TELL-Seq™ Microbial WGS Library Prep User Guide

For genome size from 1 Mb to 50 Mb

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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

Doc #130001	v1.0	Initial Release
Doc # 130001	v2.0	Removed TELL Bead option, only TELL Bead Plex is used. Added a Note and a picture with recommended mixing systems for a critical step of proper tube rotation during barcoding process to preserve high molecular weight DNA properties.

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1. Introduction

This user guide explains how to prepare indexed paired-end TELL-Seq™ microbial whole genome sequencing (WGS) libraries using a TELL-Seq Microbial Library Prep Kit from a genomic DNA sample with genome size ranges from 1 Mb to 50 Mb for subsequent sequencing on an Illumina® sequencing system.

The TELL-Seq library prep uses an innovative Transposase Enzyme Linked Long-read Sequencing (TELL-Seq™) technology† to prepare a paired-end library to generate barcode linked reads from an Illumina® sequencing system. Coupled with TELL-Seq specific analysis software, the linked reads can be easily used for genome wide variant calling, haplotype phasing, structural variation detection, metagenomic studies and *de novo* sequencing assembly.

A TELL-Seq Microbial Library Prep kit can generate TELL-Seq WGS libraries for genome size ranging from 1 Mb to 50 Mb.

- Use 0.4 ng to 0.6 ng genomic DNA input for standard procedure
- Produce barcode linked reads using an Illumina® sequencing system

A TELL-Seq Microbial Library Prep kit (Box 1 and Box 2) along with TELL-Seq Library Multiplex Primer Kits can generate different number of TELL-Seq Libraries based on the kit format.

Type of Kit	No. of TELL-Seq Libraries
Standard	24
HT	144

Genomic DNA Recommendations

High molecular weight (HMW) DNA is critical for successful TELL-seq sequencing.

- HMW DNA ranging from 100Kb to 300Kb are optimal material for best whole genome sequencing application.
- For microbial genomes, minimum input DNA size should be greater than 20Kb.
- Avoid breaking the HMW DNA during handling. Remove low molecular weight DNA (identified as a smear less than 10Kb on a gel) in the sample if they present a significant portion in the DNA sample.
- Genomic DNA should be stored in a Tris buffer with pH ranging from 7.5 - 8.0 or a low TE buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 8.0).
- Use a fluorometric-based method to quantify input DNA. If you use the Qubit dsDNA BR Assay Kit or HS Kit, use at least 2 μL of each DNA sample for a measurement. Avoid methods that only measure total nucleic acid concentration, such as NanoDrop or other UV absorbance methods.
- For accurate measurement of HMW DNA concentration, dilute the concentrated DNA to the working concentration (0.2ng/μl to 0.5ng/μl) in a Tris buffer (pH 7.5-8.0) several hours to a day before the concentration measurement and library preparation.

- For assessing the purity of a DNA sample, the ratio of absorbance measurement at 260 nm to absorbance at 280 nm can be used. This protocol is optimized for DNA with absorbance ratio values of 1.8–2.0. If there is excessive RNA in the DNA sample, it should be removed with a RNase treatment.

† Patent pending.

2. Kit Contents

TELL-Seq™ Microbial Library Prep Kit, Standard Size (2 Boxes)

Box 1 of 2: TELL-Seq™ Microbial Library Reagent Box 1, V1 RUO (PN 100045)

NOTE: Do not freeze and thaw Box 1 reagents for more than 6 times.

Component Name	Cap Color	Volume (µL)	Storage Temperature
5× Reaction Buffer	CAP Blue	120	-25°C to -15°C
Barcoding Enzyme	CAP Black	24	-25°C to -15°C
Cofactor II	CAP Amber	120	-25°C to -15°C
Exonuclease LC	CAP Yellow	24	-25°C to -15°C
Stabilizer LC	CAP Violet	24	-25°C to -15°C
Suspension Buffer EZ	CAP Natural	180	-25°C to -15°C
Tagging Enzyme LC	CAP Red	48	-25°C to -15°C
2× PCR Master Mix	CAP Pink	300	-25°C to -15°C
Enhancer	CAP Green	36	-25°C to -15°C
10× Primer I[a]	CAP White	60	-25°C to -15°C

[a] For use with 10× Primer II in any TELL-Seq Library Multiplex Primer Kit together for library amplification.

Box 2 of 2: TELL-Seq™ Microbial Library Reagent Box 2, V1 RUO (PN 100046)

Component Name	Cap Color	Volume (µL)	Storage Temperature
TELL Bead Plex	CAP Orange	76	2°C to 8°C
Wash Solution	CAP White	5000	2°C to 8°C
Stop Solution[b]	CAP Natural	960	2°C to 25°C

[b] Prior to use, if the Stop Solution is not clear or has white precipitates, warm the tube up at 37°C. Vortex to dissolve any precipitate. After the first use, store resuspended Stop Solution at room temperature for future use.

PRO TIP: One TELL-Seq™ Library Prep Kit, Standard Size including both Box 1 and Box 2 contains enough reagent for 24 library preps.

TELL-Seq™ Library Multiplex Primer (1-8) Kit (PN 100003)

Component Name	Cap Color	Volume (µL)	Storage Temperature
10× Primer II, T501	CAP Blue	15	-25°C to -15°C
10× Primer II, T502	CAP Black	15	-25°C to -15°C
10× Primer II, T503	CAP Green	15	-25°C to -15°C
10× Primer II, T504	CAP Yellow	15	-25°C to -15°C
10× Primer II, T505	CAP Violet	15	-25°C to -15°C
10× Primer II, T506	CAP Natural	15	-25°C to -15°C
10× Primer II, T507	CAP Red	15	-25°C to -15°C
10× Primer II, T508	CAP Orange	15	-25°C to -15°C

PRO TIP: **ONE** TELL-Seq Library Multiplex Primer (1-8) Kit contains enough reagent for 48 preps and can be used with **TWO** TELL-Seq™ Microbial Library Prep Kits, Standard Size.

TELL-Seq™ Library Multiplex Primer (9-16) Kit (PN 100009)

Component Name	Cap Color	Volume (µL)	Storage Temperature
10× Primer II, T509	CAP Blue	15	-25°C to -15°C
10× Primer II, T510	CAP Amber	15	-25°C to -15°C
10× Primer II, T511	CAP Green	15	-25°C to -15°C
10× Primer II, T512	CAP Yellow	15	-25°C to -15°C
10× Primer II, T513	CAP Violet	15	-25°C to -15°C
10× Primer II, T514	CAP Orange	15	-25°C to -15°C
10× Primer II, T515	CAP Red	15	-25°C to -15°C
10× Primer II, T516	CAP Natural	15	-25°C to -15°C

PRO TIP: **ONE** TELL-Seq Library Multiplex Primer (9-16) Kit contains enough reagent for 48 preps and can be used with **TWO** TELL-Seq™ Microbial Library Prep Kits, Standard Size.

TELL-Seq™ Library Multiplex Primer (17-24) Kit (PN 100010)

Component Name	Cap Color	Volume (µL)	Storage Temperature
10× Primer II, T517	CAP Amber	15	-25°C to -15°C
10× Primer II, T518	CAP Blue	15	-25°C to -15°C
10× Primer II, T519	CAP Yellow	15	-25°C to -15°C
10× Primer II, T520	CAP Green	15	-25°C to -15°C
10× Primer II, T521	CAP Black	15	-25°C to -15°C
10× Primer II, T522	CAP Violet	15	-25°C to -15°C
10× Primer II, T523	CAP Orange	15	-25°C to -15°C
10× Primer II, T524	CAP Red	15	-25°C to -15°C

PRO TIP: **ONE** TELL-Seq Library Multiplex Primer (17-24) Kit contains enough reagent for 48 preps and can be used with **TWO** TELL-Seq™ Microbial Library Prep Kits, Standard Size.

TELL-Seq™ Library Multiplex Primer C-series (1-96) Plate (PN 100043)

Contains 10× Primer II, C501 to C596 on a 96-well plate. All primers are at 10× ready-to-use concentration and 30µL per well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	C501	C509	C517	C525	C533	C541	C549	C557	C565	C573	C581	C589
B	C502	C510	C518	C526	C534	C542	C550	C558	C566	C574	C582	C590
C	C503	C511	C519	C527	C535	C543	C551	C559	C567	C575	C583	C591
D	C504	C512	C520	C528	C536	C544	C552	C560	C568	C576	C584	C592
E	C505	C513	C521	C529	C537	C545	C553	C561	C569	C577	C585	C593
F	C506	C514	C522	C530	C538	C546	C554	C562	C570	C578	C586	C594
G	C507	C515	C523	C531	C539	C547	C555	C563	C571	C579	C587	C595
H	C508	C516	C524	C532	C540	C548	C556	C564	C572	C580	C588	C596

NOTE: TELL-Seq Library Multiplex Primer C-series (1-96) Plate uses 10-base index sequences that differ from the TELL-Seq Library Multiplex Primer (1-8, 9-16, 17-24) kit (T-series) which uses 8-base index sequences.

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

NOTE: TELL-Seq Illumina sequencing primers are custom primers. Read 1, Read 2 and Index 1 primers are always needed for all Illumina sequencing systems. However, Index 2 primer may or may not be required depending on the system used.

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

TELL-Seq™ Microbial Library Prep Kit, HT **(2 Boxes)**

Box 1 of 2: TELL-Seq™ Microbial Library Reagent Box 1 HT, V1 RUO (PN 100047)

NOTE: Do not freeze and thaw Box 1 reagents for more than 6 times.

Component Name	Cap Color	Volume (µL)	Storage Temperature
5× Reaction Buffer	CAP Blue	720	-25°C to -15°C
Barcoding Enzyme	CAP Black	144	-25°C to -15°C
Cofactor II	CAP Amber	720	-25°C to -15°C
Exonuclease LC	CAP Yellow	144	-25°C to -15°C
Stabilizer LC	CAP Violet	144	-25°C to -15°C
Suspension Buffer EZ	CAP Natural	1080	-25°C to -15°C
Tagging Enzyme LC	CAP Red	288	-25°C to -15°C
2× PCR Master Mix	CAP Pink	1800	-25°C to -15°C
Enhancer	CAP Green	216	-25°C to -15°C
10× Primer I[a]	CAP White	360	-25°C to -15°C

[a] For use with 10× Primer II in the TELL-Seq Library Multiplex Primer Kit together for library amplification.

Box 2 of 2: TELL-Seq™ Microbial Library Reagent Box 2 HT, V1 RUO (PN 100048)

Component Name	Cap Color	Volume	Storage Temperature
TELL Bead Plex	CAP Orange	456 µL	2°C to 8°C
Wash Solution	CAP Blue	28.5 mL	2°C to 8°C
Stop Solution[b]	CAP White	5.76 mL	2°C to 25°C

[b] Prior to use, if the Stop Solution is not clear or has white precipitates, warm the tube up at 37°C. Vortex to dissolve any precipitate. After the first use, store resuspended Stop Solution at room temperature for future use.

PRO TIP: One TELL-Seq™ Microbial Library Prep Kit, HT including both Box 1 and Box 2 contains enough reagent for 144 library preps.

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

Component Name	Cap Color	Concentration	Volume (µL)	Storage Temperature
Read 1 Primer	CAP Black	100µM	300	-25°C to -15°C
Read 2 Primer	CAP White	100µM	300	-25°C to -15°C
Index 1 Primer	CAP Red	100µM	300	-25°C to -15°C
Index 2 Primer	CAP Yellow	100µM	300	-25°C to -15°C

NOTE: TELL-Seq Illumina sequencing primers are custom primers. Read 1, Read 2 and Index 1 primers are always needed for all Illumina sequencing systems. However, Index 2 primer may or may not be required depending on the system used.

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	24	v1 reagent: No; v1.5 reagent: Yes
HiSeq 3000/4000	12	Yes
HiSeq 2000/2500	30	No
NextSeq	48	Yes
MiSeq	96	No
MiniSeq	48	Yes

3. Consumables and Equipment (not provided)

Consumables

Consumable	Supplier
0.2 mL PCR tube or strip tube, RNase and DNase free	General lab supplier
20 µL pipette tip (standard and wide orifice)	General lab supplier
200 µL pipette tip (standard and wide orifice)	General lab supplier
Ethanol 200 proof (absolute) for molecular biology (500 mL)	Sigma-Aldrich, # E7023
Nuclease-free water	General lab supplier
AMPure XP	Beckman, # A63880
Agilent Bioanalyzer High Sensitivity DNA Analysis Kit*	Agilent, # 5067-4626
TapeStation High Sensitivity D5000 ScreenTape Assay*	Agilent, # 5067-5592, #5067-5593
Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific, # Q32851 or Q32854
Qubit Assay Tubes	Thermo Fisher Scientific, # Q32856
TE buffer, pH 8.0	General lab supplier

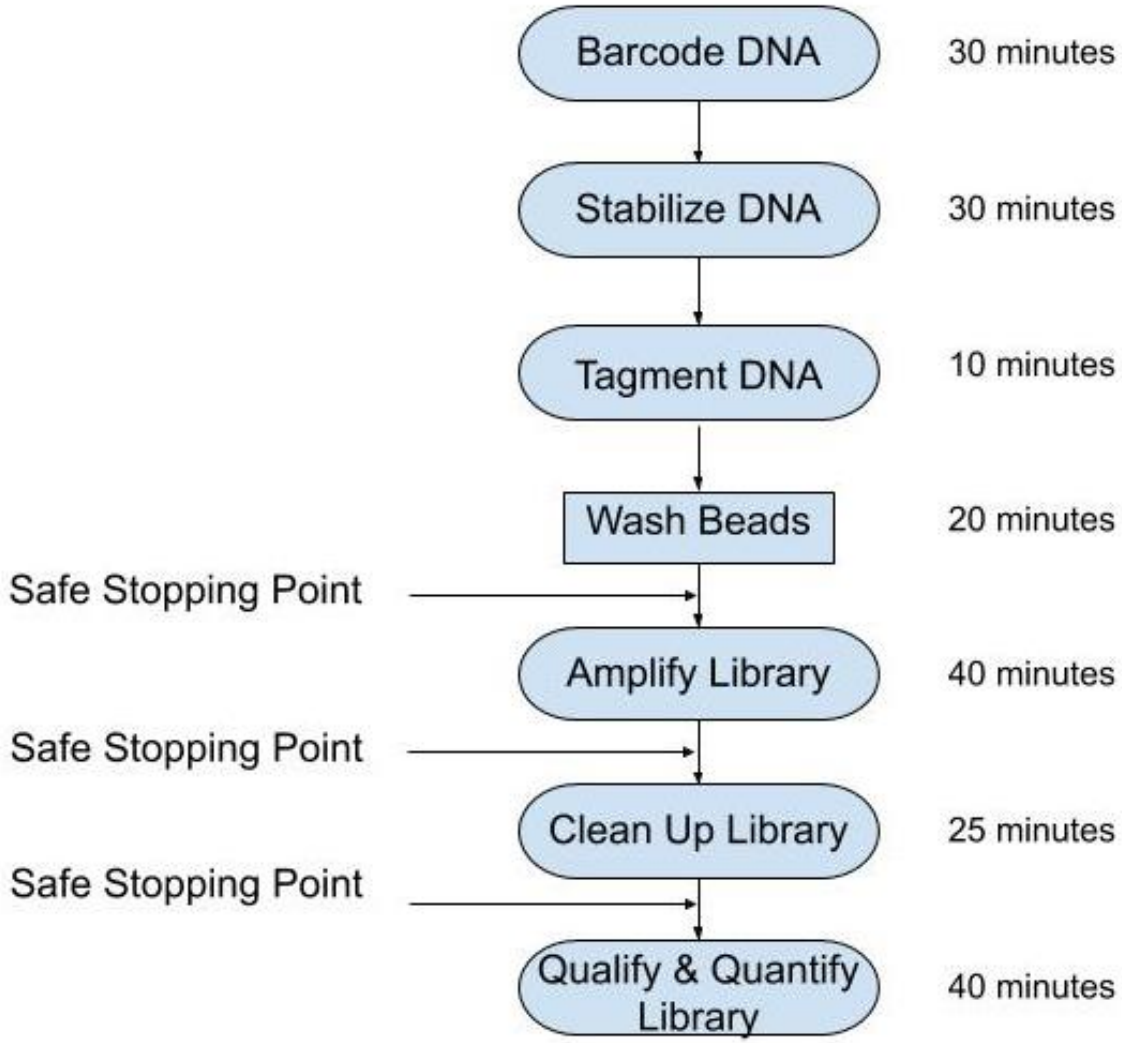
*Depends on which system is available in the user facility.

Equipment

Equipment	Supplier
Thermo Cycler	Applied Biosystems
Magnetic stand for 0.2 mL PCR tubes	General lab supplier
Tube Rotator	General lab supplier
Incubator (for 35°C)	General lab supplier
Vortexer	General lab supplier
Microcentrifuge	General lab supplier
Agilent Bioanalyzer*	Agilent
Agilent TapeStation*	Agilent
Qubit® Fluorometer 3.0 or higher	Thermo Fisher Scientific, # Q33216 or Q33218
Ice Bucket	General lab supplies

*Depends on which system is available in the user facility.

4. TELL-Seq™ Library Prep Workflow



5. Protocol

TELL-Seq microbial library prep kits are designed to generate up to 24 TELL-Seq WGS libraries using Standard Size kit and up to 144 TELL-Seq WGS libraries using HT kit for samples with genome sizes ranging from 1Mb to 50Mb. The following protocol describes library preparation procedures based on specified sample genome sizes.

CAUTION

TELL-Read pipeline v1.1 or above is required to analyze sequencing data generated from TELL-Seq libraries prepared with TELL Bead Plex.Barcode DNA

I. Samples

- Input genomic DNA (User)

Genome Size	Input Amount	Reaction Vol (µL)	Preps/ Standard Size Kit	Preps/ HT Kit
1 Mb – 20 Mb	0.4 ng	25	24	144
20 Mb – 50 Mb	0.6 ng	25	24	144

NOTE: Genomic DNA should be stored and diluted in a Tris buffer with pH ranging from 7.5 to 8.0 or a low TE buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

II. Preparation

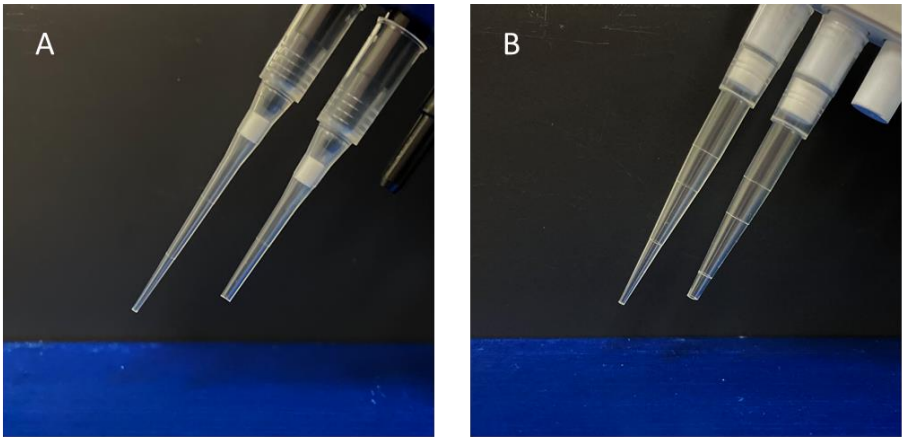
1. Prepare the following consumables:

Item	Location	Storage	Instruction
5× Reaction Buffer CAP	Box 1	-25°C to -15°C	Thaw at room temperature. Flick the tube with a finger 4 to 5 times to mix, then centrifuge briefly. Keep on ice until needed.
Cofactor II CAP	Box 1	-25°C to -15°C	Vortex to mix, then centrifuge briefly. Keep at **room temperature in the dark. Close the tube cap tightly after each use.**
Barcoding Enzyme CAP	Box 1	-25°C to -15°C	Centrifuge briefly. Keep on ice until needed.
TELL Bead Plex CAP	Box 2	2°C to 8°C	Centrifuge briefly. Keep on ice until needed. **Close the tube cap tightly after each use** to avoid any evaporation.
Suspension Buffer EZ CAP	Box 1	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep at **room temperature** until needed.
Nuclease-free water	User		Keep at room temperature.
0.2 mL PCR tube or strip tube	User		Room temperature.
20 µL wide orifice pipette tips	User		Room temperature.
200 µL wide orifice pipette tips	User		Room temperature.

2. Set up a tube rotator in a 35°C incubator.

CAUTION

Use wide orifice pipette tips to transfer and mix high molecular weight genomic DNA to avoid shearing the DNA. If wide orifice pipette tips are not available, cut 4mm-5mm off a standard pipette tip top with a sterile razor blade or a pair of sharp scissors before use.



A. 20uL pipette tips (uncut and cut) B. 200uL pipette tips (uncut and cut)

III. Procedure

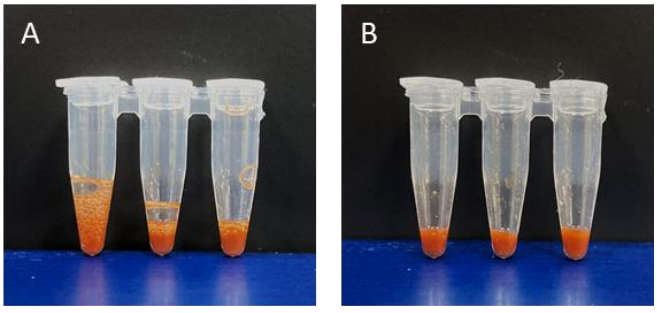
1. Vortex TELL Bead Plex vigorously for at least 30 seconds. Pulse spin (centrifuge for no more than 1 second) to bring down the bead solution present on the lid or sides of the tube. Right before use, pipet the TELL Bead or TELL Bead Plex with a 200 µL tip up and down 5 times to make sure all the beads are resuspended properly.
2. In a 0.2 mL PCR tube, assemble each reaction in the following order.

Reagent	Volume per reaction (µL)
	Total Volume = 25 µL
5× Reaction Buffer CAP	5
Nuclease-free water	3.5 – X (X is the DNA vol)
Cofactor II CAP	5
TELL Bead Plex CAP (~0.5M barcodes/µL)	3

3. Mix well by pipetting up and down for 10 times or vortexing vigorously for 5 seconds and pulse spin to bring the solution down to the bottom. Add appropriate amount of Barcoding Enzyme.

Reagent	Volume per reaction (µL)
Barcoding Enzyme CAP	1

4. Mix well by pipetting up and down for 8 times. Avoid introducing air bubbles by keeping the pipette tip at the bottom of the solution in the tube when pipetting.



A. Bubble issue B. Properly mixed

5. Using a wide orifice pipette tip, add the following reagents to the sample tube.

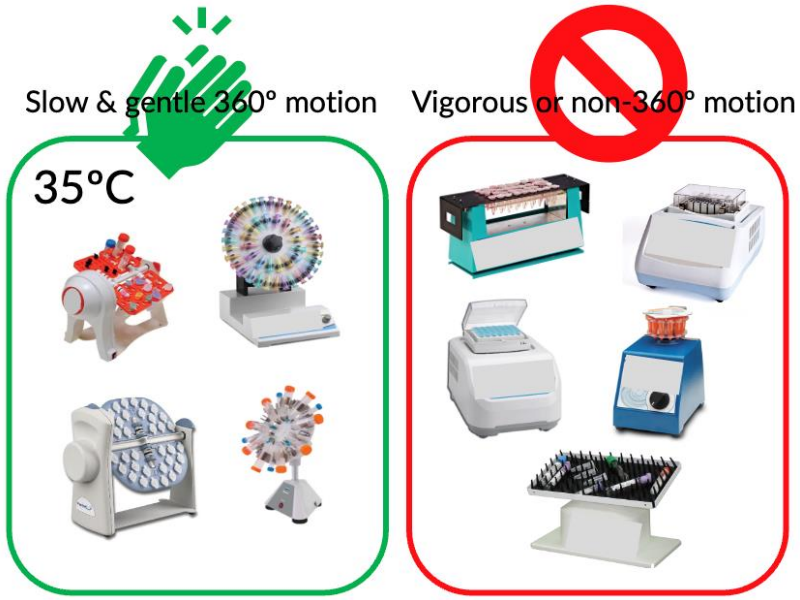
Reagent	Volume per reaction (µL)
Sample genomic DNA	X µL (>=1 AND ≤ 3.5 µL)
Suspension Buffer EZ CAP	7.5

NOTE: Suspension Buffer EZ is highly viscous. Use caution and pipette slowly to ensure that correct volume is delivered.

6. Set the pipette volume at 18µL. Using a wide orifice pipette tip, gently mix the solution by **slowly** pipetting up and down 6-8 times. Avoid introducing many air bubbles by keeping the pipette tip at the bottom of the solution in the tube when pipetting.
7. Place the sample tube on a tube rotator in the 35°C incubator and rotate slowly (10 -15 rpm) for 30 minutes.

Sample tubes placed on a Tube Rotator in a 35°C incubator.

Note: Proper tube rotation is critical to preserve HMW DNA properties and to facilitate the correct barcoding process. Recommended mixing systems are shown below (left side). Mixing systems that do not rotate or that generate vigorous shaking are incompatible with preservation of HMW DNA properties and TELL-Seq; some of these systems are also shown below (right side).



Stabilize DNA

I. Preparation

1. Prepare the following consumables:

Item	Location	Storage	Instruction
Stabilizer LC CAP	Box 1	-25°C to -15°C	Flick the tube 4 to 5 times to mix. Centrifuge briefly. Keep on ice until needed.

II. Procedure

1. Retrieve the sample tube from the 35°C incubator after incubation.
2. Add appropriate amount of Stabilizer into the tube.

Reagent	Volume per reaction (µL)
Stabilizer LC CAP	1

3. Set the pipette volume to 18 µL. Using a wide orifice pipette tip, gently mix the solution by **slowly** pipetting up and down 6-8 times. Avoid creating many bubbles.
4. Place the sample tube back on the tube rotator in the 35°C incubator and rotate it slowly (10 - 15 rpm) for 30 minutes.

Tagment DNA

I. Preparation

1. Prepare the following consumables:

Item	Location	Storage	Instruction
Tagging Enzyme LC CAP	Box 1	-25°C to -15°C	Flick the tube 4 to 5 times to mix. Centrifuge briefly. Keep on ice until needed.
Exonuclease LC CAP	Box 1	-25°C to -15°C	Flick the tube 4 to 5 times to mix. Centrifuge briefly. Keep on ice until needed.

II. Procedure

1. Retrieve the sample tube from the 35°C incubator after incubation.
2. Add Tagging Enzyme and Exonuclease into the tube.

Reagent	Volume per reaction (µL)
Tagging Enzyme LC CAP	1
Exonuclease LC CAP	1

3. Set the pipette to 18 µL. Using a wide orifice pipette tip, gently mix the solution by **slowly** pipetting up and down for 8 to 10 times. For this step, the mixing needs to be very thorough. Avoid creating many bubbles.
4. Place the sample tube back on the tube rotator in the 35°C incubator and rotate it slowly for 10 minutes.
5. Proceed to next step immediately after the incubation.

Wash Beads

I. Preparation

1. Prepare the following consumables:

Item	Location	Storage	Instruction
Stop Solution CAP Natural in the standard size kit CAP White in the HT kit	Box 2	2°C to 25°C	Check for any precipitates. If present, incubate the buffer at 37°C for 10 minutes, and vortex until they dissolve. **Store at room temperature** for future use.
Wash Solution CAP White in standard size kit CAP Blue in the HT kit	Box 2	2°C to 8°C	Bring to room temperature.
0.2 mL PCR tube or strip tube	User		Room temperature.

2. Set up a thermo cycler with the following program:
    - Set Preheat lid option to 100°C
    - 63°C forever

II. Procedure

1. Place the sample tube on a magnetic stand for 1 minute or until the solution is clear.
2. While the tube is on the magnetic stand, aspirate and discard the supernatant without disturbing beads.
3. Remove the tube from the magnetic stand. Add 60 μL Wash Solution to the sample tube. Pipet to resuspend the beads. If necessary, pulse spin to bring the solution down.
4. Place the sample tube back on the magnetic stand for 1 minute or until the solution is clear.
5. While the tube is on the magnetic stand, aspirate and discard the supernatant without disturbing beads.
6. Remove the tube from the magnetic stand. Add 40 μL of Stop Solution to the tube.
7. Pipet several times to resuspend the beads. If necessary, pulse spin to bring the solution down.
8. Incubate the tube at room temperature for 5 minutes.
9. Place the sample tube back on the magnetic stand for 1 minute or until the solution is clear.
10. While the tube is on the magnetic stand, aspirate and discard the supernatant without disturbing beads.
11. Remove the tube from the magnetic stand. Add 60 μL Wash Solution to the PCR tube. Pipet to resuspend the beads.
12. Transfer all the bead solution into a new 0.2ml PCR tube**.**
13. Incubate the tube at 63°C on the PCR thermocycler for 3 minutes.
14. Place the new sample tube on the magnetic stand at room temperature for 1 minute or until the solution is clear.
15. While the tube is on the magnetic stand, aspirate and discard the supernatant without disturbing beads.
16. Remove the tube from the magnetic stand. Add 60 μL Wash Solution to the PCR tube. Pipet to resuspend the beads. If necessary, pulse spin to bring the solution down.
17. Incubate the tube at 63°C on the PCR thermocycler for 3 minutes.
18. Place the sample tube on the magnetic stand at room temperature for 1 minute or until the solution is clear.
19. While the tube is on the magnetic stand, aspirate and discard the supernatant without disturbing beads. Use a P20 pipette to remove any remaining supernatant.
20. Remove the tube from the magnetic stand. Resuspend the beads in 20 μL of Wash Solution.

NOTE:

This is a **SAFE STOPPING POINT**. The washed beads can be stored at 2°C to 8°C for two weeks.

Amplify Library

I. Preparation

1. Prepare the following consumables:

Item	Location	Storage	Instruction
2× PCR Master Mix CAP	Box 1	-25°C to -15°C	Thaw at room temperature. Flick the tube 4 to 5 times to mix, then centrifuge briefly. Keep on ice.
10× Primer I CAP	Box 1	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
10× Primer II, T5## (T-series)	Multiplex Primer Kit	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
Enhancer CAP	Box 1	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep at room temperature.
Nuclease-free water	User	Room Temperature	Keep at room temperature.
0.2 mL PCR tube or strip tube	User		Room temperature.

CAUTION

For high throughput library preparation using Multiplex Primer C-series (1-96) Plate, please refer to the Appendix section, Usage of TELL-Seq Multiplex Primer Plate, for general guidance on plate handling to avoid cross contamination of index primers.

2. Set up the Library Amplification Program (**LAP**) on a thermo cycler as follows:
    - 63°C - 2 minutes
    - 72°C - 2 minutes
    - 98°C - 30 seconds
    - [98°C - 15 seconds, 63°C - 20 seconds, 72°C - 30 seconds] x Cycle Number
    - 72°C - 3 minutes
    - 4°C - forever

NOTE:

The deeper sequencing depth for each TELL Bead, will lead to a higher linked read density and better performance. Hence, for a fixed number of sequencing reads, the fewer TELL Beads used for library amplification, the deeper sequencing depth per bead, which will lead to a better linked read result. However, if too few TELL Beads were used for library amplification, the library complexity would be low, and sequencing read duplication level would be high.

For GC-rich genomes (GC>60%), amplify one more cycle than would be done for samples with low or medium GC content.

Genome Size (Input DNA)	Vol of Beads Used (B) for PCR	PCR Volume	Cycle Number
1 Mb – 10 Mb (0.4 ng)	5 μL	25 μL	13
10 Mb – 20 Mb (0.4 ng)	15 μL	25 μL	12
20 Mb – 50 Mb (0.6 ng)	20 μL	25 μL	11

PRO TIP: For *E. coli* (~5 Mb) samples, use 5 μL of TELL Beads and 13 cycles; for yeast (~12Mb) samples, use 15 μL of TELL Beads and 12 cycles.

II. Procedure

1. Vortex the beads vigorously for 10 seconds to resuspend. Pulse spin to bring the solution down. Using a 20 μL pipette tip, pipet the beads up and down 5 times to make sure all the beads are resuspended properly prior use. Immediately transfer the appropriate amount of bead solution (B in table above) to a new 0.2ml PCR tube.
2. If B ≤ 2 μL, proceed to Step 5 directly.
3. If B > 2 μL, place the PCR tube on a magnetic stand for 1 minute or until the solution is clear.
4. While the tube is on the magnetic stand, remove and discard (B-2) μL supernatant without disturbing beads. Remove the PCR tube from the magnet.
5. Add following reagents to the PCR tube containing the beads based on sample genome size.

Reagent	Volume per reaction (μL)
	Total Volume 25 μL
Nuclease-free water	4 μL
2× PCR Master Mix CAP	12.5 μL
10× Primer I CAP	2.5 μL
10× Primer II, T5##	2.5 μL
Enhancer CAP	1.5 μL

6. Mix well by vortexing or pipetting. Pulse spin to bring the solution down.

7. Place the tube on the thermal cycler and run the **LAP** program (Refer to step 2 of Preparation in Amplify Library section) with the appropriate number of cycles based on the genome size of the sample.
8. After PCR amplification, use 2 μL of PCR product for quality check on a Bioanalyzer or a TapeStation. See Qualify and Quantify Library section for instructions.

PRO TIP: If QC check shows the library yield is relatively low, put the tube with remaining PCR product back to the thermocycler and amplify for another one or two extra cycles before moving to Clean Up Library section.

> NOTE:
>
> This is a **SAFE STOPPING POINT**. The PCR product can be stored at -25°C to -15°C for one month.

Clean Up Library

I. Preparation

1. Prepare the following consumables:

Item	Location	Storage	Instruction
Ethanol 200 proof (absolute) for molecular biology	User	Room Temperature	
Fresh 75% (v/v) ethanol	User	Room Temperature	Require 400 μL per sample. Mix 1.5 mL Ethanol (200 proof) with 0.5 mL Nuclease-free water. Vortex to mix and keep at room temperature.
AMPure XP	User	2°C to 8°C	Bring it to room temperature for at least 20 minutes and vortex vigorously to resuspend the beads before use.
Nuclease-free water	User	Room Temperature	Keep at room temperature.
TE buffer, pH 8.0	User	Room Temperature	Keep at room temperature.
0.2 mL PCR tube or strip tube	User		Room Temperature

II. Standard Procedure (Single SPRI Cleanup)

1. Briefly centrifuge the sample PCR tube to bring all the solution down to the bottom of the tube.
2. Place the tube on the magnetic stand for 1 minute or until the solution is clear.
3. While the tube is on the magnetic stand, transfer the supernatant to a new 0.2 mL PCR tube without disturbing the beads.
4. Measure the volume of transferred supernatant (PCR product) with a pipette.
5. Add the following reagents into the PCR product tube to a total volume of 100 μL.

Reagent	Volume per reaction
PCR product	Measured Volume
Nuclease-free water	To final 100 μL total

6. Vortex vigorously to resuspend the AMPure XP solution and add 78 μL AMPure XP into the 100 μL PCR product.
7. Mix by pipetting up and down 10 times.
8. Incubate at room temperature for 5 minutes.
9. Place the tube on the magnetic stand for 1 minute or until the solution is clear.
10. Using a pipette, aspirate and discard the supernatant without disturbing AMPure beads.
11. While keeping the tube on the magnetic stand, add 200 μL of freshly prepared 75% ethanol into the tube. Let it incubate for 30 seconds.
12. Aspirate and discard the supernatant without disturbing beads.
13. Repeat steps 11-12 one more time, keeping the tube on the magnetic stand for the whole time. Use a P20 pipette to remove any remaining supernatant.
14. Keep the tube on the magnetic stand with the cap open and allow the tube to dry for 1-2 minutes to evaporate traces of ethanol. DON'T over-dry the beads.
15. Remove the tube from the magnetic stand and add 25 μL TE buffer to the beads.
16. Pipette or vortex to resuspend the beads. Incubate at room temperature for 5 minutes.
17. Place the tube on the magnetic stand for 1 minute or until the solution is clear.
18. With the tube is still on the magnetic stand, carefully transfer 23 μL of the supernatant to a new tube. Be careful not to disturb the beads.
19. The supernatant contains the TELL-Seq library. Proceed to **Qualify and Quantify Library section**.

NOTE:

This is a **SAFE STOPPING POINT**. The purified TELL-Seq library can be stored at -25°C to -15°C for six months.

Qualify and Quantify Library

I. Consumables

- Agilent High Sensitivity DNA Kit **or** TapeStation High Sensitivity D5000 ScreenTape Assay (User)
- Qubit dsDNA HS Assay Kit (User)
- Qubit assay tube (User)
- TE buffer, pH 8.0 (User)

> NOTE:
>
> Standard qPCR library quantitation assay for Illumina system works for TELL-Seq library, but it is not required.

II. Preparation

1. Prepare the necessary consumables as required by Bioanalyzer or TapeStation and Qubit.

III. Procedure

1. Use 1 μL of library for Agilent High Sensitivity DNA Kit or 2 μL of library for TapeStation High Sensitivity D5000 ScreenTape Assay.
2. Check the saved uncleaned PCR product from the Amplify Library section at the same time. Uncleaned PCR product may have a high level of primer dimer and adapter dimer. It requires a two-fold dilution with nuclease-free water before loading onto a Bioanalyzer chip or TapeStation tape to avoid interfering with lower marker signal.
3. To determine the library size, set the Region on the Bioanalyzer or TapeStation analysis software from 150 bp to 3000 bp. Record sample Average Size (bp) as Library Size. A good-sized library should have most library fragments under 1000 bp.
4. To estimate the library concentration, set the Region from 150 bp to 1000 bp. Record sample Concentration (nM) for this region (see Figure 1). This value is only used as an estimate or reference for pooling libraries when necessary. To accurately determine the library concentration, use Qubit measurement instead.

CAUTION

The concentration reading from the Bioanalyzer (or TapeStation) should be used as a starting point to make necessary dilution or library pooling for sequencing. Verify the concentration of the final diluted sequencing library or library pool with a Qubit dsDNA HS Assay kit (see Step 6).

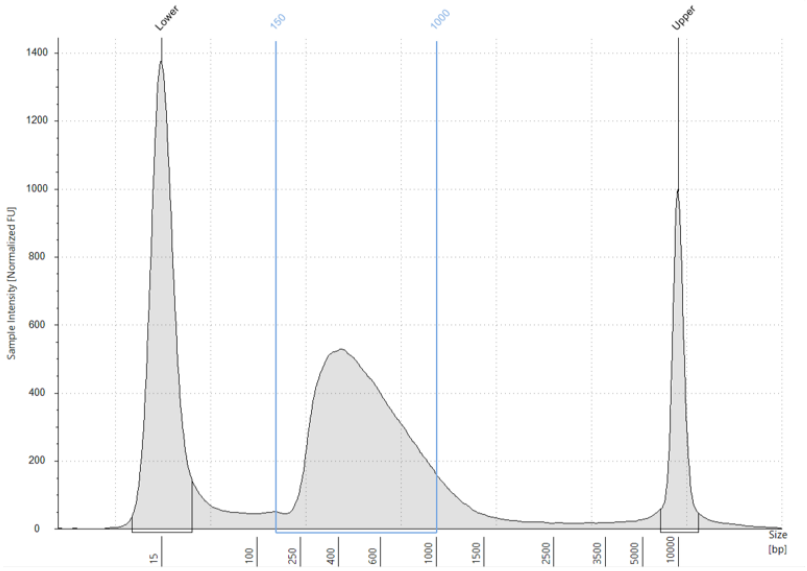


Figure 1. An example of cleaned up library profile from a TapeStation High Sensitivity D5000 ScreenTape assay.

5. Library can be sequenced immediately or stored at -25°C to -15°C.

> NOTE:
>
> Occasionally, there might be a detectable residual level of adapter dimer present in the cleaned-up library (see Figure 2). An additional round of Single SPRI Cleanup as described in Clean Up Library section is recommended in this case.

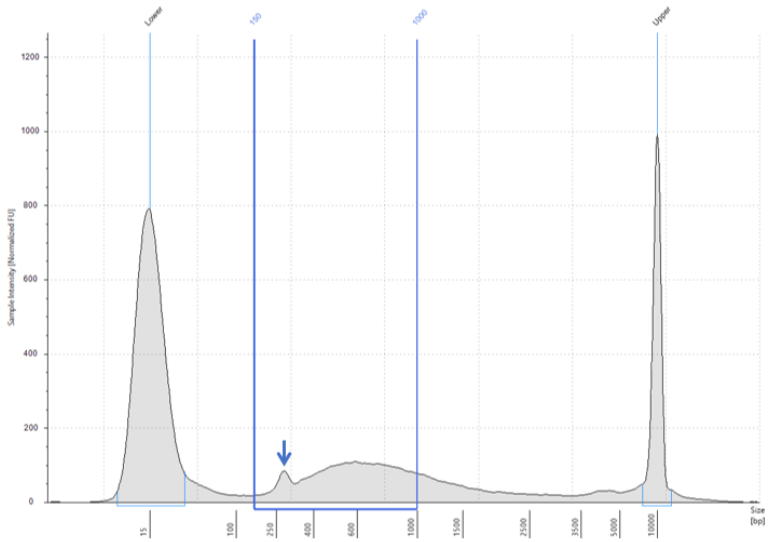


Figure 2. A library with detectable residual adapter dimer (arrow) after one round of single SPRI cleanup (TapeStation High Sensitivity D5000 ScreenTape assay).

6. When sequencing, dilute the library using TE buffer to the concentration recommended by each Illumina® sequencing system. Make a diluted library pool for sequencing if more than one library will be sequenced in the same run.
7. Use 4 μL diluted sequencing library or library pool to check the concentration with the Qubit dsDNA HS Assay Kit. Use the Library Size value measured from the Bioanalyzer (or TapeStation) for conversion of mass concentration into molar concentration.

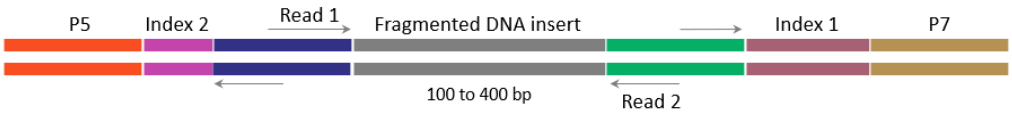
   A = Mass Concentration (ng/μL)
   S = Library Size (bp)

   Molar Concentration (nM) = (A*1,000,000)/(S*650)

8. Adjust the volume needed in the sequencing preparation if the library concentration measured by Qubit is different from the recommended concentration by more than 10%.

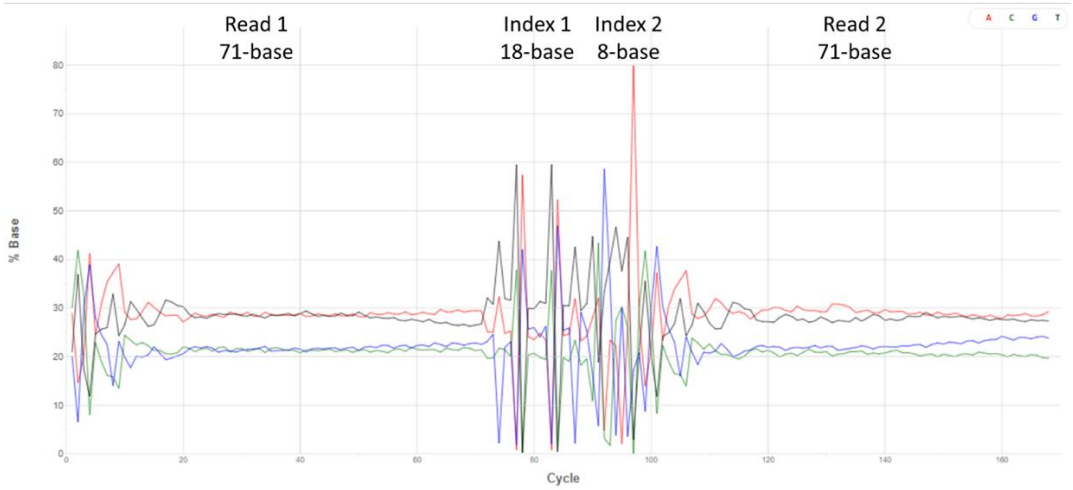
6. Appendix

TELL-Seq™ Library Structure and Sequencing Scheme



Index 1 contains 18-base TELL Bead sequences, which must be sequenced completely. Index 2 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 with TELL Bead & T-series Index II



Illumina® Sequencing Guidelines

CAUTION

TELL-Read pipeline v1.1 or above is required to analyze sequencing data generated from libraries prepared with TELL Bead Plex and works for data generated from standard TELL Bead as well.

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.

TELL-Seq Illumina Sequencing Primer Kits

Component Name	Concentration	Storage Temperature
Read 1 Primer	100 μM	-25°C to -15°C
Read 2 Primer	100 μM	-25°C to -15°C
Index 1 Primer	100 μM	-25°C to -15°C
Index 2 Primer	100 μM	-25°C to -15°C

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 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	v1 reagent: No; v1.5 reagent: Yes
HiSeq 3000/4000	Yes
HiSeq 2000/2500	No
NextSeq	Yes
MiSeq	No
MiniSeq	Yes

Illumina® Sequencing Read Length Recommendation

1. Paired end sequencing is recommended.
2. TELL-Seq library Index 1 is 18-base, Index 2 (T-series) 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 (except NovaSeq v1.5 reagents), 4-cycle for read 1 and 4-cycle for read 2 need to be deducted, respectively.
3. Recommended sequencing length is 2×96 PE with a 200-cycle kit or 2×146 PE with a 300-cycle kit for dual index run; 2x100 PE or 2x150 PE for a single sample run without need for Index 2 read.

4. For NovaSeq v1.5 reagents, there are extra reagents provided for index sequencing. Recommended sequencing length is 2×100 PE with a 200-cycle kit or 2×150 PE with a 300-cycle kit for dual index run.

Sequencing Depth Consideration

Adequate sequencing depth is required to get enough TELL Bead Plex coverage. The more TELL Beads Plex 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 Plex 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 Plex used and TELL-Seq library complexity required may depend on the genome size and application.

For *de novo* assembly of microbial genomes, approximately 100× genome coverage of the sample is recommended in general. For scaffolding application, 30× genome coverage is recommended.

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

Library Multiplex Primer	For Sample Sheet NovaSeq v1, MiSeq, HiSeq2000/2500	For Sample Sheet NovaSeq v1.5, Next Seq, MiniSeq, HiSeq3000/4000
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

Library Multiplex Primer Index Sequences (i.e., Index 2 Sequences): C-series (10-base)

Library Multiplex Primer	For Sample Sheet NovaSeq v1, MiSeq, HiSeq2000/2500	For Sample Sheet NovaSeq v1.5, Next Seq, MiniSeq, HiSeq3000/4000
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

Instructions for using the TELL-Seq Multiplex Primer Plate

When TELL-Seq Library Multiplex Primer C-series (1-96) Plate is used for high throughput library preparation, extra precautions are required to avoid cross-contamination of any primers. All primers (Primer II) are at the 10× ready-to-use concentration. The amount of primer in each well are sufficient for multiple reactions. If desirable, single use aliquots can be prepared from the TELL-Seq Library Multiplex Primer plate to avoid cross contamination.

CAUTION

C-series multiplex primers have 10-base index sequences and require 10-cycle index 2 sequencing.

Following instructions are served as a general guidance. Detailed operation procedure is required to be established by individual high throughput operator before using the plate.

1. Retrieve the Multiplex Primer C-series 96-well plate from -20°C storage.
2. Ensure that the multiplex primers (C501 – C596) are thawed with no visible ice present. Centrifuge the plate at 1000 rpm for 1 minute.
3. Carefully remove the adhesive plate seal or film.
4. Using a new pipette tip(s), pipette the required amount (depending on the sample genome size) of 10x Primer II from each well on the 96-well multiplex primer plate for the number of samples being processed.
5. Refer to the table below for individual multiplex primer locations on the plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	C501	C509	C517	C525	C533	C541	C549	C557	C565	C573	C581	C589
B	C502	C510	C518	C526	C534	C542	C550	C558	C566	C574	C582	C590
C	C503	C511	C519	C527	C535	C543	C551	C559	C567	C575	C583	C591
D	C504	C512	C520	C528	C536	C544	C552	C560	C568	C576	C584	C592
E	C505	C513	C521	C529	C537	C545	C553	C561	C569	C577	C585	C593
F	C506	C514	C522	C530	C538	C546	C554	C562	C570	C578	C586	C594
G	C507	C515	C523	C531	C539	C547	C555	C563	C571	C579	C587	C595
H	C508	C516	C524	C532	C540	C548	C556	C564	C572	C580	C588	C596

CAUTION

When preparing multiple sample libraries, ensure that appropriate multiplex primer II is added to the correctly labeled well to avoid sample mix up.

6. If processing less than 96 samples, circle the wells that are used with a marker to help indicate and visualize where the samples are located.
7. Slowly pipet up and down 5 times to mix.
8. Seal the plate with a new adhesive plate seal and centrifuge the plate at 1000 rpm for 1 minute.