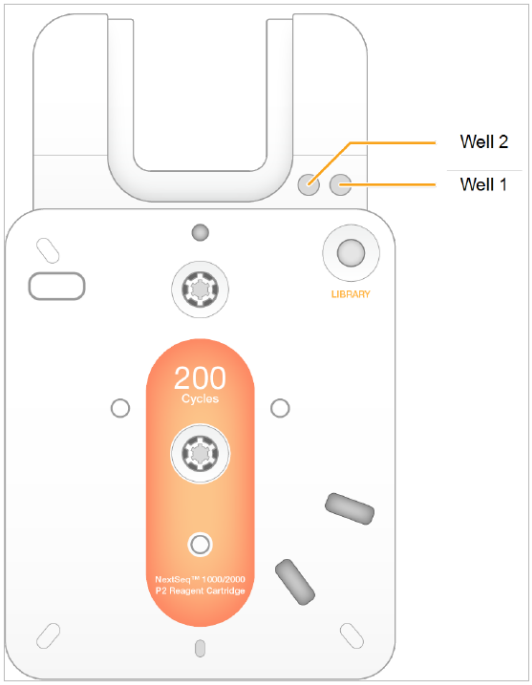
* There is no option for a custom Index 2 (i5) primer since the template uses the grafted P5 primer on the surface of the flow cell.

NovaSeq v1.5 Consumables

Kit version	Illumina Primer (name)	Cartridge Position	Total Volume (mL)	Custom primer final concentration (µM)
SP 100/200/300/500 cycles	Read 1 (VP10)	24	4	0.3
	Index 1 (i7) and Index 2 (i5) (VP14)	23	5	0.3
	Read 2 (VP11)	13	2	0.3
S1 and S2 100/200/300 cycles	Read 1 (VP10)	24	4	0.3
	Index 1 (i7) and Index 2 (i5) (VP14)	23	5	0.3
	Read 2 (VP11)	13	2	0.3
S4 200/300 cycles	Read 1 (VP10)	24	7.3	0.3
	Index 1 (i7) and Index 2 (i5) (VP14)	23	5	0.3
	Read 2 (VP11)	13	3.5	0.3

II. Setting Up a TELL-Seq™ Run on a NextSeq™ 1000/2000 System

The NextSeq™ 1000/2000 reagent cartridge has two custom wells (1 and 2, both are empty) to be used when the library requires at least one custom primer. It supports up to two custom primers (pool) for each custom well. It is essential that the Read primer and Index primer are in different wells.



TELL-Seq™ libraries require custom primers for all reads (read 1, read 2, index 1 and index 2).

When only TELL-Seq libraries are sequenced in a run

- Combine TELL-Seq read 1 and read 2 primers: use HT1 to dilute each custom read primer mix to yield 600 µl at 0.3 µM final concentration, i.e., 1.8 µl of 100 µM TELL-Seq read 1 primer and 1.8 µl of 100 µM TELL-Seq read 2 primer into 597 µl HT1. Load it into custom well 1.
- Combine TELL-Seq index 1 and index 2 primers: use HT1 to dilute each custom index primer mix to yield 600 µl at 0.6 µM final concentration, i.e., 3.6 µl of 100 µM TELL-Seq index 1 primer and 3.6 µl of 100 µM TELL-Seq index 2 primer into 593 µl HT1. Load it into custom well 2.
- Choose the proper custom primer well when setting up the sequencing run as following:

  Read 1: Custom 1

  Index 1: Custom 2

  Index 2: Custom 2

  Read 2: Custom 1

When PhiX libraries are used with TELL-Seq libraries for sequencing in a run

- Obtain a NextSeq 1000/2000 Read Primer Kit (Catalog# 20046117)

NextSeq 1000/2000 Read Primer Kit

Quantity	Acronym	Reagent Name	Cap Color
10	HP21	HP21 read primer mix	Blue
10	HT1	Hybridization Buffer 1	Clear

- Combine TELL-Seq read 1 and read 2 primers into HP21: Add each custom read primer mix to 600 µl HP21 for a 0.3 µM final concentration, i.e., 1.8 µl of 100 µM TELL-Seq read 1 primer and 1.8 µl of 100 µM TELL-Seq read 2 primer into 597 µl HP21. Load it into custom well 1.
- Combine TELL-Seq index 1 and index 2 primers: use HT1 to dilute each custom index primer mix to yield 600 µl at 0.6 µM final concentration, i.e., 3.6 µl of 100 µM TELL-Seq index 1 primer and 3.6 µl of 100 µM TELL-Seq index 2 primer into 593 µl HT1. Load it into custom well 2.
- Choose proper custom primer well when setting up the sequencing run as following:

  Read 1: Custom 1

  Index 1: Custom 2

  Index 2: Custom 2

  Read 2: Custom 1

When dual index Illumina libraries are sequenced with TELL-Seq libraries together in a run

- Obtain a NextSeq 1000/2000 Read & Index Primer Kit (Catalog# 20046115)

NextSeq 1000/2000 Read & Index Primers

Quantity	Acronym	Reagent Name	Cap Color
1	BP14	BP14 index primer mix	Yellow
1	HP21	HP21 read primer mix	Blue
2	HT1	Hybridization Buffer 1	Clear

- Combine TELL-Seq read 1 and read 2 primers into HP21: Add each custom read primer mix to 600 µl HP21 for a 0.3 µM final concentration, i.e., 1.8 µl of 100 µM TELL-Seq read 1 primer and 1.8 µl of 100 µM TELL-Seq read 2 primer into 597 µl HP21. Load it into custom well 1.
- Combine TELL-Seq index 1 and index 2 primers into BP14: Add each custom index primer mix to 600 µl BP14 for a 0.6 µM final concentration, i.e., 3.6 µl of 100 µM TELL-Seq index 1 primer and 3.6 µl of 100 µM TELL-Seq index 2 primer into 593 µl BP14. Load it into custom well 2.

- Choose proper custom primer well when setting up the sequencing run as following:

  Read 1: Custom 1

  Index 1: Custom 2

  Index 2: Custom 2

  Read 2: Custom 1

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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
C01K002 Rev. A	January 2020	Initial Release
C01K002 Rev. B	March 2020	Add Appendix
100018-USG	3.0	Add guide for NovaSeq v1.5 reagent
100018-USG	4.0	Add detailed guidance for latest NovaSeq reagents
100018-USG	5.0	DCR-210082 Add guide for NextSeq 1000/2000 system