UNIVERSAL SEQUENCING TELL-Seq Target Enrichment

UNIVERSAL SEQUENCING TELL-Seq Target Enrichment User Guide

Contents

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Specifications:

- Product Name: TELL-SeqTM Target Enrichment
- For: Research Use Only, not for diagnostic procedures
- Manufacturer: Universal Sequencing Technology Corporation
- Required Genomic DNA: 5ng
- Storage Conditions: Tris buffer pH 7.5-8.0 or low TE buffer

Product Usage Instructions

Genomic DNA Input Recommendations:
Ensure you have at least 5ng of genomic DNA for the protocol. High molecular weight DNA is crucial for successful sequencing. Quantify DNA using a fluorometric-based method like Qubit dsDNA BR Assay Kit or HS Kit. Dilute concentrated DNA to working concentration (0.4ng/l to 1ng/l) in Tris buffer before measurement. Store genomic DNA in Tris buffer with pH 7.5-8.0 or low TE buffer.

Kit Contents:

TELL-SeqTM Library Prep Kit, Standard Size consists of two boxes:

- **Box 1:** Contains various reagents including Barcoding Enzyme, Exonuclease, and more.
- **Box 2:** Contains TELL Bead Plexb, Wash Solution, and Stop Solution.

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

FAQ:

1. **Q: Can I use methods other than fluorometric-based ones to quantify DNA?**
   A: It is recommended to use a fluorometric-based method like Qubit dsDNA BR Assay Kit or HS Kit for accurate measurement. Avoid methods that only measure total nucleic acid concentration.
2. **Q: How should I store genomic DNA for optimal results?**
   A: 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) for best results.

For Research Use Only. Not for use in diagnostic procedures.
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August 2024

Revision History

Doc #100032-USG v1.0	November 2021	Initial Release
Doc #100032-USG v2.0	August 2022	Protocol update to work with updated TELL- Seq™ Lib rary Prep kit V1 with Suspension Buffer EZ and TELL Bead Plex option
Doc # 100032-USG v3.0	August 2023	Removed TELL Bead option. Only TELL Bead Plex is used moving forward. Added a Note and a picture with recommended mixing systems for a critical step of pro per tube rotation during barcoding process to preserve high molecular weight DNA properties.
Doc # 100032-USG v4.0	August 2024	Changed user guide for general Target Enrichment. Ad ded additional information about using TELL-Seq™ Lib rary Prep with other Target Enrichment systems

Introduction

This protocol explains how to prepare target enriched indexed paired-end TELL-Seq™ libraries by hybridization capture using a combination of TELL-Seq™ Library Prep Kit and Agilent® SureSelect Target Enrichment System. TELL-Seq™ Library Prep Kit system is also compatible with other Target Enrichment systems such as IDT and Twist Biosciences.
The TELL-Seq™ 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. Agilent® SureSelect Target Enrichment System allows for the enrichment of targeted regions using highly specific capture probes. Each Target Enrichment System has a unique panel of probes that allow for specific regions to be captured that is compatible with TELL-Seq™ libraries.

Genomic DNA Input Recommendations
5ng Genomic DNA is required for this protocol. High molecular weight (HMW) DNA is critical for successful TELL-Seq™ sequencing.

- For human genome, minimum sample DNA size should be greater than 40Kb.
- HMW DNA ranging from 100Kb to 300Kb are optimal material for best human phasing application.
- 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.

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 (0.4ng/µl to 1ng/µl) in a Tris buffer (pH 7.5-8.0) several hours to a day before the concentration measurement and library preparation. 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). 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.

Kit Contents

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

Box 1 of 2: TELL-Seq™ Library Reagent Box 1 V1 (PN 100035)

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	CAP Yellow	12	-25°C to -15°C
Stabilizer	CAP Violet	12	-25°C to -15°C
Suspension Buffer EZ	CAP Natural	180	-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 the TELL-Seq™ Library Multiplex Primer Kit together for library amplification.

Box 2 of 2: TELL-Seq™ Library Reagent Box 2 V1 (PN 100036)

Component Name	Cap Color	Volume (mL)	Storage Temperature
TELL Bead Plex[b]	CAP CAP Orange	76	2°C to 8°C
Wash Solution	CAP CAP White	4500	2°C to 8°C
Stop Solution[c]	CAP CAP Natural	960	2°C to 25°C

b TELL Bead Plex works well on both Illumina and non-Illumina Sequencing Systems.
c 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.

⚠ CAUTION

TELL-Read pipeline v1.1 or above is required to analyze sequencing data generated from TELL-Seq™ libraries

prepared with TELL Bead Plex.

TELL-Seq™ Library Prep Kit, HT24 (2 Boxes)
Box 1 of 2: TELL-Seq™ Library Reagent Box 1 V1, HT24 (PN 100037)

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	Blue	720	-25°C to -15°C
Barcoding Enzyme	Black	144	-25°C to -15°C
Cofactor II	Amber	720	-25°C to -15°C
Exonuclease	Yellow	72	-25°C to -15°C
Stabilizer	Violet	72	-25°C to -15°C
Suspension Buffer EZ	Natural	1080	-25°C to -15°C
Tagging Enzyme	Red	144	-25°C to -15°C
2× PCR Master Mix	Pink	900	-25°C to -15°C
Enhancer	Green	108	-25°C to -15°C
10× Primer I[a]	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™ Library Reagent Box 2 V1, HT24 (PN 100038)

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

b TELL Bead Plex works well on both Illumina and non-Illumina Sequencing Systems.
c Prior to use, if the Stop Solution is not clear or has white precipitates, warm the tube up at 37 ºC. Vortex to dissolve any precipitate. After the first use, store resuspended Stop Solution at room temperature for future use.

PRO TIP: TWO TELL-Seq™ Library Prep Kits, HT24 including both Box 1 and Box 2 can pair with of any TELL-Seq™ Library Multiplex Primer Kits.

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

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

Component Name	Cap Color	Volume (µL)	Storage Temperature
10× Primer II, T501	Blue	15	-25°C to -15°C
10× Primer II, T502	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.

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 (mL)	Storage Temperature
Read 1 Primer	CAP Black	100mM	50	-25°C to -15°C

Read 2 Primer	CAP White	100mM	50	-25°C to -15°C
Index 1 Primer	CAP Red	100mM	50	-25°C to -15°C
Index 2 Primer	CAP Yellow	100mM	50	-25°C to -15°C

TELL-Seq™ Target Blocker (PN 100019)

Component Name	Cap Color	Volume (mL)	Storage Temperature
TELL-Seq™ Target Blocker	CAP White	40	-25°C to -15°C

Consumables and Equipment (not provided)

Consumables

Consumable	Supplier
0.2 mL PCR tube or strip tube	General lab supplier
20 mL pipette tip (standard and wide orifice)	General lab supplier
200 mL 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
Dynabeads MyOne Streptavidin T1	Thermo Fisher Scientific, # 65601, 6 5602 or 65603
Agilent SureSelect XT HS Capture Library Human All Exon 7	Agilent, # G9704N, G9705N or G970 6N
Agilent SureSelect XT HS Target Enrichment Kit, ILM Hyb Module (Post PCR), 16 Rxn	Agilent, #G9916B
TE buffer, pH 8.0	General lab supplier

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

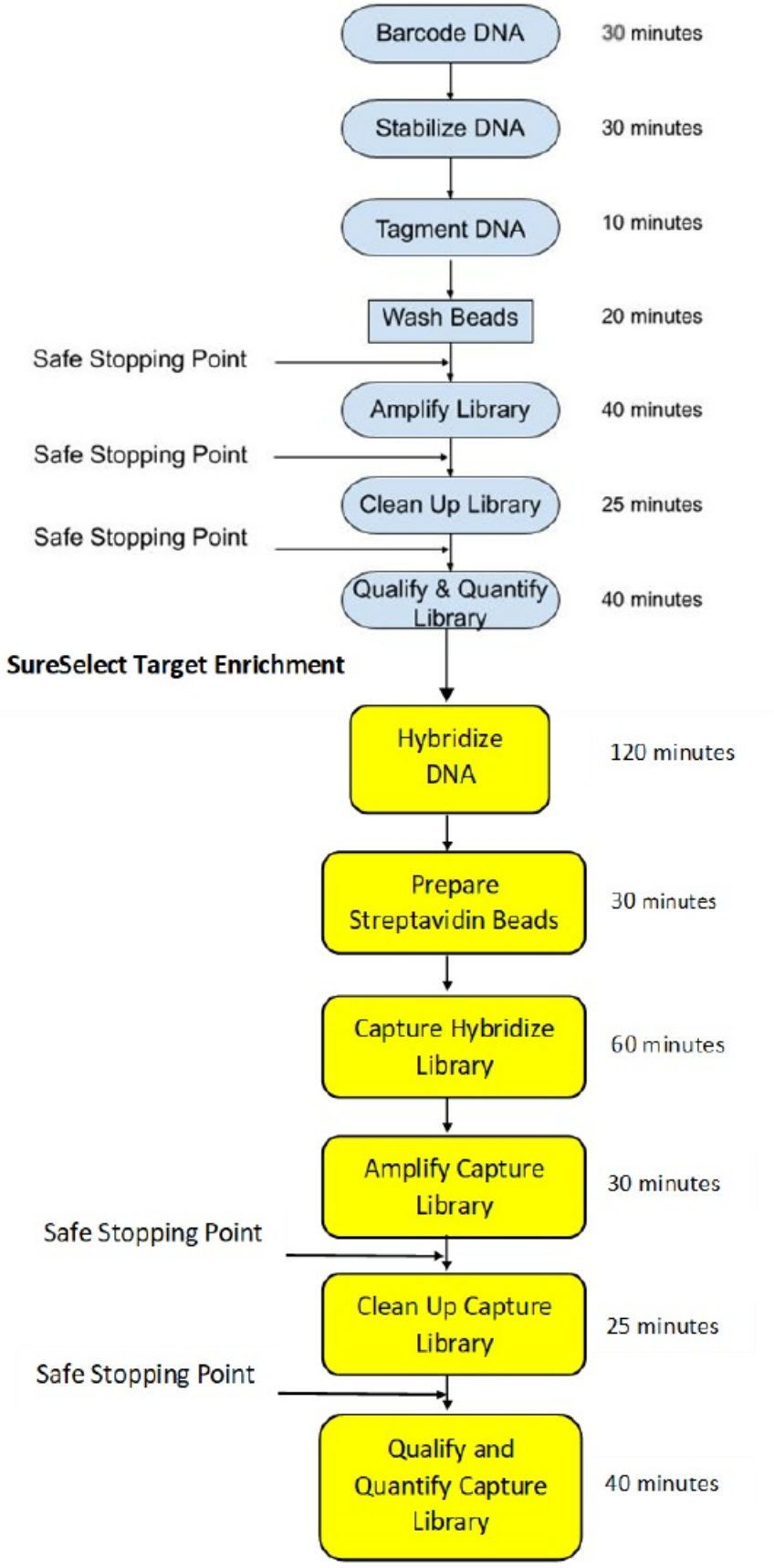
Equipment

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
SpeedVac Vacuum Concentrators (Optional)	Thermo Fisher Scientific

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

TELL-Seq™ Human Exome Capture Workflow

TELL-Seq™ WGS Library Prep



Protocol

TELL-Seq™ WGS Library Prep

The following protocol describes a modified TELL-Seq™ whole genome sequencing library preparation procedure with TELL-Seq™ Library Prep kit using human DNA samples. Different genomic DNA sample types can also be substituted following the same protocol. TELL-Seq™ libraries are compatible with other Target Enrichment systems, but Agilent SureSelectXT HS Target Enrichment System using SureSelect Human All Exon V8 capture probes is used as an example in protocol. For use in other Target Enrichment Systems please follow protocol for

TELL-Seq™ Library Prep (pg. 10-22) to generate appropriate libraries. TELL-Seq™ libraries can be used as DNA Input following the protocol of each specific Target Enrichment System along with TELL-Seq™ Target Blockers being used in addition to specified blockers.

Barcode DNA

Consumables
➢ Input genomic DNA (User)

Genome Size	Input Amount	Reaction Vol (mL)	Preps/Kit
Large (Human)	5 ng	150	4

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).

➢ 5× Reaction Buffer (Kit Box 1, 

Blue)
➢ Cofactor II (Kit Box 1, 

Amber)
➢ Barcoding Enzyme (Kit Box 1, 

Black)
➢ TELL Bead or TELL Bead Plex (Kit Box 2, 

Orange)
➢ Suspension Buffer EZ (Kit Box 1, 

Natural)
➢Nuclease-free water (User)
➢ 0.2 mL PCR tube or strip tube (User)
➢20 µL and 200 µL wide orifice pipette tips (User)

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 in the dark. Close the tube cap tightly after each use.**
Barcoding Enzyme CAP	-25°C to -15°C	Flick the tube 4 to 5 times to mix. Centrifuge briefly. Keep on ice.
TELL Bead Plex 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 EZ 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 (see Step 7 of the Procedure section).

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 2mm-3mm off a standard pipette tip top with a clean razor blade before use.

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 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 (mL)**
	Large Genome (150 mL)
5× Reaction Buffer CAP	30
Nuclease-free water Cofactor II CAP	20 – Z (Z is the DNA vol)30
TELL Bead Plex CAP (0.5M barcodes/mL)	19

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 Barcoding Enzyme.

Reagent	Volume per reaction (mL)
	Large Genome
Barcoding Enzyme CAP	6 mL

4. Mix well by pipetting up and down for 8 times. Avoid introducing air bubbles when pipetting by keeping the pipette tip at the bottom of the solution in the tube.
5. Use a wide orifice pipette tip, add following reagent.

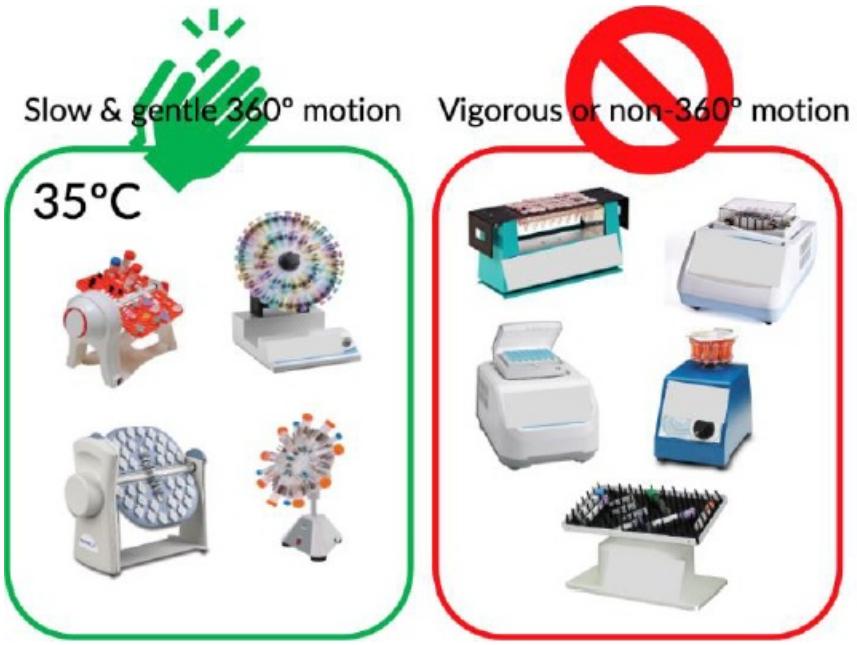
Reagent	Volume per reaction (µL)
	Large Genome
Sample genomic DNA	Z (≤ 15)
Suspension Buffer EZ CAP	45

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

6. Set pipette volume at 110 µL. Use a wide orifice pipette tip, gently mix the solution by slowly pipetting up and down 6-8 times. Avoid introducing many air bubbles when pipetting by keeping the pipette tip at the bottom of the solution in the tube.
7. Place the sample tube on a tube rotator in a 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

Consumables

➢ Stabilizer (Kit Box 1, CAP Violet)

Preparation

1. Prepare the following consumables:

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

Procedure

1. Retrieve the sample tube from the 35°C incubator.
2. Add Stabilizer into the tube.

Reagent	Volume per reaction (mL)
	Large Genome
Stabilizer CAP	3

3. Set pipette volume at 110 µL. Use 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

Consumables

➢ Tagging Enzyme (Kit Box 1, CAP Red)

➢ Exonuclease (Kit Box 1, CAP Yellow)

Preparation

1. Prepare the following consumables:

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

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

Procedure

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

Reagent	Volume per reaction (mL)
	Large Genome
Tagging Enzyme CAP	2
Exonuclease CAP	3

3. Set pipette volume at 110 µL. Use a wide orifice pipette tip, gently mix the solution by slowly pipetting up and down for 8 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. 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, up to 6µL Tagging Enzymes can be used in the reaction.
5. Proceed to next step immediately after the incubation.

Wash Beads

Consumables

- Stop Solution (Kit Box 2, CAP Natural or stored at room temperature after the first use)
- Wash Solution (Kit Box 2, CAP White)

- 0.2 mL PCR tube or strip tube (User)

Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
Stop Solution CAP	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	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

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 120 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 80 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 120 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 120 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

Consumables

- 2× PCR Master Mix (Kit Box 1, CAP Pink)
- 10× Primer I (Kit Box 1, CAP White)
- 10× Primer II, T50# (Multiplex Primer Kit)
- Nuclease-free water (User)
- 0.2 mL PCR tube or strip tube (User)

Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
2× PCR Master Mix CAP	-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	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
10× Primer II, T50#	-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:
Cycle number is flexible based on downstream applications and requirement. Recommend starting with 13 cycles. Higher cycle number will generate more TELL-Seq library as an input for Hybridization and Capture, but higher duplication rates for the final sequencing analysis. Lower cycle number may decrease duplication rate, but lower DNA inputs may result in decreased capture efficiency and reduced library complexity. Please refer to the end of Qualify and Quantify Library for Hybridization and Capture for further considerations when determining appropriate cycle number.

Genome Size	Vol of Beads Used (B) for PCR	PCR Volume	Cycle Number
Large	20 mL	75 mL	12-14

Procedure

1. Vortex beads vigorously for 10 seconds to resuspend the beads. Pulse spin to bring solution down. Using a 20 µL tip, pipet the beads up and down 5 times to make sure all the beads are resuspended properly. Immediately transfer entire bead solution amount of to a new PCR tube.
2. Place the PCR tube on a magnetic stand for 1 minute or until the solution is clear.
3. While the tube is on the magnetic stand, remove 20 µ L supernatant without disturbing beads. Remove the PCR tube from the magnet.
4. Add following reagents to the PCR tube.

Reagent	Volume per reaction (µL)
	Large Genome (75 µL)
Nuclease-free water	16 mL
2× PCR Master Mix CAP	37.5 mL
10× Primer I CAP	7.5 mL
10× Primer II, T50#	7.5 mL
Enhancer CAP Green	4.5 mL

5. Mix well by vortexing or pipetting. Pulse spin to bring solution down.
6. Place the tube on the thermal cycler and run the LAP program (see above) with proper number of cycles.
7. After PCR amplification, use 2 µL PCR product for quality check on a Bioanalyzer or a TapeStation. See Qualify and Quantify Library section for instruction.

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

Consumables

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

Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
Fresh 75% (v/v) ethanol	Room Temperature	Require 400 mL per sample. Mix 1.5 mL Ethanol (200 p roof) with 0.5 mL Nuclease-free water. Vortex to mix an d 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.

Procedure

1. Briefly centrifuge the sample PCR tube to bring all solution down.
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 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	75 mL
Nuclease-free water	To final 100 mL 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. 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. Keep 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. Remove the tube from the magnetic stand and add 20 µL nuclease-free water 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 18 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 a month.

Qualify and Quantify Library for Target Enrichment

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.

Preparation

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

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. A good-sized library should have most library fragments under 1000 bp (Figure 1).

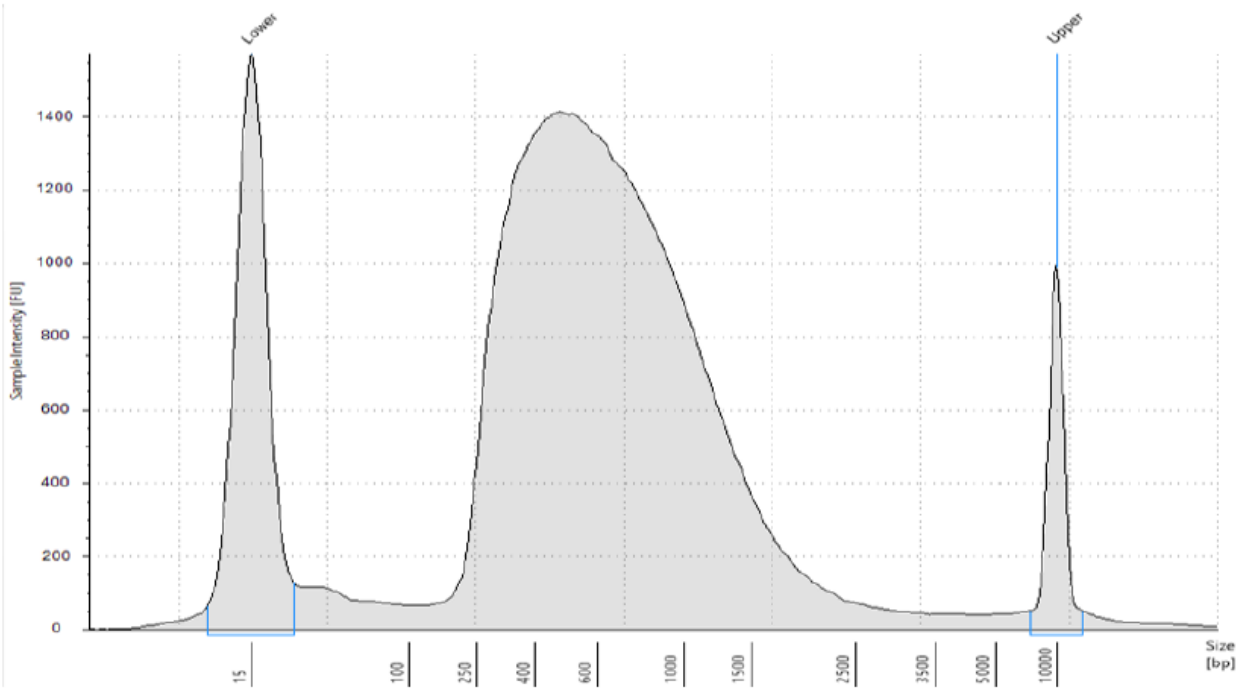


Figure 1. An example of cleaned up library profile from a TapeStation High Sensitivity D5000 Screen Tape assay.

4. Library can be stored at -25°C to -15°C.
5. Make a 10-fold diluted TELL-Seq™ library sample: dilute 2 µL of TELL-Seq™ library with 18 µL of nuclease-free water. Use 4 µL diluted library to check the concentration with the Qubit dsDNA HS Assay Kit.
6. Use the concentration (ng/µL) and volume to calculate total mass of each TELL-Seq™ Library going into the SureSelectXT HS Target Enrichment System process. 500 ng -1,000 ng TELL-Seq™ library per sample is recommended for optimal results, but library inputs as low as 250 ng per sample are possible though performance can be negatively impacted.

   NOTE:

   There are volume constraints for the Hybridization and Capture protocol. 12 µL is the max volume allowed for DNA input. Careful consideration should be used to ensure that the DNA library volume falls within this range. Ideally use all available TELL-Seq library material after QC into the Hybridization and Capture reaction.
7. (Optional) TELL-Seq™ libraries can be concentrated using SpeedVac Vacuum Concentrator. If a SpeedVac will be used for concentrating library, TELL-Seq™ library after AMPure XP cleanup can be eluted from XP beads with at least 30 µL nuclease-free water for better recovery. After QC, the clean TELL-Seq™ library can be concentrated to desired volume for Hybridization and Capture.

NOTE:
Other Target Enrichment Systems can be used like IDT and Twist Biosciences using TELL-Seq™ libraries created. Follow specified Target Enrichment protocols using TELL-Seq™ libraries as DNA Input. Use 5ul of TELL-Seq™ TargetSeq Blocker in addition to detailed blockers in each protocol.

SureSelect Target Enrichment
The following protocol is a modification of the Hybridization and Capture portion SureSelect XT HS Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library Protocol (Agilent, G9702-90000). Modifications allow for compatibility of TELL-Seq™ WGS Library Prep with Agilent SureSelect XT HS Target Enrichment System and All Exon V8 Capture Probes. Agilent reagents for just Hybridization and Capture can be purchased separately in 16-reaction format (Agilent Part Number G9916B). If using other Target Enrichment Systems follow the specified protocols for each system and substituting TELL-Seq™ libraries as DNA Input. 5ul of TELL-Seq™ Target Blockers is also needed in addition to detailed blockers in each Target Enrichment protocol

Hybridize TELL-Seq™ Library to the Exome Capture Panel

Consumables

- SureSelect XT HS and XT Low Input Blocker Mix, 16 Rxn (Agilent, SureSelect XT HS Target Enrichment Kit, ILM Hyb Module, Box 2 (Post PCR), 

 Blue)
- SureSelect RNase Block, 16 Rxn (Agilent, SureSelect XT HS Target Enrichment Kit ILM Hyb Module, Box 2 (Post PCR), 

 Violet)
- SureSelect Fast Hybridization Buffer, 16 Rxn (Agilent, SureSelect XT HS Target Enrichment Kit ILM Hyb Module, Box 2 (Post PCR), Bottle)
- TELL-Seq™ Target Blocker (UST, TELL-Seq™ Target Blocker Box, 

 White)
- SSel XT HS and XT Low Input Human All Exon V7 (Agilent, SSel XT HS and XT Low Input Human All Exon V7, 

 Red)
- Nuclease-free water (User)
- 0.2 mL PCR tube or strip tube (User)
- TELL-Seq™ Library (User)

Input Amount	Reaction Vol (mL)
500 -1,000 ng	Up to 12 mL

Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
SureSelect XT HS and XT Low Input Blocker Mix CAP	-25°C to -15°C	Thaw on ice. Vortex to mix, then centrifuge briefly. Keep on ice.
SureSelect RNase Block CAP	-25°C to -15°C	Thaw on ice. Vortex to mix, then centrifuge briefly. Keep on ice.
SureSelect Fast Hybridization Buffer	-25°C to -15°C	Thaw and keep at room temperature
TELL-Seq™ TargetSeq Blocker	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
TELL-Seq™ DNA Library	-25°C to -15°C	Thaw and keep at ice

SSel XT HS and XT Low Input Human All Exon V8 CAP	-85°C to -75°C	Thaw on ice. Vortex to mix, then centrifuge briefly. Keep on ice.

2. Set up the Hybridization Program (HP) on a thermo cycler (with the heated lid ON) with the program below. Start the program, then immediately press the Pause button, allowing the heated lid to reach temperature while you set up the reactions.

Segment Number	Number of Cycles	Temperature	Time
1	1	95°C	5 minutes
2	1	65°C	10 minutes
3	1	65°C	1 minute
4	60	65°C 37°C	1 minute 3 seconds
5	1	65°C	Hold

Procedure

1. Place 500–1000 ng of each prepared TELL-Seq™ library sample into the strip tube wells and then bring the final volume in each well to 12 µl using nuclease-free water if needed. Ideally make the total amplified TELL-Seq™ library DNA, within the 500–1000 ng range and use all for the hybridization reaction.
2. To each TELL-Seq™ library sample well, add 5 µl of SureSelect XT HS and XT Low Input Blocker Mix and add 5 µl of TELL-Seq™ Target Blocker. Cap the wells then vortex at high speed for 5 seconds. Spin the strip tube

briefly to collect the liquid release any bubbles.

3. Transfer the tubes to the thermal cycler and press the Play button to resume the HP program set up.

   NOTE:

   The thermal cycler must be paused during Segment 3 (see HP) to allow additional reagents to be added to the Hybridization wells, as described in step 6. During Segments 1 and 2 of the thermal cycling program, begin preparing the additional reagents as described in step 4 and step 5. If needed, you can finish these preparation steps after pausing the thermal cycler in Segment 3.

4. Prepare a 25% solution of SureSelect RNase Block (containing 1 volume of RNase Block with 3 volume of water), according to the table below. Prepare the amount required for the number of hybridization reactions in the run, plus excess.

Reagent	**Volume per reaction (mL)**

Volume for 1 Reaction		Volume for 8 reactions (inc ludes excess)	Volume for 24 reactions (inc ludes excess)
SureSelect RNase Block CAP	0.5 mL	4.5 mL	12.5 mL
Nuclease-free water	1.5 mL	13.5 mL	37.5 mL

5. Prepare the Capture Library Hybridization Mix in the following order.

Reagent	**Volume per reaction (mL)**		
	Volume for 1 Reaction	Volume for 8 reactions (includes excess)	Volume for 24 reactions (includes excess)
25% RNase Block solution	2 mL	18 mL	50 mL
SSel XT HS and XT Low Input Human All Exon V7 CAP	5 mL	45 mL	125 mL
SureSelect Fast Hybridization Buffer	6 mL	54 mL	150 mL

   Combine the listed reagents at room temperature. Mix well by vortexing at high speed for 5 seconds then spin down briefly. Proceed immediately to step 6.

6. Once the thermal cycler starts Segment 3 of the HP (1 minute at 65°C), press the Pause button. With the cycler paused, and while keeping the DNA + Blocker samples in the cycler, transfer 13 µl of the room- temperature Capture Library Hybridization Mix from step 6 to each sample well. Mix well by pipetting up and down slowly 8 to 10 times. The hybridization reaction wells now contain approximately 35 µl
7. Make sure that all wells are completely sealed. Vortex briefly, then spin strip tube briefly to remove any bubbles from the bottom of the wells. Immediately return the strip tube to the thermal cycler.
8. Press the Play button to resume the thermal cycling program to allow hybridization of the prepared DNA samples to the Capture Library.

Prepare Streptavidin-coated Magnetic Beads

Consumables

- Dynabeads MyOne Streptavidin T1 (User)
- SureSelect Binding Buffer (Agilent, SureSelect XT HS Target Enrichment Kit ILM Hyb Module, Box 1 (Post PCR), CAP)

Preparation

Prepare the following consumables:

Item	Storage	Instruction
Dynabeads MyOne Streptavidin T1	2°C to 8°C	Centrifuge vigorously. Keep at room temperature.
SureSelect Binding Buffer, CAP	Room Temperature	Keep at room temperature.

Procedure

1. Vigorously resuspend the Dynabeads MyOne Streptavidin T1 magnetic beads on a vortex mixer. The magnetic beads settle during storage.
2. For each hybridization sample, add 50 µl of the resuspended beads to wells of a fresh PCR plate or a strip tube.
3. Wash the beads by adding 200 µl of SureSelect Binding Buffer. Mix by pipetting up and down 20 times or cap the wells and vortex at high speed for 5–10 seconds.
4. Put the plate or strip tube into a magnetic separator device.
5. Wait at least 5 minutes or until the solution is clear, then remove and discard the supernatant.
6. Repeat Steps 3-5 two more times for a total of 3 washes.
7. Resuspend the beads in 200 µl of SureSelect Binding Buffer.

Capture the Hybridized DNA using Streptavidin-coated Beads

Consumables

- SureSelect Wash Buffer 1, 16 Rxn (Agilent, SureSelect XT HS Target Enrichment Kit ILM Hyb Module, Box 1 (Post PCR), CAP)
- SureSelect Wash CAP Buffer 2, 16 Rxn (Agilent, SureSelect XT HS Target Enrichment Kit ILM Hyb Module, Box 1 (Post PCR), CAP)

Preparation

Prepare the following consumables:

Item	Storage	Instruction
SureSelect Wash Buffer 1, CAP	Room Temperature	Keep at room temperature.
SureSelect Wash Buffer 2, CAP	Room Temperature	Heat 200 µl aliquots at 70°C. See Step 4

Procedure

1. After the hybridization step is complete and the thermal cycler reaches the 65°C hold step, transfer the samples to room temperature.
2. Immediately transfer the entire volume (approximately 30 µl) of each hybridization mixture to wells containing 200 µl of washed streptavidin beads using a multichannel pipette. Pipette up and down 5–8 times to mix.
3. Incubate the capture strip tube on a 96- well plate mixer, mixing vigorously (at 1400–1800 rpm) or rotator, for 30 minutes at room temperature. Make sure the samples are properly mixing in the wells.
4. Proceed to next step immediately after the incubation. During the 30-minute incubation for capture, prewarm SureSelect Wash Buffer 2 at 70°C as described below. Place 200 µl aliquots of Wash Buffer 2 in wells of a fresh 96- well plate or strip tubes. Aliquot 6 wells of buffer for each DNA sample in the run. Cap the wells and then incubate in the thermal cycler, with heated lid ON, held at 70°C until used in
5. When the 30-minute incubation period initiated in Step 3 is complete, spin the samples briefly to collect the liquid.
6. Place the strip tube in a magnetic separator to collect the beads. Wait until the solution is clear, then remove and discard the supernatant.
7. Resuspend the beads in 200 µl of SureSelect Wash Buffer 1. Mix by pipetting up and down 15–20 times, until beads are fully resuspended.
8. Place the strip tube in the magnetic separator. Wait for the solution to clear (approximately 1 minute), then remove and discard the supernatant.
9. Remove the strip tubes from the magnetic separator and transfer to a rack at room temperature. Resuspend the beads in 200 µl of 70°C prewarmed Wash Buffer 2. Pipette up and down 15–20 times, until beads are fully resuspended. Seal the wells with fresh caps and then vortex at high speed for 8 seconds. Spin the plate or strip tube briefly to collect the liquid without pelleting the beads.
10. Incubate the samples for 5 minutes at 70°C on the thermal cycler with the heated lid on.
11. Place the strip tube in the magnetic separator at room temperature. Wait 1 minute for the solution to clear, then remove and discard the supernatant.
12. Repeat Steps 9-11 five more times, for a total of 6 washes.
13. After verifying that all wash buffer has been removed, add 25 µl of nuclease-free water to each sample well. Pipette up and down 8 times to resuspend the beads. Samples can be stored on ice before amplification

Amplify Captured Library

Consumables

- 5× Herculase II Reaction Buffer (Agilent, SureSelect XT HS Target Enrichment Kit, ILM Hyb Module Box 2 (Post PCR), CAP Clear)
- Herculase II Fusion DNA Polymerase (Agilent, SureSelect XT HS Target Enrichment Kit, ILM Hyb Module Box 2

(Post PCR), CAP Red)

- 100 mM dNTP Mix, (Agilent, SureSelect XT HS Target Enrichment Kit, ILM Hyb Module Box 2 (Post PCR), CAP Green)
- SureSelect Post-Capture Primer Mix (Agilent, SureSelect XT HS Target Enrichment Kit, ILM Hyb Module Box 2 (Post PCR), CAP Clear)

Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
5× Herculase II Reaction Buffer CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
Herculase II Fusion DNA Polymerase CAP	-25°C to -15°C	Centrifuge briefly. Keep on ice.
100 mM dNTP Mix CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.
SureSelect Post-Capture Primer Mix CAP	-25°C to -15°C	Thaw at room temperature. Vortex to mix, then centrifuge briefly. Keep on ice.

2. Set up following program on a thermo cycler as following:
    - 98°C 2 minutes
    - [98°C 30 seconds, 63°C 30 seconds, 72°C 1 minute] x 9
    - 72°C 5 minutes
    - 4°C forever

Procedure

1. Prepare the appropriate volume of PCR reaction mix. Add following reagents to the PCR tube based on number of reactions.

Reagent	Volume per reaction (µL)		
	Volume for 1 Reaction	Volume for 8 reactions (includes excess)	Volume for 24 reactions (includes excess)
Nuclease-free water	12.5 µL	112.5 µL	312.5 µL
5× Herculase II Reaction Buffer CAP	10 µL	90 µL	250 µL
Herculase II Fusion DNA Polymerase CAP	1 µL	9 µL	25 µL
100 mM dNTP Mix CAP	0.5 µL	4.5 µL	12.5 µL
SureSelect Post-Capture Primer Mix CAP	1 µL	9 µL	25 µL

2. Prepare the appropriate volume of PCR reaction mix. Add following reagents to the PCR tube based on number of reactions. Add 25 µl of the PCR reaction mix prepared in Table 29 to each sample well containing 25 µl of bead- bound target- enriched DNA (prepared on page 26 and held on ice).
3. Mix the PCR reactions well by pipetting up and down until the bead suspension is homogeneous. Avoid splashing samples onto well walls; do not spin the samples at this step.
4. Place the tube on the thermal cycler and run the program (see above) with proper number of cycles.
5. When the PCR amplification program is complete, spin the strip tube briefly. Remove the streptavidin- coated beads by placing the plate or strip tube on the magnetic stand at room temperature. Wait 2 minutes for the solution to clear, then remove each supernatant (approximately 50 µl) to wells of a strip tube.

Clean Up Captured Library

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)

Preparation

Prepare the following consumables:

Item	Storage	Instruction
Fresh 75% (v/v) ethanol	Room Temperature	Require 400 mL per sample. Mix 1.5 mL Ethanol (200 p roof) with 0.5 mL Nuclease-free water. Vortex to mix an d 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.

Procedure

1. Bring solution down with a quick ~1 second spin in the centrifuge.
2. Vortex vigorously to resuspend the AMPure XP solution and add 50 µL AMPure XP into each PCR product.
3. Mix by pipetting up and down 10 times.
4. Incubate at room temperature for 5 minutes.
5. Place the tube on the magnetic stand for 1 minute or until the solution is clear.
6. Aspirate and discard the supernatant without disturbing AMPure beads.
7. While keeping the tube on the magnetic stand, add 200 µL freshly prepared 75% ethanol into the tube. Let it sit for 30 seconds.
8. Aspirate and discard the supernatant without disturbing beads.
9. Repeat steps 7-8 one more time, keeping the tube on the magnetic stand for the whole time.
10. 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.
11. Remove the tube from the magnetic stand and add 25 µL TE buffer to the beads.
12. Pipette or vortex to resuspend the beads. Let it sit for 5 minutes.
13. Place the tube on the magnetic stand for 1 minute or until the solution is clear.
14. Recover 23 µL of the supernatant to a new tube. Be careful not to disturb the beads.
15. The supernatant contains the captured TELL-Seq™ library.

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

Qualify and Quantify Captured Library for Sequencing

Consumables

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

Preparation

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

Procedure

1. Use 1 µL of library for Agilent Bioanalyzer High Sensitivity DNA Kit or 2 µL of library for TapeStation High Sensitivity D5000 ScreenTape Assay.
2. 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 2). To determine the library size, set the Region from 150 bp to 3000 bp. Record sample Average Size (bp) as Library Size.

   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).

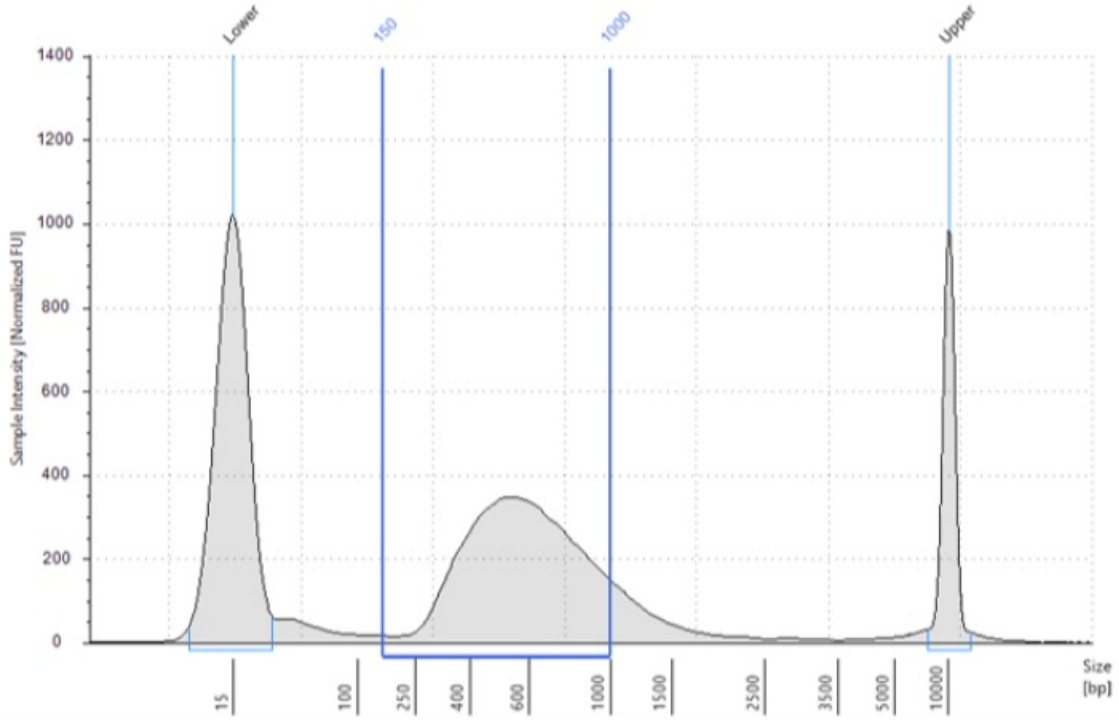
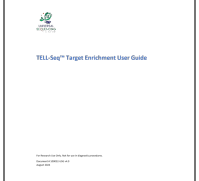


   Figure 2. An example of exome captured library profile from a Tape Station High Sensitivity D5000 Screen Tape assay.
3. Library can be sequenced immediately or stored at -25°C to -15°C.
4. When sequencing, dilute the library using TE buffer to the concentration recommended by each Illumina® sequencing system. Make diluted library pool for sequencing if more than one library will be sequenced in the same run.
5. Measure the library concentration with the Qubit dsDNA HS Assay Kit. Use the Average Size value from the Bioanalyzer (or TapeStation) measurement as the library size 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)
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%.

Documents / Resources

	**UNIVERSAL SEQUENCING TELL-Seq Target Enrichment** [pdf] User Guide TELL-Seq Target Enrichment, TELL-Seq, Target Enrichment, Enrichment

References

- **User Manual**