TELL-Seq™ Library Sequencing User Guide

For all Illumina® sequencing systems except iSeq 100

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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.

UNIVERSAL SEQUENCING TECHNOLOGY DOES NOT ASSUME ANY LIABILITY OCCURING AFTER INCORRECT USE OF THE TELL-SEQ™ KIT.

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	Add guide for NextSeq 1000/2000 system
100018-USG	6.0	Add 96 Multiplex primers (C-series) information
100018-USG	7.0	Add 384 Multiplex primers (C,D,E,F-series) information. Update Index 2 Primer information
100018-USG	8.0	Doubled the volumes of primers in TELL-Seq™ Illumina® Sequencing Primer Kit (PN 100004 and PN 100013)

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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	100	-25°C to -15°C
Read 2 Primer	CAP White	100μM	100	-25°C to -15°C
Index 1 Primer	CAP Red	100μM	100	-25°C to -15°C
Index 2 Primer	CAP Yellow	100μM	100	-25°C to -15°C

TELL-Seq™ Illumina® Sequencing Primer Kit, HT **(PN 100013)**

Component Name	Cap Color	Concentration	Volume (μL)	Storage Temperature
Read 1 Primer	CAP Black	100μM	600	-25°C to -15°C
Read 2 Primer	CAP White	100μM	600	-25°C to -15°C
Index 1 Primer	CAP Red	100μM	600	-25°C to -15°C
Index 2 Primer	CAP Yellow	100μM	600	-25°C to -15°C

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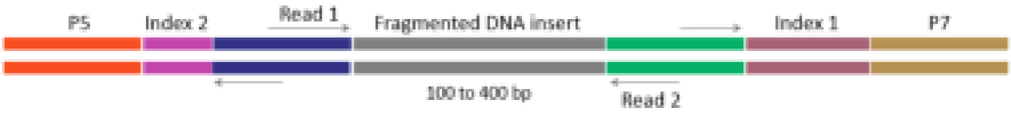
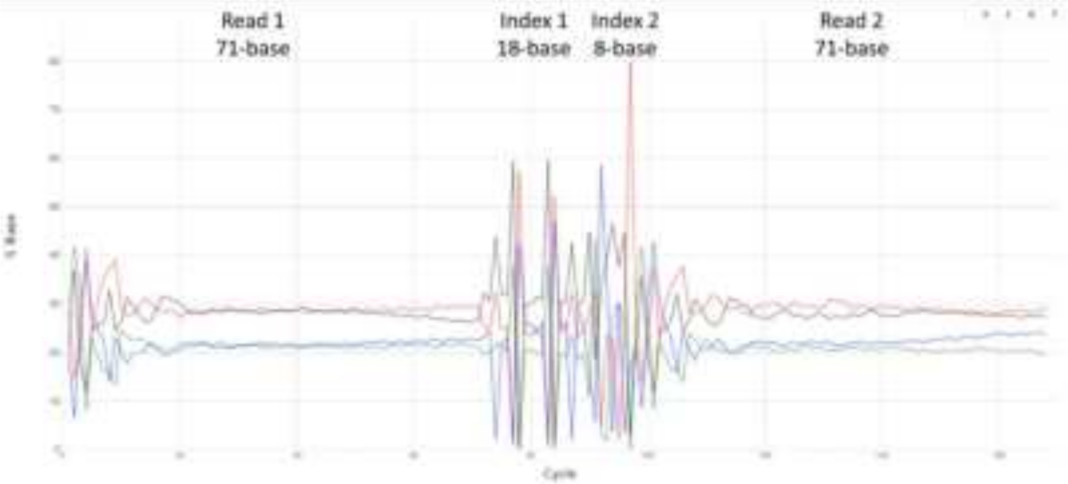
† Patent pending.

3. TELL-Seq™ Library Structure and Sequencing Scheme



Index 1 (i.e., I7 index) contains **<u>18-base</u>** TELL Bead sequences, which MUST be sequenced completely. Index 2 (i.e., I5 index) contains 8-base (T-series) or 10-base (C-series) 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. **Currently only for MiSeq, custom Index 2 Primer is not required. Also for**

non supported systems like HiSeq 2000/2500 and NovaSeq v1 reagents, 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	Is custom Index 2 Primer required?
NovaSeq X/X Plus	Yes
NovaSeq 6000	Yes
HiSeq 3000/4000	Yes
NextSeq	Yes
MiSeq	No
MiniSeq	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 or 10-base (C-series) 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 a 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	5003	NNNNNNNN		

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 (T-series) or 10-base (C-series). There are total 26-base (T-series) or 28-base (C-series) for both indexes compared to total 16-base for standard Illumina dual index.
3. 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.
4. 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 60x 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.
3. For human phasing, at least 500 million cluster reads per sample which is ~40x depth is recommended.
4. For targeted sequencing, minimum 100x coverage is recommended.

8. Library Multiplex Primer Index Sequences (i.e., Index 2 Read): T-series (8-base)

Library Multiplex Primer (T-series)	For Sample Sheet MiSeq	For Sample Sheet NovaSeq, NovaSeq X, NextSeq, MiniSeq
T501	TGAACCTT	AAGGTTCA
T502	TGCTAAGT	ACTTAGCA
T503	TGTTCTCT	AGAGAACA
T504	TAAGACAC	GTGTCTTA
T505	CTAATCGA	TCGATTAG
T506	CTAGAACA	TGTTCTAG
T507	TAAGTTCC	GGAACTTA
T508	TAGACCTA	TAGGTCTA
T509	CATCCGAA	TTCGGATG
T510	TTATGAGT	ACTCATAA
T511	AGAGGCGC	GCGCCTCT
T512	TAGCCGCG	CGCGGCTA
T513	ACGAATAA	TTATTCGT
T514	TTCGTAGG	CCTACGAA
T515	GATCTGCT	AGCAGATC
T516	CGCTCCGC	GCGGAGCG
T517	AGGCTATA	TATAGCCT
T518	GCCTCTAT	ATAGAGGC
T519	AGGATAGG	CCTATCCT
T520	TCAGAGCC	GGCTCTGA
T521	CTTCGCCT	AGGCGAAG
T522	TAAGATTA	TAATCTTA
T523	AGTAAGTA	TACTTACT
T524	GACTTCCT	AGGAAGTC

9. Library Multiplex Primer Index Sequences (i.e. Index 2 Read): C,D,E,F-series (10-base)

Library Multiplex Primer (C-series)	For Sample Sheet MiSeq	For Sample Sheet NovaSeq, NovaSeq X, NextSeq, MiniSeq
C501	ACGTACGTAC	GTACGTACGT
C502	CATGCATGCA	TGCATGCATG
C503	GTACGTACGT	ACGTACGTAC
C504	TGCATGCATG	CATGCATGCA
C505	ATGCTGATCA	TGATCAGCAT
C506	CACAGCTGTG	CACAGCTGTG
C507	GCTGATCAGC	GCTGATCAGC
C508	TGATCAGCAT	ATGCTGATCA
C509	ATTCAATACT	AGTATTGAAT
C510	CTAGCGCTAG	CTAGCGCTAG
C511	GCTAGTAGTA	TACTACTAGC
C512	TCCAATCAAG	CTTGATTGGA
C513	AATATTGCTG	CAGCAATATT
C514	CGTCGTTACG	CGTAACGACG
C515	GATTGATTCC	GGAATCAATC
C516	TCTAACAATG	CATTGTTAGA
C517	AGAATTGTCA	TGACAATTCT
C518	CTCAGCAATT	AATTGCTGAG
C519	GGTCCTTGTC	GACAAGGACC
C520	AGGCCTGACA	TGTCAGGCCT
C521	CTCCTAGTGG	CCACTAGGAG
C522	GGTTACAGCT	AGCTGTAACC
C523	CTGATTGGCG	CGCCAATCAG

C524	ATTGGTTAGA	TCTAACCAAT
C525	CCATTCAACT	AGTTGAATGG
C526	CAGTATTGAC	GTCAATACTG
C527	GAGTCCTCAA	TTGAGGACTC
C528	AGCTACTACT	AGTAGTAGCT
C529	TAGCTAGCGC	GCGCTAGCTA
C530	GATGCAACAC	GTGTTGCATC
C531	CCTCAGTACA	TGTACTGAGG
C532	CGGTAATTCA	TGAATTACCG
C533	CGCAATGGCT	AGCCATTGCG
C534	GTACGTTGAA	TTCAACGTAC
C535	TTGATCAGTA	TACTGATCAA
C536	GGCCTAACAA	TTGTTAGGCC
C537	GTTGTTGGAA	TTCCAACAAC
C538	TACGTTGGAC	GTCCAACGTA
C539	ACACCATGCA	TGCATGGTGT
C540	GCAATAGTAC	GTACTATTGC
C541	ACGCAGCCAG	CTGGCTGCGT
C542	CGAGTTGACG	CGTCAACTCG
C543	CGTGGCTGAA	TTCAGCCACG
C544	TCTCAAGGAC	GTCCTTGAGA
C545	CCTAGGCACT	AGTGCCTAGG
C546	CTGCGGTAAT	ATTACCGCAG
C547	GGCACTACCA	TGGTAGTGCC
C548	GCTCAATCAA	TTGATTGAGC
C549	AGGCACACAC	GTGTGTGCCT
C550	CCTGGCAAGA	TCTTGCCAGG

C551	TAATTGGTAG	CTACCAATTA
C552	GCCAACAAGT	ACTTGTTGGC
C553	ATGGCTTATA	TATAAGCCAT
C554	GCATGGCCTT	AAGGCCATGC
C555	ACAATACTGG	CCAGTATTGT
C556	GGATTGGACT	AGTCCAATCC
C557	ACTGTACTAT	ATAGTACAGT
C558	CAGCTGTGAG	CTCACAGCTG
C559	CTTGAGGACC	GGTCCTCAAG
C560	GGTACAATAG	CTATTGTACC
C561	CTGACTACTA	TAGTAGTCAG
C562	TCAACCATGG	CCATGGTTGA
C563	ATTATAACCG	CGGTTATAAT
C564	ACTAGTCCTT	AAGGACTAGT
C565	ACTTGGACGT	ACGTCCAAGT
C566	ATGGTTAGGA	TCCTAACCAT
C567	ATGGTACCAA	TTGGTACCAT
C568	GAATTGACTC	GAGTCAATTC
C569	AGCAACCAGG	CCTGGTTGCT
C570	TACTGTGCTG	CAGCACAGTA
C571	CAACAACGTC	GACGTTGTTG
C572	CAGTAGCGCT	AGCGCTACTG
C573	ATTACCAATC	GATTGGTAAT
C574	TAAGGACCGC	GCGGTCCTTA
C575	ACACGTACCG	CGGTACGTGT
C576	CAACGTTGTT	AACAACGTTG
C577	ATTGTGCTGA	TCAGCACAAT

C578	GTACCAACAG	CTGTTGGTAC
C579	TTGTCAAGGA	TCCTTGACAA
C580	CTTGTACGTA	TACGTACAAG
C581	TGCCTTGTAA	TTACAAGGCA
C582	TAGTAGCTTA	TAAGCTACTA
C583	GACCGCAATG	CATTGCGGTC
C584	CTACTAGCTT	AAGCTAGTAG
C585	AGCACACGTT	AACGTGTGCT
C586	TGTTATAAGC	GCTTATAACA
C587	GTTGCCAAGT	ACTTGGCAAC
C588	CTGGCAACCG	CGGTTGCCAG
C589	TTAGGCCTTA	TAAGGCCTAA
C590	CGCAGCACAG	CTGTGCTGCG
C591	CTAGGCACAA	TTGTGCCTAG
C592	TGTTGTACAG	CTGTACAACA
C593	CTAACGTGGC	GCCACGTTAG
C594	GCGTACTGGT	ACCAGTACGC
C595	GGCCTGAATT	AATTCAGGCC
C596	CATGCTCGAG	CTCGAGCATG

Library Multiplex Primer (D-series)	**For Sample Sheet MiSeq**	**For Sample Sheet NovaSeq, NovaSeq X, NextSeq, MiniSeq**
D501	AGCACTGTAA	TTACAGTGCT
D502	CCGAAGTACT	AGTACTTCGG
D503	GTTACAAGTA	TACTTGTAAC
D504	TGATTGCGTG	CACGCAATCA
D505	ATCTTAGGCA	TGCCTAAGAT

D506	CACGCAAGTT	AACTTGCGTG
D507	GAGCTTAGGA	TCCTAAGCTC
D508	TATTGCTCGA	TCGAGCAATA
D509	AACATCTGAG	CTCAGATGTT
D510	CAACTCAGCA	TGCTGAGTTG
D511	GAACTATATG	CATATAGTTC
D512	TAATAGTCTA	TAGACTATTA
D513	AAGATTGACG	CGTCAATCTT
D514	CACGTAGCCG	CGGCTACGTG
D515	GACTCGATCA	TGATCGAGTC
D516	TAATCCTCGC	GCGAGGATTA
D517	AAGCTCCTGG	CCAGGAGCTT
D518	CAGATTGTTG	CAACAATCTG
D519	GATGCTATTC	GAATAGCATC
D520	TAATCCTCGC	GCGAGGATTA
D521	AAGTATACCT	AGGTATACTT
D522	CAGTAGCCAA	TTGGCTACTG
D523	GCAAGGACTC	GAGTCCTTGC
D524	TACTACTGTA	TACAGTAGTA
D525	AATCCTAGCT	AGCTAGGATT
D526	CATTGGCAAG	CTTGCCAATG
D527	GCCTGAGAAT	ATTCTCAGGC
D528	TACTAGCAAG	CTTGCTAGTA
D529	ACAGCTAGTC	GACTAGCTGT
D530	CCAACCAGTA	TACTGGTTGG
D531	GCGCTCAATT	AATTGAGCGC
D532	TAGCGCGGAC	GTCCGCGCTA

D533	ACCTATAGTT	AACTATAGGT
D534	CCACGTCAGG	CCTGACGTGG
D535	GCTACGCAAT	ATTGCGTAGC
D536	TAGTTGCTGC	GCAGCAACTA
D537	ACGGTACAAC	GTTGTACCGT
D538	CCGAACTTCG	CGAAGTTCGG
D539	GCTCCTAATT	AATTAGGAGC
D540	TATCCTCGAT	ATCGAGGATA
D541	ACTCGATTCT	AGAATCGAGT
D542	CCTGGTAATT	AATTACCAGG
D543	GCTTAGTTGG	CCAACTAAGC
D544	TCAGCAAGGA	TCCTTGCTGA
D545	ACTGGTAGCA	TGCTACCAGT
D546	CGATCAACCT	AGGTTGATCG
D547	GGAAGTAGCA	TGCTACTTCC
D548	TCAGCTGCGG	CCGCAGCTGA
D549	ACTGGTAGCA	TGCTACCAGT
D550	CGATCAACCT	AGGTTGATCG
D551	GGCAAGATGA	TCATCTTGCC
D552	TCCGCCAATG	CATTGGCGGA
D553	AGAATCTTAT	ATAAGATTCT
D554	CGCTAGCAAC	GTTGCTAGCG
D555	GGCCTTATAT	ATATAAGGCC
D556	TCGAATACTA	TAGTATTCGA
D557	AGCAATGGTA	TACCATTGCT
D558	CGGTCTGATC	GATCAGACCG
D559	GGTCCGCCAT	ATGGCGGACC

D560	TCGAATACTA	TAGTATTCGA
D561	AGCACTGTAA	TTACAGTGCT
D562	CGTACTACCG	CGGTAGTACG
D563	GGTTCATCGG	CCGATGAACC
D564	TCGGAGCTGC	GCAGCTCCGA
D565	AGGACGTTAG	CTAACGTCCT
D566	CGTTGACAAT	ATTGTCAACG
D567	GTACTGACAC	GTGTCAGTAC
D568	TCTAAGATAG	CTATCTTAGA
D569	AGTGCTCAAC	GTTGAGCACT
D570	CTAAGAACCT	AGGTTCTTAG
D571	GTATAATTAC	GTAATTATAC
D572	TGATTGCGTG	CACGCAATCA
D573	ATATTCGACG	CGTCGAATAT
D574	CTATAACGAC	GTCGTTATAG
D575	GTCGTTAGGT	ACCTAACGAC
D576	TGCCACTGAT	ATCAGTGGCA
D577	ATCGGCTAAG	CTTAGCCGAT
D578	CTCAAGTTGC	GCAACTTGAG
D579	GTGTGTTCGA	TCGAACACAC
D580	TGCTATAGTC	GACTATAGCA
D581	ATGAGCTATT	AATAGCTCAT
D582	CTCACAACGA	TCGTTGTGAG
D583	TGCTCGATTG	CAATCGAGCA
D584	ATGTTGGACT	AGTCCAACAT
D585	CTGATGTTAG	CTAACATCAG
D586	TGGTAACCAG	CTGGTTACCA

D587	ATTCGAAGTA	TACTTCGAAT
D588	CTTAGCTCCT	AGGAGCTAAG
D589	TGTAATCGAT	ATCGATTACA
D590	CTTGGACTTC	GAAGTCCAAG
D591	TGTACTTGAA	TTCAAGTACA
D592	TTAAGCTCAG	CTGAGCTTAA
D593	TTACAACCAG	CTGGTTGTAA
D594	TTCTCAGCCA	TGGCTGAGAA
D595	TTGCATGGCT	AGCCATGCAA
D596	TTGGCGGTAC	GTACCGCCAA

Library Multiplex Primer (E-series)	For Sample Sheet MiSeq	For Sample Sheet NovaSeq, NovaSeq X, NextSeq, MiniSeq
E501	AGCCACGGTC	GACCGTGGCT
E502	CCGCCTGAGT	ACTCAGGCGG
E503	GTTATATACC	GGTATATAAC
E504	CTTCCTGGCA	TGCCAGGAAG
E505	ATCGAATTGC	GCAATTCGAT
E506	CACGTGTTGT	ACAACACGTG
E507	GAGCTGTGTT	AACACAGCTC
E508	TATTACAGAT	ATCTGTAATA
E509	AACAATGGCG	CGCCATTGTT
E510	CAACTCGCTA	TAGCGAGTTG
E511	GAATTGCTCA	TGAGCAATTC
E512	GTGTGTTCGA	TCGAACACAC
E513	CGATTCGAGC	GCTCGAATCG
E514	GTCAACGTTA	TAACGTTGAC

E515	GACAATCCTA	TAGGATTGTC
E516	TAATCAGACT	AGTCTGATTA
E517	AAGCTTATAT	ATATAAGCTT
E518	CATATTGTAA	TTACAATATG
E519	GATAGGACTT	AAGTCCTATC
E520	ATGCTAGGTT	AACCTAGCAT
E521	GCAAGATTAG	CTAATCTTGC
E522	CAGCTGTATA	TATACAGCTG
E523	GCAAGTCTAA	TTAGACTTGC
E524	TACTAGCAAG	CTTGCTAGTA
E525	AATACTGTTA	TAACAGTATT
E526	CATATCTGAC	GTCAGATATG
E527	GCCACTAAGG	CCTTAGTGGC
E528	CTGGCAATTC	GAATTGCCAG
E529	ACAATAGGCG	CGCCTATTGT
E530	CCATGCTAAG	CTTAGCATGG
E531	GCGAGCGATC	GATCGCTCGC
E532	TAGGTAGTTC	GAACTACCTA
E533	ACCTTGGACC	GGTCCAAGGT
E534	TGACTAATAC	GTATTAGTCA
E535	GCTATATCTG	CAGATATAGC
E536	TCTAGTTCAG	CTGAACTAGA
E537	ACGGTTGAGA	TCTCAACCGT
E538	CTGATTCCGG	CCGGAATCAG
E539	TGGTAACCAG	CTGGTTACCA
E540	GACTCAGACA	TGTCTGAGTC
E541	TTAGCCAATA	TATTGGCTAA

E542	CCTTAAGGCG	CGCCTTAAGG
E543	CGTAGCAATC	GATTGCTACG
E544	CATGGCTAAT	ATTAGCCATG
E545	ACTCGACAAT	ATTGTCGAGT
E546	CGAGTCTAGG	CCTAGACTCG
E547	GGACTCAGCT	AGCTGAGTCC
E548	TCAGCAAGGA	TCCTTGCTGA
E549	CGATTCCTTA	TAAGGAATCG
E550	ATTAGGCAAT	ATTGCCTAAT
E551	TTGTCGAAGG	CCTTCGACAA
E552	TCCAAGTAGC	GCTACTTGGA
E553	AGATAGTCCG	CGGACTATCT
E554	CGCTAACATA	TATGTTAGCG
E555	GGCTAATTGT	ACAATTAGCC
E556	AGGTACGTCA	TGACGTACCT
E557	TTCCAAGTTC	GAACTTGGAA
E558	CGGAGCTCCG	CGGAGCTCCG
E559	GGTACTAATA	TATTAGTACC
E560	TCGAAGGCAA	TTGCCTTCGA
E561	CTCCTTGATG	CATCAAGGAG
E562	GCAATTCCGG	CCGGAATTGC
E563	ACTACGCAGC	GCTGCGTAGT
E564	AGGAATGGCC	GGCCATTCCT
E565	AGGTTAGCAT	ATGCTAACCT
E566	CGTCCTAGAC	GTCTAGGACG
E567	GCGTGGCAAT	ATTGCCACGC
E568	TCTGATCGAC	GTCGATCAGA

E569	AGTTGTCAGG	CCTGACAACT
E570	CTACGCCAAT	ATTGGCGTAG
E571	GTAGTTGCGT	ACGCAACTAC
E572	TGAGCGCTAT	ATAGCGCTCA
E573	ATACCTTAGT	ACTAAGGTAT
E574	GCACACCATT	AATGGTGTGC
E575	GTCTAAGGAG	CTCCTTAGAC
E576	CATTCCGCGG	CCGCGGAATG
E577	GCGTACCTAG	CTAGGTACGC
E578	CTCAATAGCA	TGCTATTGAG
E579	GTGCTAAGCC	GGCTTAGCAC
E580	TGCCACTATC	GATAGTGGCA
E581	GCTGTTGAAT	ATTCAACAGC
E582	CGGCTAATCC	GGATTAGCCG
E583	AACTACCGCA	TGCGGTAGTT
E584	ATGCATCAAG	CTTGATGCAT
E585	CTGACTTGCA	TGCAAGTCAG
E586	TGGTACCTAT	ATAGGTACCA
E587	ATTAGCTAAC	GTTAGCTAAT
E588	TGGTACCTAT	ATAGGTACCA
E589	TGTTGCTACC	GGTAGCAACA
E590	CTTATATAAG	CTTATATAAG
E591	TCAAGTCTGT	ACAGACTTGA
E592	TGAGCGCTAT	ATAGCGCTCA
E593	TTAGGCGTAC	GTACGCCTAA
E594	TTCTCATCAG	CTGATGAGAA
E595	CTAGGTATTG	CAATACCTAG

E596	TTGCTGACTA	TAGTCAGCAA

Library Multiplex Primer (F-series)	For Sample Sheet MiSeq	For Sample Sheet NovaSeq, NovaSeq X, Next Seq, MiniSeq
F501	AGCCAGTAGC	GCTACTGGCT
F502	CCGATGAGTT	AACTCATCGG
F503	GTTCAACTTC	GAAGTTGAAC
F504	TTGAGCATCA	TGATGCTCAA
F505	ATCAACAGTG	CACTGTTGAT
F506	CACACGGTAG	CTACCGTGTG
F507	GAGGATTGCT	AGCAATCCTC
F508	TATCTTGGCC	GGCCAAGATA
F509	AACTAGTGGC	GCCACTAGTT
F510	CAATCCTGGT	ACCAGGATTG
F511	GAAGGTTACG	CGTAACCTTC
F512	CTAAGAACCT	AGGTTCTTAG
F513	CACACGGTAG	CTACCGTGTG
F514	AATTAGTCTG	CAGACTAATT
F515	GACCGTGTTA	TAACACGGTC
F516	TAATTCGAGG	CCTCGAATTA
F517	AAGATTGACG	CGTCAATCTT
F518	CAACTCGCTA	TAGCGAGTTG
F519	GATTCGAACT	AGTTCGAATC
F520	GCCTTGAGTT	AACTCAAGGC
F521	CACAGATTAG	CTAATCTGTG
F522	CAGCTAGGCC	GGCCTAGCTG
F523	GCAAGGTATA	TATACCTTGC

F524	TACCTAGGTA	TACCTAGGTA
F525	AATCCTTGAA	TTCAAGGATT
F526	CATCGAACCT	AGGTTCGATG
F527	GCCTGCGTAA	TTACGCAGGC
F528	CATCGAACCT	AGGTTCGATG
F529	ACAGTGCCAT	ATGGCACTGT
F530	CCAACTATTG	CAATAGTTGG
F531	GCGTACCGCA	TGCGGTACGC
F532	TAGGAGCGAT	ATCGCTCCTA
F533	ACCAATTGGT	ACCAATTGGT
F534	AATACTGTTA	TAACAGTATT
F535	GCTGTTGAAT	ATTCAACAGC
F536	TTACAACCAG	CTGGTTGTAA
F537	ACGTCAAGTC	GACTTGACGT
F538	CAATTCAATG	CATTGAATTG
F539	TGAATTCAGC	GCTGAATTCA
F540	ACGTACAGCT	AGCTGTACGT
F541	TGACTACCAT	ATGGTAGTCA
F542	CCTGGTGTGA	TCACACCAGG
F543	GACTAATCGC	GCGATTAGTC
F544	ACGTTAGTGC	GCACTAACGT
F545	ACTCGATTCT	AGAATCGAGT
F546	CGACAAGATT	AATCTTGTCG
F547	GGAATTGGCT	AGCCAATTCC
F548	TCACAGCCGA	TCGGCTGTGA
F549	CTGTACTGTA	TACAGTACAG
F550	TCGCTCGAGG	CCTCGAGCGA

F551	ACCTTAGCAA	TTGCTAAGGT
F552	TCCGGACTAC	GTAGTCCGGA
F553	AGAACTTGCA	TGCAAGTTCT
F554	CGCAGGACTT	AAGTCCTGCG
F555	GGCAAGATGA	TCATCTTGCC
F556	GCCTAGCGCG	CGCGCTAGGC
F557	GACATCGCTA	TAGCGATGTC
F558	CGGCTAATCC	GGATTAGCCG
F559	GGTTGAGCTT	AAGCTCAACC
F560	TCGCTCAGGC	GCCTGAGCGA
F561	GCTGATTATA	TATAATCAGC
F562	GCGCTAGTCC	GGACTAGCGC
F563	TATGTCTGAA	TTCAGACATA
F564	AAGCTTATAT	ATATAAGCTT
F565	AGGAATGGCC	GGCCATTCCT
F566	CGTAGCGCGC	GCGCGCTACG
F567	GCCTGCGTAA	TTACGCAGGC
F568	TCTTAGCGAT	ATCGCTAAGA
F569	AGTTCTAAGA	TCTTAGAACT
F570	CTAGGTATTG	CAATACCTAG
F571	GTAGTACTAC	GTAGTACTAC
F572	TGACAAGTAG	CTACTTGTCA
F573	ATAGTCTGAG	CTCAGACTAT
F574	CTGTGTTACA	TGTAACACAG
F575	GTCTAGCCAC	GTGGCTAGAC
F576	AATCAAGGTT	AACCTTGATT
F577	CATAATTATG	CATAATTATG

F578	CTCGAGCTAT	ATAGCTCGAG
F579	GTGCCTGATG	CATCAGGCAC
F580	TGCTCGATTG	CAATCGAGCA
F581	GATTCGAACT	AGTTCGAATC
F582	TCGTTGAGGA	TCCTCAACGA
F583	AGCCAGGCGT	ACGCCTGGCT
F584	ATGTCAGATT	AATCTGACAT
F585	CTGTATATAC	GTATATACAG
F586	TGGTGTCAAC	GTTGACACCA
F587	ATTGCATGCT	AGCATGCAAT
F588	AGTATTCATA	TATGAATACT
F589	TGTAATTCCG	CGGAATTACA
F590	CTTCCTGGCA	TGCCAGGAAG
F591	TCCGCGCGGA	TCCGCGCGGA
F592	CACTGTTCGG	CCGAACAGTG
F593	TTAGCCAATA	TATTGGCTAA
F594	TTCGAGCTGA	TCAGCTCGAA
F595	GTACCGCGCG	CGCGCGGTAC
F596	TTGAGCATCA	TGATGCTCAA

10. 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 for T-series primer and 10-base for C-series primer.

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.

Each Illumina® sequencer has different final concentration and total volume so refer to document below to ensure that appropriate amount of TELL-Seq™ Illumina® Sequencing Primers are being directly added.

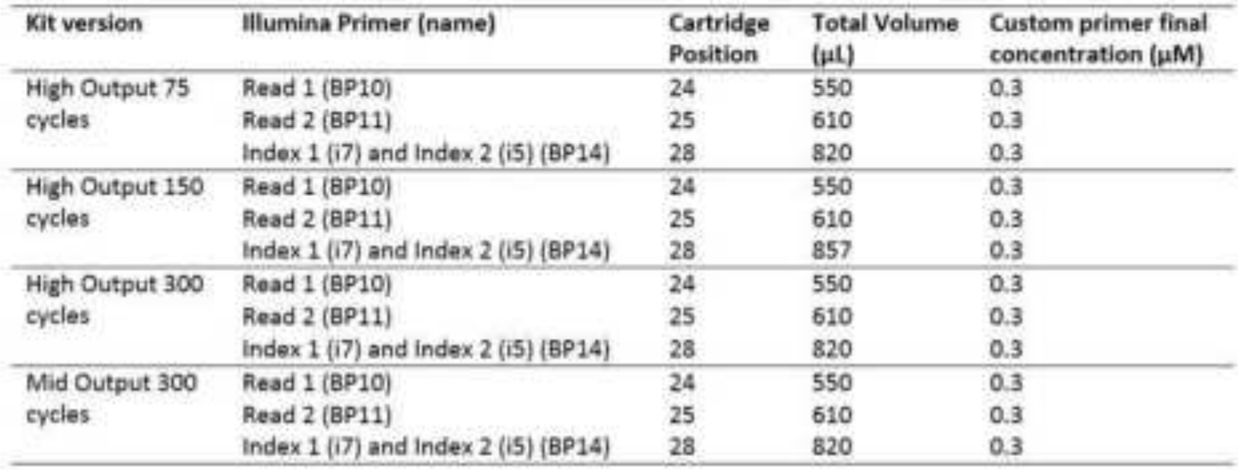
*Note: Index 1 and Index 2 along with Read 1 and Read 2 primers are often pooled together in reagent cartridge well. Please ensure that final concentration custom primer for the well is of each primer (eg. 0.3μM of Index 1 and Index 2 for a total of 0.6μM)

<https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000001542>

Below are

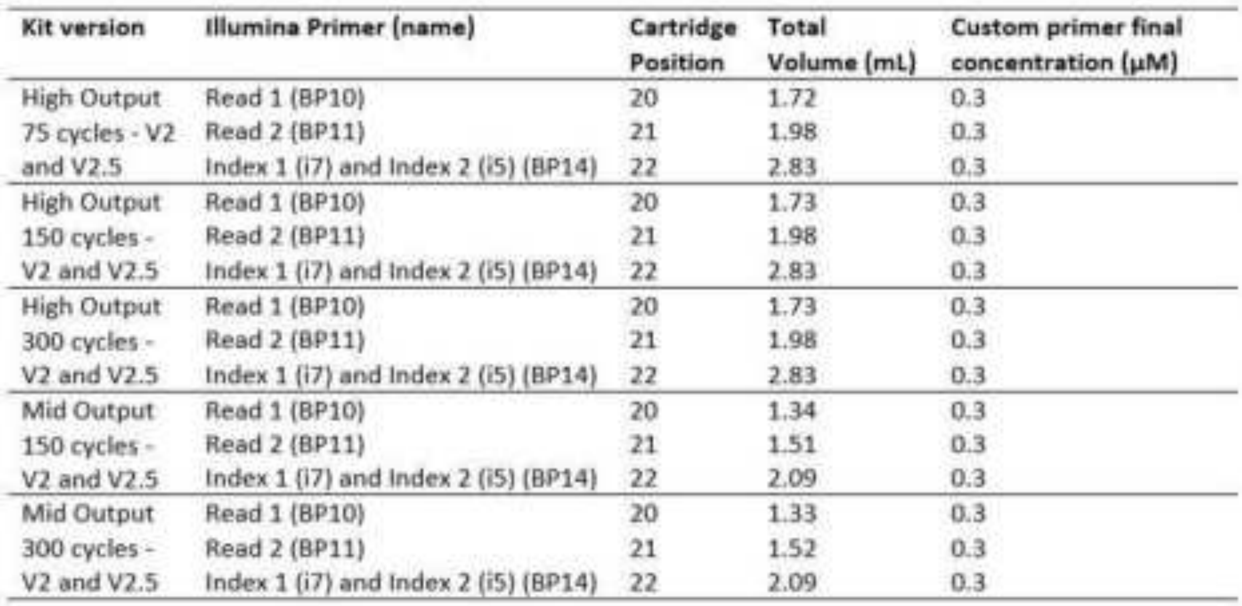
iSeq100
Due to the construction of the iSeq100 cartridge, it is not possible to load and use custom primers.

MiniSeq



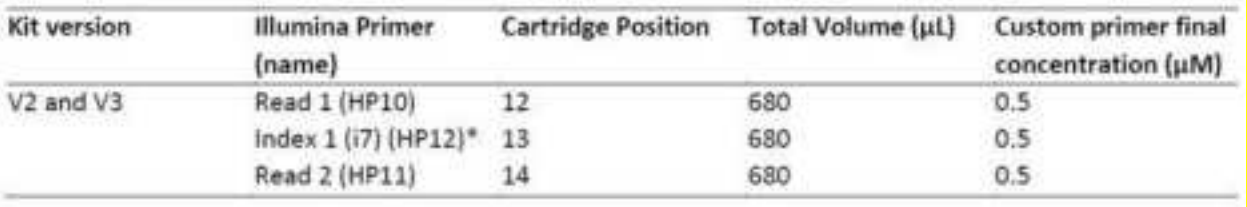
Kit version	Illumina Primer (name)	Cartridge Position	Total Volume (μL)	Custom primer final concentration (μM)
High Output 75 cycles	Read 1 (BP10)	24	550	0.3
	Read 2 (BP11)	25	610	0.3
	Index 1 (i7) and Index 2 (i5) (BP14)	28	820	0.3
High Output 150 cycles	Read 1 (BP10)	24	550	0.3
	Read 2 (BP11)	25	610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

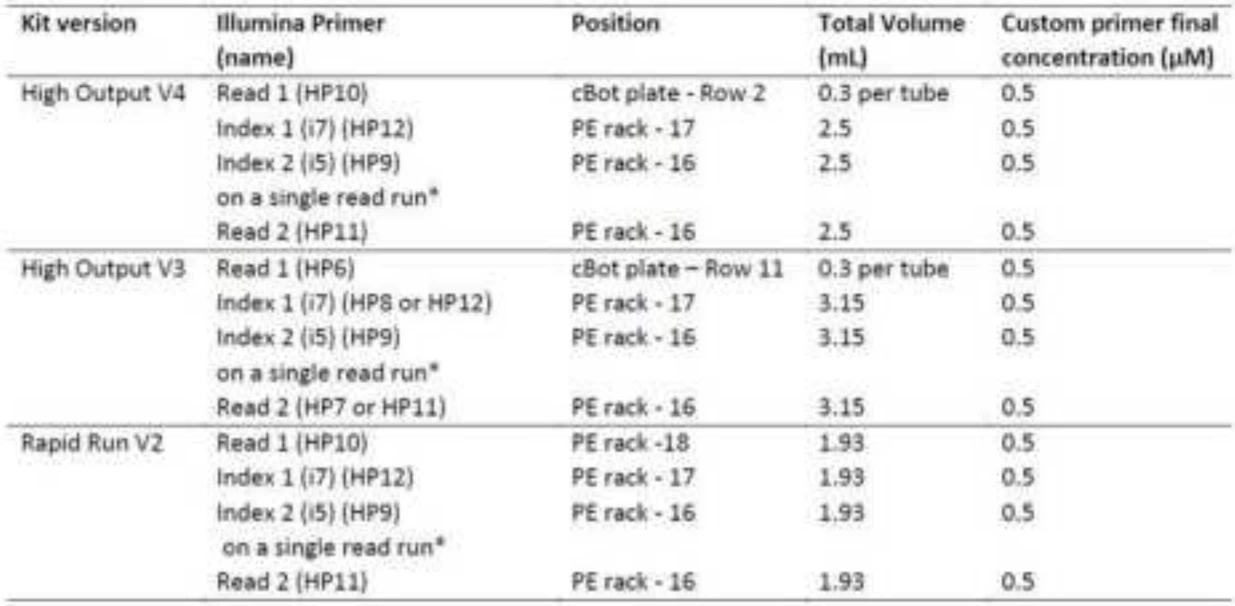
MiSeq



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.

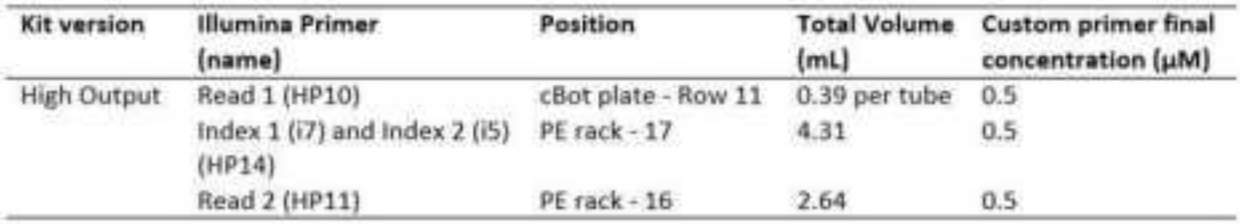
HiSeq 1000/2000 - 1500/2300



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) on a single read run*	PE rack - 16	2.5	0.5
	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) on a single read run*	PE rack - 16	3.15	0.5
	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) on a single read run*	PE rack - 16	1.93	0.5
	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

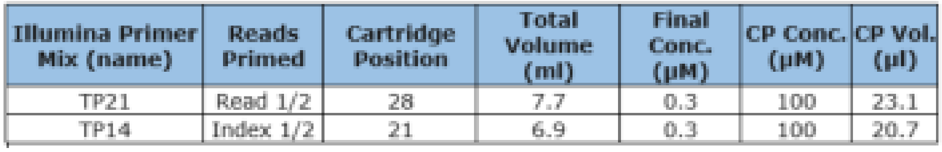
Kit version	Illumina Primer (name)	Cartridge Position	Total Volume (mL)	Custom primer final concentration (µM)
SP 100/200/300/500 cycles	Read 1 (BP10)	24	4	0.3
	Index 1 (i7) (BP14)*	23	5	0.3
	Read 2 (BP11)	13	2	0.3
S1 and S2 100/200/300 cycles	Read 1 (BP10)	24	4	0.3
	Index 1 (i7) (BP14)*	23	5	0.3
	Read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

NovaSeq X/X Plus



Illumina Primer Mix (name)	Reads Primed	Cartridge Position	Total Volume (ml)	Final Conc. (µM)	CP Conc. (µM)	CP Vol. (µl)
TP21	Read 1/2	28	7.7	0.3	100	23.1
TP14	Index 1/2	21	6.9	0.3	100	20.7

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

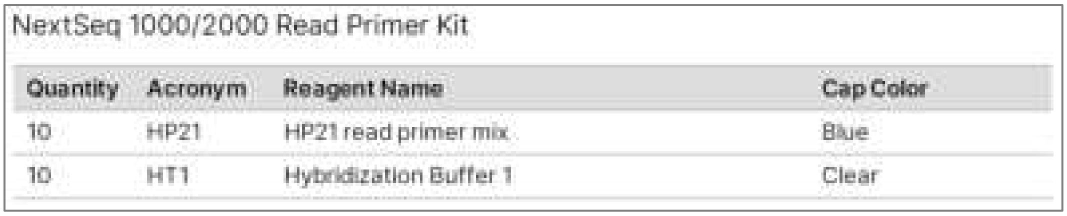
  Index 1: Custom 2

  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

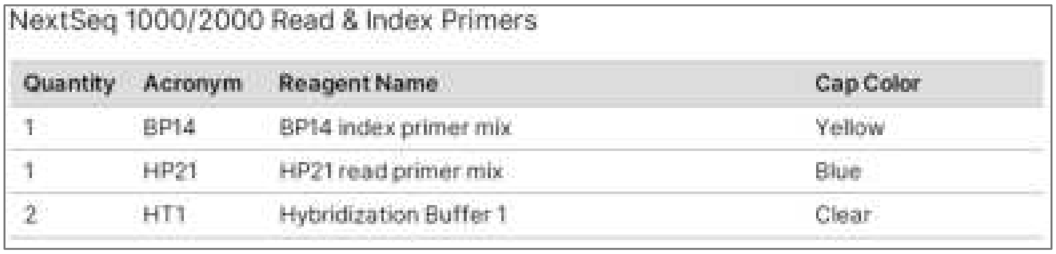
Index 1: Custom 2

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