UNIVERSAL SEQUENCING
innovation for all

TELL-Seq™ WGS Library Prep User Guide

For genome size from 1 Mb to 5 Gb

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

Document # 100005 v6
September 2021

Table of Contents

1. Introduction

This protocol explains how to prepare indexed paired-end TELL-Seq™ whole genome sequencing (WGS) libraries using a TELL-Seq WGS Library Prep Kit from a genomic DNA sample with genome size ranges from 1 Mb to 5 Gb for subsequent sequencing on an Illumina® sequencing system.

The TELL-Seq WGS library prep kit 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™ WGS Library Prep kit and protocol can:

- Generate TELL-Seq WGS libraries for genome size ranging from 1 Mb to 5 Gb
    - Standard Size kit

  12 paired end TELL-Seq libraries from samples with small genomes (1 Mb to 200 Mb)
  or 6 paired end TELL-Seq libraries from samples with medium sized genomes (200 Mb to 1 Gb)
  or 4 paired end TELL-Seq libraries from samples with large sized genomes (1 Gb to 5 Gb)
    - HT24 kit

  72 paired end TELL-Seq libraries from samples with small genomes (1 Mb to 200 Mb)
  or 36 paired end TELL-Seq libraries from samples with medium sized genomes (200 Mb to 1 Gb)
  or 24 paired end TELL-Seq libraries from samples with large sized genomes (1 Gb to 5 Gb)

- Use 0.5 ng to 5 ng genomic DNA input for standard input procedure
- Use as little as 0.1 ng DNA for ultralow input applications (Appendix)
- Produce barcode linked reads using an Illumina® sequencing system

Genomic DNA Input Recommendations

Genomic DNA inputs range from 0.1 ng to 5 ng based on the genome size. Use a fluorometric-based method to quantify input DNA. Avoid methods that only measure total nucleic acid, such as NanoDrop or other UV absorbance methods. If you use the Qubit dsDNA BR Assay Kit or HS Kit, use at least 2 µL of each DNA sample with 198 µL of the Qubit Working Solution. Genomic DNA should be stored in a Tris buffer with pH ranging from 7.5 - 8.0.

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

For linked read analysis, the longer the input genomic DNA length, the better the results for phasing and assembly. High molecular weight genomic DNA (greater than 20 Kb) is preferred in this protocol. Avoid breaking the HMW DNA during handling. Remove small molecular weight DNA (identified as a smear less than 10 Kb on a gel).

† Patent pending.

2. Kit Contents

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

Box 1 of 2: TELL-Seq™ WGS Library Reagent Box 1 (PN 100001)

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	48	-25°C to -15°C
Barcoding Enzyme	CAP Black	24	-25°C to -15°C
Cofactor II	CAP Amber	48	-25°C to -15°C
Exonuclease	CAP Yellow	12	-25°C to -15°C
Stabilizer	CAP Violet	12	-25°C to -15°C
Suspension Buffer	CAP Natural	36	-25°C to -15°C
Tagging Enzyme	CAP Red	24	-25°C to -15°C
2× PCR Master Mix	CAP Pink	150	-25°C to -15°C
Enhancer	CAP Green	18	-25°C to -15°C
10× Primer I[a]	CAP White	30	-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™ WGS Library Reagent Box 2 (PN 100002)

Component Name	Cap Color	Volume (µL)	Storage Temperature
TELL-Bead	CAP Orange	76	2°C to 8°C
Wash Solution	CAP White	4500	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, 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: **FOUR** TELL-Seq™ WGS Library Prep Kits, Standard Size including both Box 1 and Box 2 can pair with **ONE** of any TELL-Seq Library Multiplex Primer Kits.

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 primers to be used with **FOUR** TELL-Seq™ WGS 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 primers to be used with **FOUR** TELL-Seq™ WGS 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 primers to be used with **FOUR** TELL-Seq™ WGS Library Prep Kits, Standard Size.

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

TELL-Seq™ WGS Library Prep Kit, HT24 **(2 Boxes)**

Box 1 of 2: TELL-Seq™ WGS Library Reagent Box 1, HT24 (PN 100011)

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	288	-25°C to -15°C
Barcoding Enzyme	CAP Black	144	-25°C to -15°C
Cofactor II	CAP Amber	288	-25°C to -15°C
Exonuclease	CAP Yellow	72	-25°C to -15°C
Stabilizer	CAP Violet	72	-25°C to -15°C
Suspension Buffer	CAP Natural	216	-25°C to -15°C
Tagging Enzyme	CAP Red	144	-25°C to -15°C
2× PCR Master Mix	CAP Pink	900	-25°C to -15°C
Enhancer	CAP Green	108	-25°C to -15°C
10× Primer I[a]	CAP White	180	-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™ WGS Library Reagent Box 2, HT24 (PN 100012)

Component Name	Cap Color	Volume	Storage Temperature
TELL-Bead	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, 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: **TWO** TELL-Seq™ WGS Library Prep Kits, HT24 including both Box 1 and Box 2 can pair with **THREE** of any TELL-Seq Library Multiplex Primer Kits.

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

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	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 High Sensitivity DNA 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
TE buffer, pH 8.0	General lab supplier

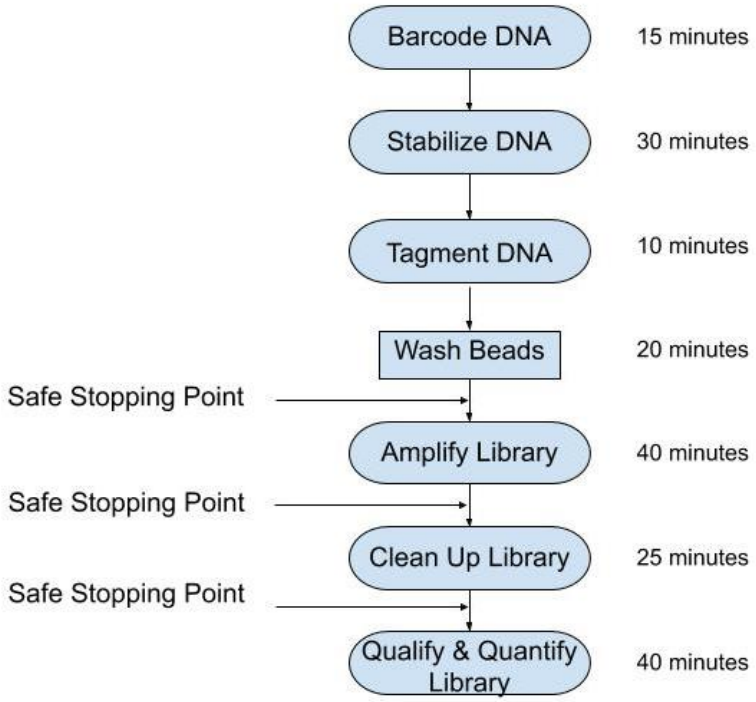
*Depends on which system is available in the user facility.

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	Thermo Fisher Scientific, # Q33216, Q33217 or Q33218
Ice Bucket	General lab supplier

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

4. TELL-Seq™ Library Prep Workflow



5. Protocol

TELL-Seq WGS library prep kits are designed to generate up to 12 TELL-Seq libraries using Standard Size kit and up to 72 TELL-Seq libraries using HT24 kit (see table below). The following protocol describes library preparation procedures based on specified sample genome sizes. All other unspecified conditions will apply to all genome sizes.

Barcode DNA

I. Consumables

- Input genomic DNA (User)

Genome Size	Input Amount	Reaction Vol (µL)	Preps/ Standard Size Kit	Preps/ HT24 Kit
1 Mb – 50 Mb	0.5 ng	22	12	72
50 Mb – 100 Mb	1 ng	22	12	72
100 Mb – 200 Mb	1.5 ng	22	12	72
200 Mb – 1 Gb	2 – 3 ng	44	6	36
1 Gb – 5 Gb	3 – 5 ng	66	4	24

PRO TIP: Use 5 ng of input DNA for a human genome.

NOTE:

1. 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).

2. The recommended amount of input genomic DNA is based on an average fragment size of ⩾ 30 Kb. If the average size of genomic DNA is ~15 Kb only, reduce the amount of input per reaction to half of the recommended value.

- 5× Reaction Buffer (Kit Box 1, CAP Blue)
- Cofactor II (Kit Box 1, CAP Amber)
- Barcoding Enzyme (Kit Box 1, CAP Black)
- TELL Bead (Kit Box 2, CAP Orange)
- Suspension Buffer (Kit Box 1, CAP Natural)
- Nuclease-free water (User)
- 0.2 mL PCR tube or strip tube (User)
- 20 µL and 200 µL wide orifice pipette tips (User)

II. Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
5× Reaction Buffer CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
Cofactor II CAP	-25°C to -15°C	Vortex to mix, then centrifuge briefly. Keep at **room temperature** but **avoid light.** **Close the tube cap tightly after each use.**
Barcoding Enzyme CAP	-25°C to -15°C	Centrifuge briefly. Keep on ice.
TELL Bead CAP	2°C to 8°C	Centrifuge briefly. Keep on ice. **Close the tube cap tightly after each use** to avoid any evaporation.
Suspension Buffer CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep at **room temperature**.
Nuclease-free water	Room Temperature	Keep at 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 breaking the DNA. If wide orifice pipette tips are not available, cut 4mm-5mm off a standard pipette tip top with a clean razor blade before use.

III. Procedure

1. Vortex TELL Bead vigorously for at least 30 seconds. Then centrifuge for no more than 1 second to bring down the solution. Right before use, pipet the TELL Bead 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)		
	Small Genome (22 µL)	Medium Genome (44 µL)	Large Genome (66 µL)
5× Reaction Buffer CAP	4	8	12
Nuclease-free water	3 – X (X is the DNA vol)	6 – Y (Y is the DNA vol)	8 – Z (Z is the DNA vol)
*Cofactor II CAP	4	8	12
TELL Bead CAP (0.5M barcodes/µL)	6	12	19

* Note that the volume of Cofactor II used is different from the volume of Cofactor in the early version of kit.

3. Mix well by pipetting or vortexing vigorously and centrifuge for 1 second to bring the solution down to bottom. Add Barcoding Enzyme.

Reagent	Volume per reaction (µL)		
	Small Genome	Medium Genome	Large Genome
Barcoding Enzyme CAP	2	4	6

4. Mix well by pipetting and avoid making bubbles during mixing.
5. Use a wide orifice pipette tip, add following reagent.

Reagent	Volume per reaction (µL)		
	Small Genome	Medium Genome	Large Genome
Sample genomic DNA	X µL (≤ 3 µL)	Y µL (≤ 6 µL)	Z µL (≤ 8 µL)
Suspension Buffer CAP	3	6	9

6. Use a wide orifice pipette tip, set pipette volume based on the genome size of the sample (small-15 µL, medium-30 µL, large-45 µL); gently mix the solution by **slowly** pipetting up and down 6-8 times. Avoid creating many bubbles.
7. Put the tube on a tube rotator in a 35°C incubator and rotate slowly (10 - 15 rpm) for 15 minutes.

Stabilize DNA

I. Consumables

- Stabilizer (Kit Box 1, CAP Violet)

II. Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
Stabilizer CAP	-25°C to -15°C	Centrifuge briefly. Keep on ice.

2. Use the same tube rotator in the 35°C incubator.

III. Procedure

1. Take the PCR tube out of the 35°C incubator.
2. Add Stabilizer into the tube.

Reagent	Volume per reaction (µL)		
	Small Genome	Medium Genome	Large Genome
Stabilizer CAP	1	2	3

3. Use a wide orifice pipette tip, set pipette volume based on the genome size of the sample (small-15 µL, medium-30 µL, large-45 µL); gently mix the solution by **slowly** pipetting up and down 6-8 times. Avoid creating many bubbles.
4. Put the tube back on the tube rotator in the 35°C incubator and rotate it slowly (10 - 15 rpm) for 30 minutes.

Tagment DNA

IV. Consumables

- Tagging Enzyme (Kit Box 1, CAP Red)
- Exonuclease (Kit Box 1, CAP Yellow)

V. Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
Tagging Enzyme CAP	-25°C to -15°C	Centrifuge briefly. Keep on ice.
Exonuclease CAP	-25°C to -15°C	Centrifuge briefly. Keep on ice.

2. Use the same tube rotator in the 35°C incubator.

VI. Procedure

1. Take the PCR tube out of the 35°C incubator.
2. Add Tagging Enzyme and Exonuclease into the tube.

Reagent	Volume per reaction (µL)		
	Small Genome	Medium Genome	Large Genome
Tagging Enzyme CAP	1	2	3
Exonuclease CAP	1	2	3

3. Use a wide orifice pipette tip, set volume based on the genome size of the sample (small-15 µL, medium-30 µL, large-45 µL); gently mix the solution by **slowly** pipetting up and down for 8 times. For this step, the mixing needs to be very thorough and the pipetting does NOT need to be as gentle as in the Barcoding and Stabilizing DNA steps.
4. Put the tube back on the tube rotator in the 35°C incubator and rotate it slowly for 10 minutes. When necessary, different amount of Tagging Enzyme can be used to adjust the library size.

> NOTE: If a longer insert library is preferred, less amount of Tagging Enzyme can be used in the reaction. On the other hand, if a shorter insert library is preferred, twice as much as of Tagging Enzymes can be used in the reaction.

5. Proceed to next step immediately after the incubation.

Wash Beads

I. Consumables

- Stop Solution (Kit Box 2, CAP Natural in the standard size kit, CAP White in the HT24 kit, or stored at room temperature after the first use)
- Wash Solution (Kit Box 2, CAP White in the standard size kit, CAP Blue in the HT24 kit)
- 0.2 mL PCR tube or strip tube (User)

II. Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
Stop Solution	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. Keep at room temperature for future use.
Wash Solution	2°C to 8°C	Bring to room temperature.

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

III. Procedure

1. Put the PCR 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 125 µL Wash Solution to the PCR tube. Pipet to resuspend the beads. If necessary, bring solution down with a quick ~1 second spin in the centrifuge.
4. Put the PCR 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 80 µL of Stop Solution to the tube.
7. Pipet several times to resuspend the beads. If necessary, bring solution down with a quick ~1 second spin in the centrifuge.
8. Incubate the tube at room temperature for 5 minutes.
9. Put the PCR 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 125 µL Wash Solution to the PCR tube. Pipet to resuspend the beads.

12. **Transfer all the bead solution into a new PCR tube.**
13. Incubate the tube at 63°C on the PCR thermocycler for 3 minutes.
14. Put the PCR 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 125 µL Wash Solution to the PCR tube. Pipet to resuspend the beads. Centrifuge for 1 second to bring all solution down when necessary.
17. Incubate the tube at 63°C on the PCR thermocycler for 3 minutes.
18. Put the PCR 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.
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. Consumables

- 2× PCR Master Mix (Kit Box 1, CAP Pink)
- 10× Primer I (Kit Box 1, CAP White)
- 10× Primer II, T5## (Multiplex Primer Kit)
- Enhancer (Kit Box 1, CAP Green)
- Nuclease-free water (User)
- 0.2 mL PCR tube or strip tube (User)

II. Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
2× PCR Master Mix CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
10× Primer I CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
10× Primer II, T5##	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
Enhancer CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep at room temperature.

Nuclease-free water	Room Temperature	Keep at room temperature.

2. Set up Library Amplification Program (**LAP**) on a thermo cycler as following:
    - 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 read output, 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 GC content.

Genome Size	Vol of Beads Used (B) for PCR	PCR Volume	Cycle Number
1 Mb – 50 Mb	1 – 12 μL	25 μL	14 – 11
50 Mb – 100 Mb	6 – 10 μL	25 μL	12 – 10
100 Mb – 200 Mb	10 – 20 μL	25 μL	12 – 10
200 Mb – 1 Gb	15 – 20 μL	50 μL	11 – 9
1 Gb – 5 Gb	12 – 20 μL	75 μL	10 – 9

PRO TIP:

a) For *E. coli* (4.6Mb) use 1.5 μL of TELL Beads and 13 cycles.
b) For human (3Gb) use 20 μL of TELL Beads and 9 cycles.

III. Procedure

1. Vortex beads vigorously for 10 seconds to resuspend the beads. Bring solution down with a quick ~1 second spin in the centrifuge. Using a 20 μL tip, pipet the beads up and down 5 times to make sure all the beads are resuspended properly. Immediately transfer the proper amount of bead solution (B in table above) to a new PCR tube.
2. If B ≤ 2 μL, go to Step 5 directly.
3. If B > 2 μL, put 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)		
	Small Genome (25 µL)	Medium Genome (50 µL)	Large Genome (75 µL)
Nuclease-free water	4 µL	10 µL	16 µL
2× PCR Master Mix CAP	12.5 µL	25 µL	37.5 µL
10× Primer I CAP	2.5 µL	5 µL	7.5 µL
10× Primer II, T5##	2.5 µL	5 µL	7.5 µL
Enhancer CAP	1.5 µL	3 µL	4.5 µL

6. Mix well by vortexing or pipetting. Bring solution down with a quick ~1 second spin in the centrifuge.
7. Place the tube on the thermal cycler and run the **LAP** program (see above) with proper number of cycles.
8. After PCR amplification, save 2 µL PCR product for quality check on a Bioanalyzer or a TapeStation. See Qualify and Quantify Library section for instruction.

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. Consumables

- AMPure XP (User)
- Ethanol 200 proof (absolute) for molecular biology (User)
- Nuclease-free water (User)
- TE buffer, pH 8.0 (User)
- 0.2 mL PCR tube or strip tube (User)

II. Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
Fresh 75% (v/v) ethanol	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	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	Room Temperature	Keep at room temperature.
TE buffer, pH 8.0	Room Temperature	Keep at room temperature.

III. Procedure

1. Bring solution down with a quick ~1 second spin in the centrifuge.
2. Put the PCR 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 beads.
4. Measure the volume of transferred supernatant (PCR product) with a pipette.
5. Add following reagents into the PCR product to a total volume of 100 μL.

Reagent	Volume per reaction
PCR product	20 to 75 μL
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. Put the tube on the magnetic stand for 1 minute or until the solution is clear.
10. Aspirate and discard the supernatant without disturbing AMPure beads.
11. While keeping the tube on the magnetic stand, add 200 μL freshly prepared 75% ethanol into the tube. Let it sit 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.
14. Leave the tube on the magnetic stand with cap open and allow the tube to dry for 1-2 minutes to evaporate traces of ethanol. DON'T over dry the beads.
15. Take the tube off the magnetic stand and add 25 μL TE buffer to the beads.
16. Pipette or vortex to resuspend the beads. Let it sit for 5 minutes.
17. Put the tube on the magnetic stand for 1 minute or until the solution is clear.
18. Recover 23 μL of the supernatant to a new tube. Be careful not to disturb the beads.
19. The supernatant contains the TELL-Seq library.

> 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)
- 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 concentration, set the Region on the Bioanalyzer or TapeStation analysis software from 150 bp to 1000 bp. Record sample Concentration (nM) for this region (see Figure 1). To determine the library size, set the Region 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.

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).

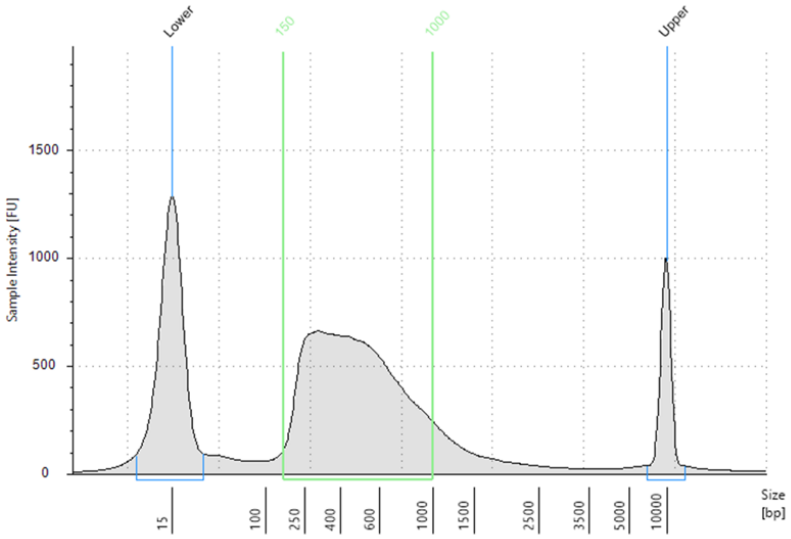


Figure 1. An example of cleaned up library profile from a TapeStation High Sensitivity D5000 ScreenTape assay.

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

> NOTE:
>
> Occasionally, there is a detectable residual level of adapter dimer in a cleaned-up library (see Figure 2). An additional round of AMPure XP cleanup as described in Clean Up Library section is recommended in this case.

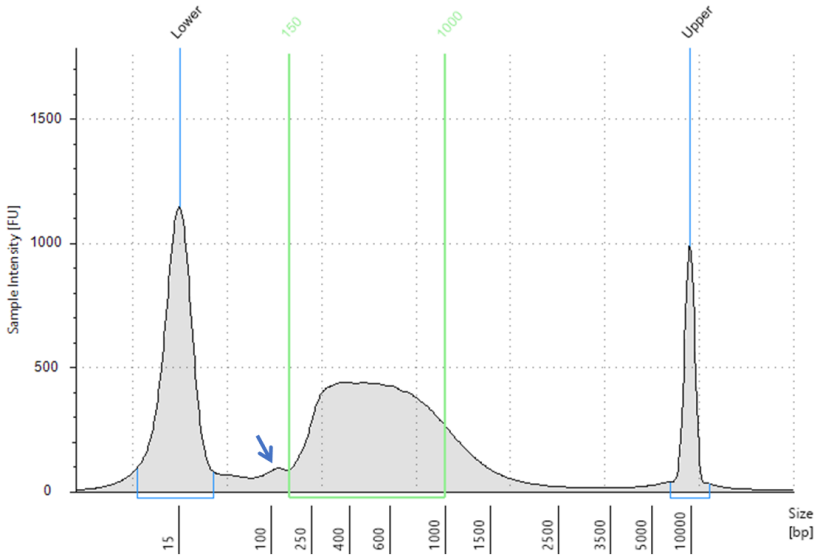


Figure 2. A library with detectable residual adapter dimer (arrow) after cleanup (TapeStation High Sensitivity D5000 ScreenTape assay).

5. When sequencing, dilute the library using TE buffer to the concentration recommended by each Illumina® sequencing system. Make diluted library pool for sequencing if more than one library will be sequenced in the same run.
6. 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 (nM).

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

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

7. 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

Ultralow Input TELL-Seq™ Library Preparation

In a standard input TELL-Seq library procedure above, there will be 3 to 8 high molecular weight DNA fragments captured by one TELL Bead on average. When available sample DNA is rare or some cases, such as, mixed samples or targeted sequencing, prefer one HMW DNA fragment input to one TELL Bead (i.e. one unique barcode), the standard library protocol can be adjusted with lower input DNA and/or more TELL Beads to decrease the HMW DNA to TELL Bead ratio. Below are modified conditions for ultralow input TELL-Seq library preparation for small genome. All the other steps should follow the standard input library preparation procedure for small genome without changes.

- DNA input for small genome

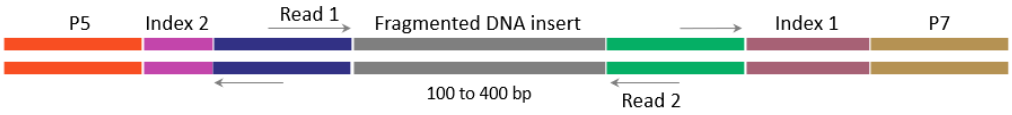
Genome Size	Input Amount	Reaction Vol (μL)
1 Mb – 15 Mb	0.1 ng	22
16 Mb – 30 Mb	0.2 ng	22

- Volume of TELL Beads used in library amplification for small genome

Genome Size	Vol of Beads Used (B) for PCR	PCR Volume	Cycle Number
1 Mb – 15 Mb	5 - 20 μL	25 μL	14
16 Mb – 30 Mb	10 – 20 μL	25 μL	13

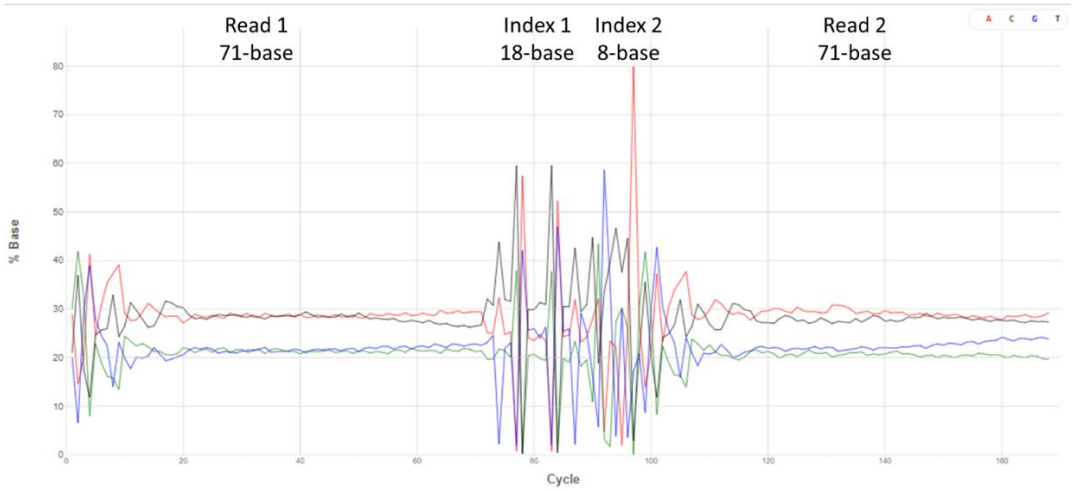
PRO TIP: For *E. coli* (4.6Mb) use 7.5 μL of TELL Beads and 14 cycles.

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



Illumina® Sequencing Guide

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 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.

Sequencing Depth Consideration

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.

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.

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

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.

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

Revision History

Doc #C01K001 Rev. A	October 2019	Initial Release
Doc #CO1K001 Rev. B	December 2019	Added storage instructions
Doc #CO1K001 Rev. C	February 2020	Added storage instructions
Doc #100005 v4	June 2020	Reaction volume changes
Doc #100005 v5	August 2020	Included high throughput kits and library amplification cycle number modification
Doc #100005 v6	September 2021	Updated sequencing primer instruction for NovaSeq v1.5 reagents