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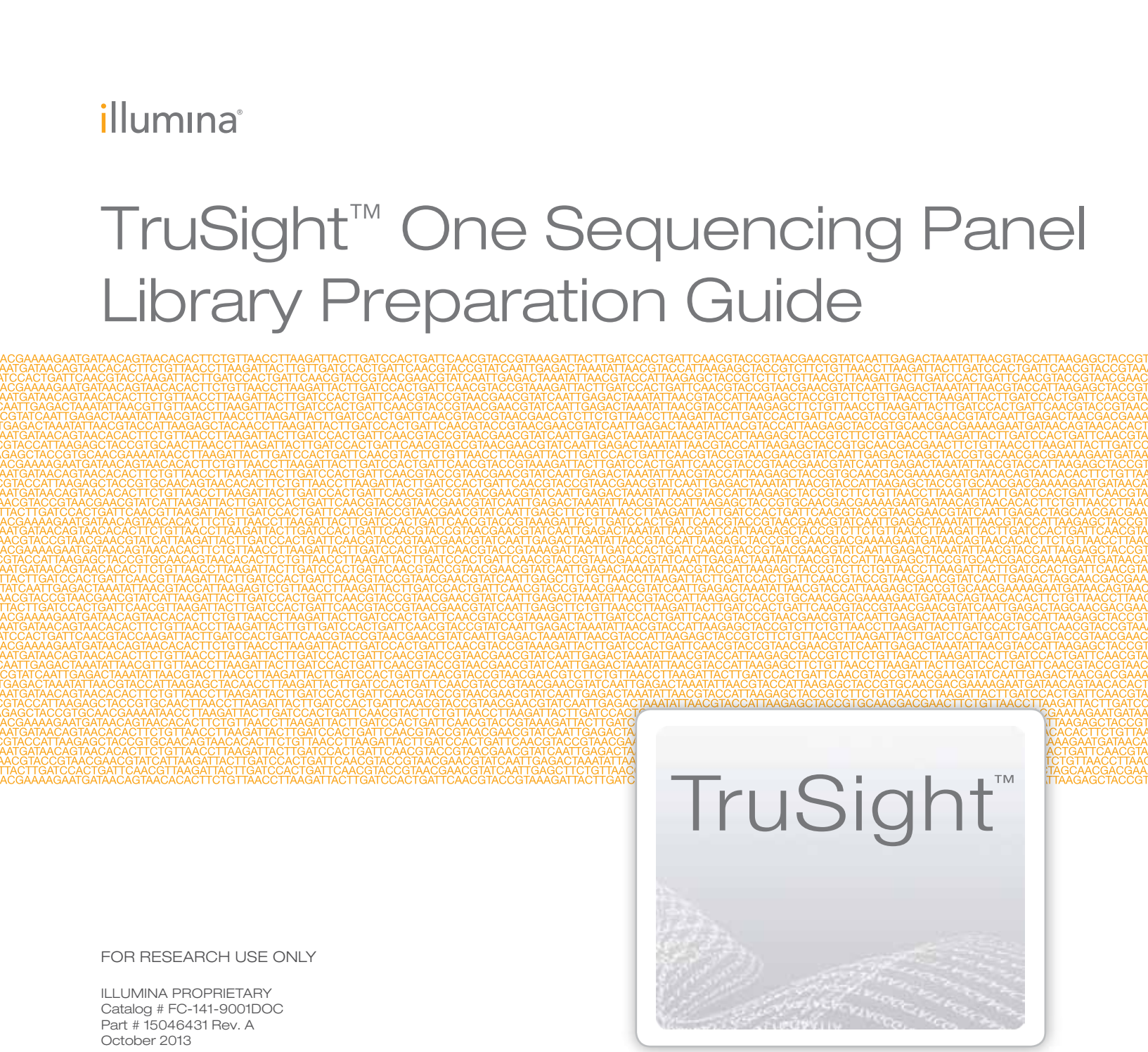
TruSight™ One Sequencing Panel Library Preparation Guide



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Revision History

Part #	Revision	Date	Description of Change
15046433	A	October 2013	Initial Release

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Overview

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Introduction

This protocol explains how to prepare up to 36 indexed, paired-end libraries, followed by enrichment using the TruSight One™ Sequencing Panel and reagents provided in an Illumina TruSight One Sequencing Panel kit. The goal of this protocol is to fragment and add adapter sequences onto template DNA to generate indexed libraries that can be carried through enrichment for targeted resequencing applications.

The TruSight One Sequencing Panel protocol offers:

- Fast and easy sample preparation
  - Prepare up to 36 enriched libraries in approximately 1.5 days, with approximately 5 hours of hands-on time
  - High throughput, automation-friendly procedures with no fragmentation bottlenecks
- Low DNA input and excellent data quality
  - Excellent data quality with low input of 50 ng
  - Access precious samples with no affect on performance
  - Ability to archive samples for subsequent analysis
- High enrichment rates, low duplicates, and exceptional coverage uniformity
  - Efficient use of sequencing
  - Reliable variant calling
  - Reduced hands-on time with the most cost-effective, high-throughput workflow

DNA Input Recommendations

TruSight One library preparation uses an enzymatic DNA fragmentation step and thus can be more sensitive to DNA input compared to mechanical fragmentation methods. The ultimate success of enrichment strongly depends on using an accurately quantified amount of input DNA. Therefore, accurate quantitation of the gDNA is essential.

Illumina recommends quantifying the starting gDNA using a fluorometric-based method specific for double-stranded DNA (dsDNA) and running samples in triplicate to obtain more confident measurements. Methods that measure total nucleic acid content (e.g. nanodrop or other UV absorbance methods) should be avoided because common contaminants such as ssDNA, RNA, and oligos are not substrates for the TruSight One Sequencing Panel.

The TruSight One protocol has been optimized for 50 ng of total gDNA. A higher mass input of gDNA may result in incomplete tagmentation and larger insert sizes, which can impact enrichment performance. Conversely, a low mass input of gDNA or low quality gDNA in the tagmentation reaction may generate smaller than expected insert sizes, which can be lost during subsequent clean-up steps resulting in lower diversity.

To minimize gDNA sample input variability into the tagmentation step, Illumina strongly recommends a two-step method of gDNA normalization. After the initial quantification, gDNA samples are first normalized to 10 ng/µl. Samples are then re-quantified using a similar fluorometric-based method and normalized to a final 5 ng/ul.

Critical Steps for Successful Enrichment and Coverage

To ensure robust performance from the TruSight One Sequencing Panel, Illumina recommends using a microheating system with a MIDI plate insert for the Enrichment Wash steps. The Enrichment Wash steps reduce non-specific DNA binding and require that samples are maintained at the indicated temperature. Too low or too high temperatures can result in lower percent enrichments and decreased yields. If a microheating system is not available, a thermal cycler can be used with some modifications. See *Alternative Thermal Cycler Steps for Successful Enrichment* on page 59 for instructions using a thermal cycler.

Obtaining Desired Reads Per Sample:

The number of resulting reads for each sample of a pool are dependent on the following factors:

- Accurate quantification of tagmented samples before pooling for enrichment. Inaccurate quantification can lead to uneven pooling between samples in the enrichment and can result in less than expected reads for a given sample.
- Accurate quantification of final enriched library pools. Illumina recommends using the same dilution of final library for both quantification and clustering. Inaccurate quantification can result in lower than targeted cluster densities, less reads passing filter and/or inefficient demultiplexing if overclustered. Illumina recommends targeting 1200k-1400k clusters/mm2 (raw density) on MiSeq v3® runs, though the optimal cluster density can vary between instruments.

Additional Resources

The following resources are available for TruSight One Sequencing Panel protocol guidance and sample tracking. Access these and other resources on the Illumina website at support.illumina.com/sequencing/kits.ilmn. Then, select **TruSight One Sequencing Panel Support**.

Resource	Description
Best Practices	Provides best practices specific to this protocol. Review this before starting sample preparation. Topics include: • Consistency • Handling Magnetic Beads • Avoiding Cross-Contamination • Washing During SPB Clean-Up • Freeze/thawing for Small Number of Samples • Preventing PCR Product Contamination Click **Best Practices** on the **TruSight One Sequencing Panel Support** page.
TruSight One Sequencing Panel Experienced User Card and Lab Tracking Form (part # 15046433)	Provides protocol instructions, but with less detail than what is provided in this user guide. **New or less experienced users are strongly advised to follow this user guide and not the EUC and LTF.** Click **Documentation & Literature** on the **TruSight One Sequencing Panel Support** page.
Illumina Experiement Manager (IEM) IEM TruSight One or TruSight Rapid Capture Quick Reference Card (part # 15048138)	Enables you to create and edit appropriate sample sheets for Illumina sequencers and analysis software and record parameters for your sample plate. To download the software, click **Downloads** on the **TruSight One Sequencing Panel Support** page. To download the documentation, click **Documentation & Literature** on the **TruSight One Sequencing Panel Support** page.

Protocol

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Introduction

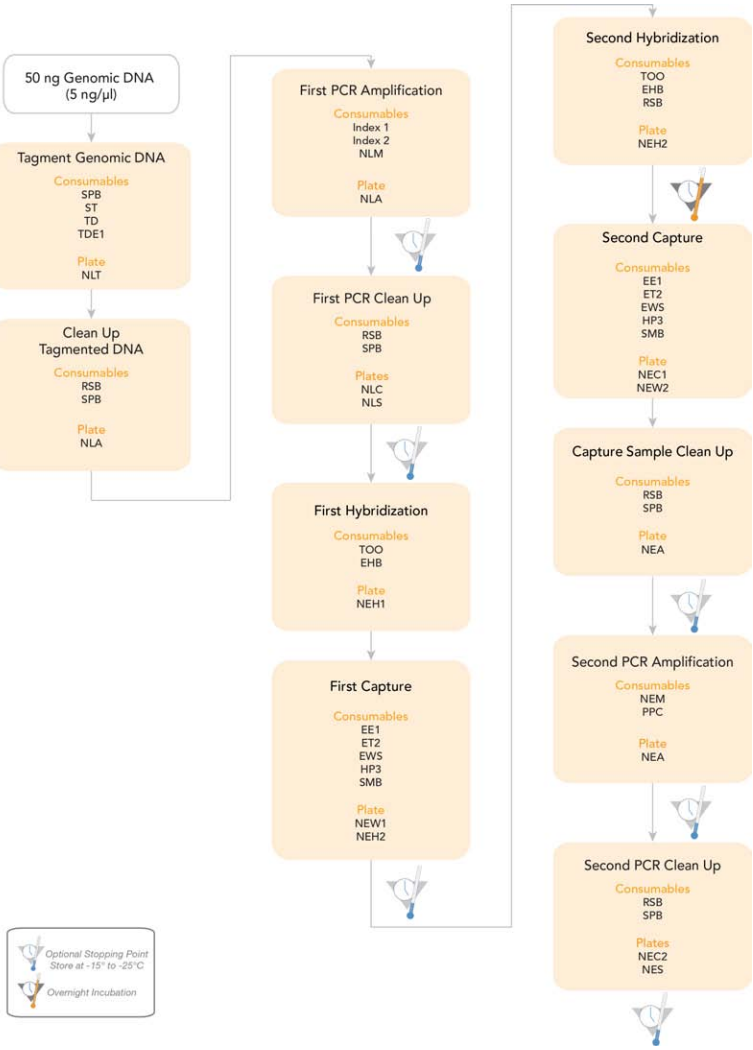
This chapter describes the TruSight One protocol.

- Review Best Practices before proceeding. See *Additional Resources* on page 5 for information on how to access TruSight One Sequencing Panel Best Practices on the Illumina website.
- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- If you are pooling, record information about your samples before beginning library preparation for later use in data analysis.
  - Use IEM to create and edit well formed sample sheets for Illumina sequencers and analysis software. Detailed procedures on how to create a sample sheet for the TruSight One Sequencing Panel are available in an IEM quick reference card. See *Additional Resources* on page 5 for information on how to download IEM software and TruSight One appropriate IEM documentation from the Illumina website.
  - Each column should contain a common index. This will facilitate pipetting operations when dispensing indexed adapters and pooling indexed libraries later in the protocol.

Library Prep Workflow

The following diagram illustrates the workflow using a TruSight One Sequencing Panel kit. Safe stopping points are marked between steps.

Figure 1 TruSight One Workflow



Tagment Genomic DNA

This process tagments (tags and fragments) the gDNA by the Nextera transposome. The Nextera transposome simultaneously fragments the gDNA and adds adapter sequences to the ends, allowing amplification by PCR in subsequent processes.

Consumables

Item	Quantity	Storage	Supplied By
Sample Purification Beads (SPB)	1 tube	2°C to 8°C	Illumina
Stop Tagment Buffer (ST)	1 tube	15°C to 30°C	Illumina
Tagment DNA Buffer (TD)	1 tube	-15°C to -25°C	Illumina
Tagment DNA Enzyme (TDE1)	1 tube	-15°C to -25°C	Illumina
96-well MIDI plate	1	15°C to 30°C	User
gDNA (5 ng/µl)	50 ng	-15°C to -25°C	User
Ice bucket	1	15°C to 30°C	User
Microseal 'B' adhesive seals	2	15°C to 30°C	User
PCR-grade water	10 µl per sample	15°C to 30°C	User
RNase/DNase-free eight-tube strips and caps (for multi-sample processing)	3	15°C to 30°C	User
RNase/DNase-free reagent reservoir (for multi-sample processing)	1	15°C to 30°C	User
Tris-HCl 10 mM, pH 8.5	As needed	15°C to 30°C	User

Preparation

- Remove the Tagment DNA Buffer, Tagment DNA Enzyme 1, and gDNA from -15°C to -25°C storage and thaw on ice.

- After thawing, make sure that all reagents are adequately mixed. Gently invert the tubes 3–5 times, followed by a brief spin in a microcentrifuge.

- Remove the Sample Purification Beads from 2°C to 8°C storage and let stand to bring to room temperature.
- Make sure that the Stop Tagment Buffer has no precipitate. If there is precipitate, vortex until all particulates are resuspended.
- For multi-sample processing:
  - Use a multichannel pipette.
  - Distribute the Stop Tagment Buffer, Tagment DNA Buffer, and Tagment DNA Enzyme 1 into separate eight-tube strips, dispensing equal volumes into each of the wells.
  - Pour the Sample Purification Beads into a multichannel reagent reservoir.
- Place a MIDI plate insert on the microheating system.
- Pre-heat the microheating system to 58°C.
- Label a new 96-well MIDI plate **NLT** (Nextera Library Tagment) with a smudge resistant pen.
- Use the Illumina Experiment Manager to determine the index primers to be used. For more information on IEM, see *Additional Resources* on page 5.

WARNING
Allowing the Tagment DNA Enzyme 1 to warm up to room temperature might result in decreased activity.

Procedure

NOTE
Make sure that the reaction is assembled in the order described for optimal kit performance. It is not necessary to assemble the reaction on ice.

1 Perform the following steps to normalize your gDNA samples:
   a Quantify your gDNA samples using a fluorometric method such as QuantiFluor or Qubit.
   b Normalize your gDNA samples in Tris-HCl 10 mM, pH 8.5 to 10 ng/µl.
   c Requantify the 10 ng/µl normalized sample using the same fluorometric quantification method.
   d Based on the quantification, further dilute your gDNA samples in Tris-HCl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl (50 ng total).

2 Add 10 µl gDNA at 5 ng/µl (50 ng total) to each well of the new 96-well MIDI plate labeled NLT.

3 Add 25 µl Tagment DNA Buffer to each well of the NLT plate.

4 Add 5 µl Tagment DNA Enzyme 1 to each well of the NLT plate.

5 Add 10 µl PCR-grade water to each well of the NLT plate.

6 Mix thoroughly as follows:
   a Seal the NLT plate with a Microseal 'B' adhesive seal.
   b Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute.

7 Centrifuge the NLT plate to 280 × g for 1 minute.

8 Place the sealed NLT plate on the pre-heated microheating system. Close the lid and incubate at 58°C for 10 minutes.

9 Remove the NLT plate from the microheating system.

10 Remove the adhesive seal from the NLT plate.

11 Add 15 µl Stop Tagment Buffer to each well of the NLT plate.

12 Mix thoroughly as follows:
   a Seal the NLT plate with a Microseal 'B' adhesive seal.
   b Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute.

13 Centrifuge the NLT plate to 280 × g for 1 minute.

14 Incubate the NLT plate at room temperature for 4 minutes.

15 Proceed to *Clean Up Tagmented DNA* on page 13.

Clean Up Tagmented DNA

This process purifies the tagmented DNA from the Nextera transposome. It is critical, because the Nextera transposome can bind tightly to DNA ends and interfere with downstream processes when it is not removed.

Consumables

Item	Quantity	Storage	Supplied By
Resuspension Buffer (RSB)	1 tube	-15°C to -25°C	Illumina
Sample Purification Beads (SPB)	1 tube	2°C to 8°C	Illumina
96-well hard-shell plate (HSP)	1	15°C to 30°C	User
Freshly prepared 80% ethanol (EtOH)	400 µl per sample	15°C to 30°C	User
Microseal 'B' adhesive seals	2	15°C to 30°C	User
RNase/DNase-free reagent reservoirs (for multi-sample processing)	3	15°C to 30°C	User

Preparation

- Remove the Resuspension Buffer from -15°C to -25°C storage and thaw at room temperature.

NOTE
The Resuspension Buffer can be stored at 2°C to 8°C after the initial thaw.

- Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 5 for information on how to access TruSight One Best Practices on the Illumina website.
- Make sure that the Sample Purification Beads are at room temperature.
- For multi-sample processing:
  - Use a multichannel pipette.
  - Pour the Resuspension Buffer, Sample Purification Beads, and 80% EtOH into separate multichannel reagent reservoirs.

- Label a new 96-well HSP plate **NLA** (Nextera Library Amplification) with a smudge resistant pen.

Procedure

1. Remove the adhesive seal from the NLT plate.
2. Vortex the room temperature Sample Purification Beads until they are well dispersed.

   NOTE
   Keep the Sample Purification Beads tube at room temperature for later use in the protocol.

3. Add 65 µl well-resuspended Sample Purification Beads to each well of the NLT plate.
4. Mix thoroughly as follows:
   a. Seal the NLT plate with a Microseal 'B' adhesive seal.
   b. Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute.
5. Incubate the NLT plate at room temperature for 8 minutes.
6. Centrifuge the NLT plate to 280 × g for 1 minute.
7. Remove the adhesive seal from the NLT plate.
8. Place the plate on the magnetic stand for 2 minutes or until the liquid appears clear.
9. Using a 200 µl single channel or multichannel pipette set to 130 µl, remove and discard all of the supernatant from each well of the NLT plate.

   NOTE
   Leave the NLT plate on the magnetic stand while performing the following 80% EtOH wash steps (10–12).

10. With the NLT plate on the magnetic stand, slowly add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads. Incubate the plate at room temperature for 30 seconds.
11. Remove and discard the 80% EtOH from each well of the NLT plate.
12. Repeat steps 10 and 11 one time for a total of two 80% EtOH washes.
13. Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NLT plate without disturbing the beads.

14 With the NLT plate on the magnetic stand, incubate the plate at room temperature for 10 minutes to dry.

15 Remove the NLT plate from the magnetic stand.

16 Add 22.5 µl Resuspension Buffer to each well of the NLT plate. Do not touch the beads with the pipette tips.

17 Mix thoroughly as follows:
   a Seal the NLT plate with a Microseal 'B' adhesive seal.
   b Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute

18 Incubate the NLT plate at room temperature for 2 minutes.

19 Centrifuge the NLT plate to 280 × g for 1 minute.

20 Place the NLT plate on the magnetic stand for 2 minutes or until the liquid appears clear.

21 Remove the adhesive seal from the NLT plate.

22 Transfer 20 µl of clear supernatant from each well of the NLT plate to the corresponding well of the new HSP plate labeled NLA. Take care not to disturb the beads.

NOTE
Illumina recommends using a 20 µl single channel or multichannel pipette set to 10 µl to perform two consecutive transfers of 10 µl. This technique reduces sample loss by making sure that all of the liquid is transferred without disturbing the beads.

First PCR Amplification

This process amplifies the purified tagmented DNA via a 10-cycle PCR program. It also adds index 1 (i7) and index 2 (i5) sequences needed for sequencing, as well as common adapters (P5 and P7) required for cluster generation and sequencing. It is critical to use the full amount of recommended input DNA. It is imperative that no extra cycles are added to the PCR process, to ensure the generation of libraries that produce high-quality sequencing results.

Consumables

Item	Quantity	Storage	Supplied By
Index 1 primers (i7, N701–N712)	1 tube each index	-15°C to -25°C	Illumina
Index 1 Tube Caps, Orange	1 per Index 1 primer tube	15°C to 30°C	Illumina
Index 2 primers (i5, E502–E505)	1 tube each index	-15°C to -25°C	Illumina
Index 2 Tube Caps, White	1 per Index 2 primer tube	15°C to 30°C	Illumina
Nextera Library Amplification Mix (NLM)	1 tube	-15°C to -25°C	Illumina
1.7 ml microcentrifuge tubes	1 per index primer tube	15°C to 30°C	User
Microseal 'A' film	1	15°C to 30°C	User
Microseal 'B' adhesive seal	1	15°C to 30°C	User
RNase/DNase-free eight-tube strips and caps (for multi-sample processing)	1	15°C to 30°C	User
[Optional] TruSeq® Index Plate Fixture Kit	1	15°C to 30°C	User

Preparation

- Remove the Nextera Library Amplification Mix from -15°C to -25°C storage and thaw on ice.
- Remove the following from -15°C to -25°C storage and thaw at room temperature:
  - Index 1 primers (i7, N7xx) (only remove primers being used)
  - Index 2 primers (i5, E5xx) (only remove primers being used)

NOTE
TruSight One Sequencing Panel kits are designed to work only with Index 2 primers with the "E" prefix. Do not use Index 2 primers from other sample prep kits.

- For multi-sample processing:
  - Use a multichannel pipette.
  - Dispense the Nextera Library Amplification Mix in equal volumes into each of the wells of an eight-tube strip.
- Pre-program the thermal cycler with the following program and save as **NLM AMP**:
  - Choose the pre-heat lid option and set to 100°C
  - 72°C for 3 minutes
  - 98°C for 30 seconds
  - **10 cycles** of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C for 5 minutes
  - Hold at 10°C

NOTE
Illumina has optimized the number of recommended PCR cycles for enrichment assays based on the level of pre-enrichment sample pooling and the size of the oligonucleotide set. Do not add or reduce the cycles of PCR, as it can compromise data quality.

Setup Index Primers

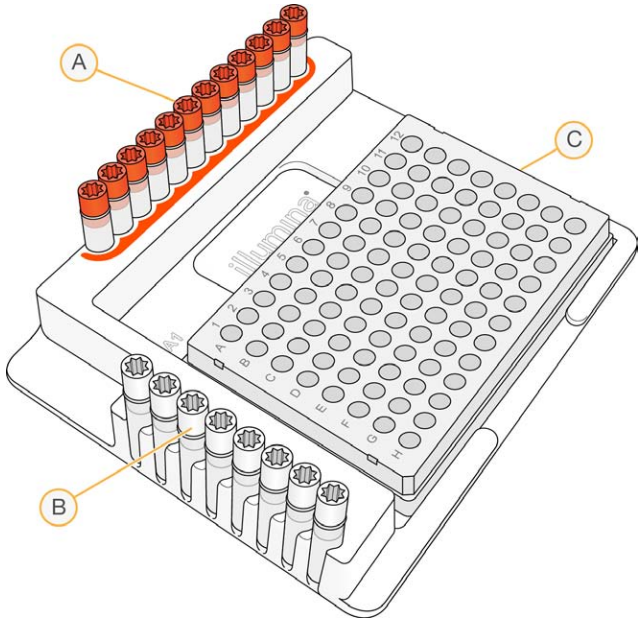
1. Vortex the index primer tubes for 5 seconds.
2. Centrifuge the index primer tubes to 600 × g for 5 seconds. Use empty 1.7 ml microcentrifuge tubes as tube adapters for the microcentrifuge.

3 Arrange the index primers in a rack on ice (i.e. the TruSeq Index Plate Fixture) using the following arrangement:

a Arrange the Index 1 Primer tubes (orange caps) vertically, aligned with columns 1-12.

b Arrange the Index 2 Primer tubes (white caps) horizontally, aligned with rows A-H.

Figure 2 Index Plate Fixture



A Index 1 Primer tubes (orange caps)
B Index 2 Primer tubes (white caps)
C NLA plate

Procedure

NOTE

When pooling libraries before enrichment with the 9 sample kit, it is recommended to pool libraries so all Index 1 (i7) indices are unique. Choose Index 1 and Index 2 primers for PCR accordingly. For pooling 3 samples in one enrichment for sequencing on a MiSeq, Illumina recommends using Index 1 Primers N701, N705, and N709, along with either Index 2 Primer for all three samples. For pooling with the 36 sample kit make sure that each sample has a unique combination of Index 1 and Index 2 sequences.

1. Add 5 µl Index 1 primer to each well of the NLA plate.
2. Add 5 µl Index 2 primer to each well of the NLA plate.
3. Add 20 µl Nextera Library Amplification Mix to each well of the NLA plate.
4. Mix thoroughly as follows:
   a. Seal the NLA plate with a Microseal 'A' film.
   b. Shake the NLA plate on a microplate shaker at 1200 rpm for 1 minute
5. Centrifuge the NLA plate to 280 × g for 1 minute.
6. Place the sealed NLA plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NLM AMP** program using a heated lid.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *First PCR Clean Up* on page 20, the NLA plate can remain on the thermocycler overnight. If you are stopping, replace the Microseal 'A' with a Microseal 'B' adhesive seal and store the NLA plate at 2°C to 8°C for up to two days.

First PCR Clean Up

This process uses Sample Purification Beads to purify the library DNA and remove unwanted products.

Consumables

Item	Quantity	Storage	Supplied By
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
Sample Purification Beads (SPB)	1 tube	2°C to 8°C	Illumina
96-well HSP plate	1	15°C to 30°C	User
96-well MIDI plate	1	15°C to 30°C	User
Freshly prepared 80% ethanol (EtOH)	400 µl per sample	15°C to 30°C	User
Microseal 'B' adhesive seals	3	15°C to 30°C	User
RNase/DNase-free reagent reservoirs (for multi-sample processing)	3	15°C to 30°C	User

Preparation

- Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 5 for information on how to access TruSight One Best Practices on the Illumina website.
- Make sure that the Resuspension Buffer and Sample Purification Beads are at room temperature.
- For multi-sample processing:
  - Use a multichannel pipette.
  - Pour the Resuspension Buffer, Sample Purification Beads, and 80% EtOH into separate multichannel reagent reservoirs.
- Label a new 96-well MIDI plate **NLC** (Nextera Library Clean Up) with a smudge resistant pen.

- Label a new 96-well HSP plate **NLS** (Nextera Library Sample) with a smudge resistant pen.

Procedure

1. Remove the NLA plate from the thermocycler and centrifuge to 280 × g for 1 minute.
2. Remove the adhesive seal from the NLA plate.
3. Transfer 50 µl of clear supernatant from each well of the NLA plate to the corresponding well of the new 96-well MIDI plate labeled NLC.
4. Vortex the Sample Purification Beads until the beads are well dispersed.
5. Add 90 µl well-resuspended Sample Purification Beads to each well of the NLC plate.
6. Mix thoroughly as follows:
   a. Seal the NLC plate with a Microseal 'B' adhesive seal.
   b. Shake the NLC plate on a microplate shaker at 1800 rpm for 1 minute.
7. Incubate the NLC plate at room temperature for 10 minutes.
8. Centrifuge the NLC plate to 280 × g for 1 minute.
9. Remove the adhesive seal from the NLC plate.
10. Place the NLC plate on the magnetic stand for 2 minutes or until the liquid appears clear.
11. Using a 200 µl single channel or multichannel pipette set to 140 µl, remove and discard all of the supernatant from each well of the NLC plate.

    NOTE
    Leave the NLC plate on the magnetic stand while performing the following 80% EtOH wash steps (12–14).

12. With the NLC plate on the magnetic stand, slowly add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads. Incubate at room temperature for 30 seconds.
13. Remove and discard the 80% EtOH from each well of the NLC plate.
14. Repeat steps 12 and 13 one time for a total of two 80% EtOH washes.
15. Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NLC plate without disturbing the beads.

16 Let the NLC plate stand at room temperature for 10 minutes to dry on the magnetic stand.

17 Remove the NLC plate from the magnetic stand.

18 Add 27.5 µl Resuspension Buffer to each well of the NLC plate. Do not touch the beads with the pipette tips.

19 Mix thoroughly as follows:
   a Seal the NLC plate with a Microseal 'B' adhesive seal.
   b Shake the NLC plate on a microplate shaker at 1800 rpm for 1 minute.

20 Incubate the NLC plate at room temperature for 2 minutes.

21 Centrifuge the NLC plate to 280 × g for 1 minute.

22 Remove the adhesive seal from the NLC plate.

23 Place the NLC plate on the magnetic stand for 2 minutes or until the liquid appears clear.

24 Transfer 25 µl of clear supernatant from each well of the NLC plate to the corresponding well of the new HSP plate labeled NLS. Take care not to disturb the beads.

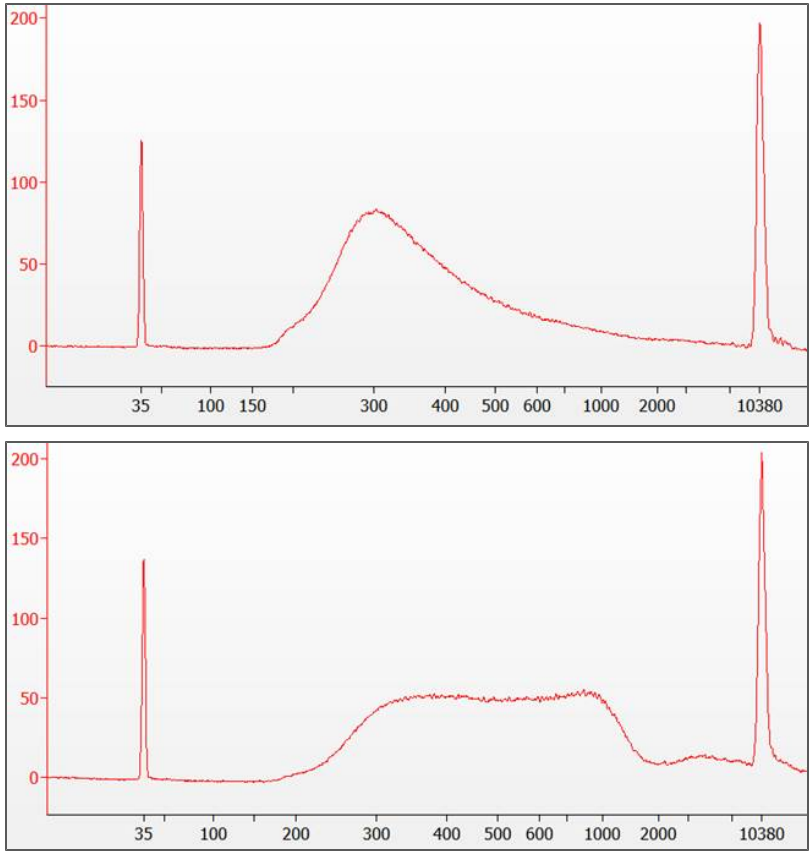
NOTE
Illumina recommends using a 20 µl single channel or multichannel pipette set to 12.5 µl to perform two consecutive transfers of 12.5 µl. This technique reduces sample loss by making sure that all of the liquid is transferred without disturbing the beads.

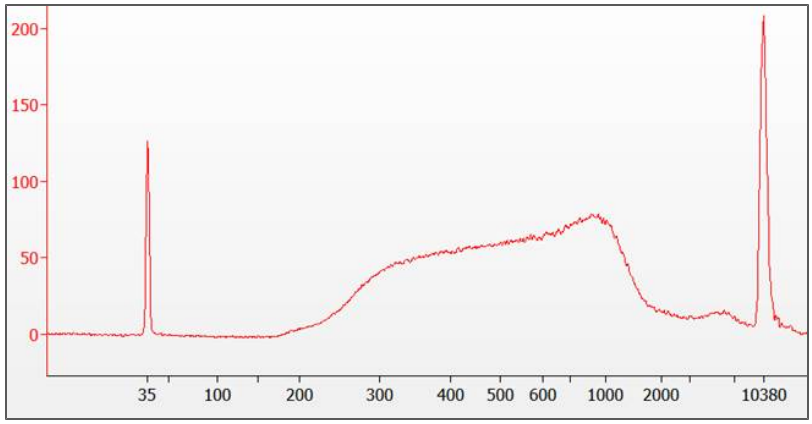
25 Quantify the library in the NLS plate using a fluorometric quantification method that uses dsDNA binding dyes.

NOTE
Accurate library quantification is needed to achieve even pooling for enrichment. Inaccurate quantitation and pooling can result in higher representation of some samples compared to others in the same pool.

26 [Optional] Load 1 µl of the library on an Agilent Technologies 2100 Bioanalyzer using an Agilent DNA 1000 Chip. Check the size of the library for a distribution of DNA fragments with a size range from approximately 300 bp–1 kb.
It is not necessary to have a sharp peak, but rather more important that most of the fragments fall within the 300 bp–1 kb range. The traces can be variable from prep to prep. The traces show some examples of possible distributions, but is not inclusive of successful preps.

Figure 3 Example TruSight One Post-PCR, Pre-Enriched Library Distributions





SAFE STOPPING POINT

If you do not plan to proceed immediately to *First Hybridization* on page 25, you can safely stop the protocol here. If you are stopping, seal the NLS plate with a Microseal 'B' adhesive seal and store it at -15°C to -25°C for up to 14 days.

First Hybridization

This process mixes the DNA library with capture probes to targeted regions of interest. The recommended hybridization time makes sure that targeted regions bind to the capture probes thoroughly. This process also describes how to combine multiple libraries with different indices into a single pool before enrichment.

Consumables

Item	Quantity	Storage	Supplied By
TruSight One Oligos (TOO)	1 tube	-15°C to -25°C	Illumina
Enrichment Hybridization Buffer (EHB)	1 tube	-15°C to -25°C	Illumina
96-well HSP plate	1	15°C to 30°C	User
[Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa)	1 per pooled sample	15°C to 30°C	User
Microseal 'B' adhesive seal	1	15°C to 30°C	User
RNase/DNase-free eight-tube strips and caps (for multi-sample processing)	2	15°C to 30°C	User

Preparation

- Remove the following from -15°C to -25°C storage and thaw them at room temperature:
  - Enrichment Hybridization Buffer
  - TruSight One Oligos
- For multi-sample processing:
  - Use a multichannel pipette.
  - Distribute the TruSight One Oligos and Enrichment Hybridization Buffer into separate eight-tube strips, dispensing equal volumes into each of the wells.
- Remove the NLS plate from -15° to -25°C storage, if it was stored at the conclusion of *First PCR Clean Up* and thaw on ice.

- Centrifuge the thawed NLS plate to 280 × g for 1 minute.
- Remove the adhesive seal from the thawed NLS plate.

▸ Pre-program the thermal cycler with the following program and save as **NRC HYB**:

a Choose the pre-heat lid option and set to 100°C
b 95°C for 10 minutes
c 18 incubations of 1 minute each, starting at 94°C, then decreasing 2°C per incubation
d 58°C for forever

▸ Label a new 96-well HSP plate **NEH1** (Nextera Enrichment Hyb 1) with a smudge resistant pen.

Pool Libraries

1 Reference Table 1 for the amount of DNA library to use for enrichment. Illumina recommends using 500 ng of each DNA library, quantified by a fluorometric dsDNA quantification method. See *DNA Input Recommendations* on page 3.

- If you are pooling libraries, combine 500 ng of each DNA library. Ensure that each library in the pool has a unique index.
- If the total volume is greater than 40 µl, concentrate the pooled sample. Use either a vacuum concentrator or Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) according to the manufacturer's instructions.
  - If you are using a vacuum concentrator, Illumina recommends concentrating samples with a no heat and medium drying rate setting.
  - If you are using an Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa), it is not required to pre-rinse the device before use. Most of the volume filters through in 5 minutes, but up to 30 minutes can be required, depending on the starting volume.
- If the pooled sample volume after concentrating is less than 40 µl, bring the volume up to 40 µl with Resuspension Buffer.

2 The recommended pre-enrichment pooling strategy is to pool libraries so that each contains a unique Index 1/i7 index. With this pooling approach, samples can be sequenced using a single index read workflow, as described in the HiSeq® and GAIIx user guides.

- If Index1/i7 indices are not unique, make sure that libraries with different Index 2/i5 indices are included (e.g. N703/E503 and N703/E504). With this approach,

sequence samples using a dual index read workflow, as described in the HiSeq and GAIIx user guides.

Table 1 DNA Libraries for Enrichment

Library Pool Complexity	Total DNA Library Mass (ng)
1-plex	500
2-plex	1000
3-plex	1500
4-plex	2000
5-plex	2500
6-plex	3000
7-plex	3500
8-plex	4000
9-plex	4500
10-plex	5000
11-plex	5500
12-plex	6000

NOTE
The 9 sample TruSight One kit is intended to support 3 samples per enrichment. The 36 sample TruSight One kit can support up to 12 samples per enrichment.

Procedure

1 Thoroughly vortex the Enrichment Hybridization Buffer tube until the solution is completely resuspended. Visually make sure that no crystal structures are present.

NOTE
If crystals and cloudiness are observed, vortex the Enrichment Hybridization Buffer tube until it appears clear.

2 Add the following reagents in the order listed to each well of the new 96-well HSP plate labeled NEH1:

Reagent	Volume (µl)
DNA library sample or library pool from NLS plate	40
Enrichment Hybridization Buffer	50
TruSight One Oligos	10
Total Volume per Sample	**100**

3 Mix thoroughly as follows:
   a Seal the NEH1 plate with a Microseal 'B' adhesive seal. Make sure that the plate is tightly sealed to prevent potential evaporation. Use an adhesive seal roller to apply force to the seal and make sure that the seal is secured.
   b Shake the NEH1 plate on a microplate shaker at 1200 rpm for 1 minute.

4 Centrifuge the NEH1 plate to 280 × g for 1 minute.

5 Place the sealed NEH1 plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NRC HYB** program.
Incubate the plate at the 58°C holding temperature for at least 90 minutes and up to a maximum of 24 hours. Do not remove the plate from 58°C incubation until you are ready to proceed to *First Capture* on page 29.

First Capture

This process uses streptavidin beads to capture probes hybridized to the targeted regions of interest. Two heated wash procedures remove non-specific binding from the beads. The enriched library is then eluted from the beads and prepared for a second round of hybridization. The use of the correct equipment and temperatures is needed to ensure removal of non-specific DNA as well as retention of the target regions.

Consumables

Item	Quantity	Storage	Supplied By
2N NaOH (HP3)	1 tube	-15°C to -25°C	Illumina
Elute Target Buffer 2 (ET2)	1 tube	2°C to 8°C	Illumina
Enrichment Elution Buffer 1 (EE1)	1 tube	-15°C to -25°C	Illumina
Enrichment Wash Solution (EWS)	1 tube	-15°C to -25°C	Illumina
Streptavidin Magnetic Beads (SMB)	1 tube	2°C to 8°C	Illumina
1.7 ml microcentrifuge tube	1	15°C to 30°C	User
96-well HSP plate	1	15°C to 30°C	User
96-well MIDI plate	1	15°C to 30°C	User
Microseal 'B' adhesive seals	6	15°C to 30°C	User

Preparation

- Remove the HP3, Enrichment Elution Buffer 1, and Enrichment Wash Solution from -15°C to -25°C storage and thaw at room temperature.
- Remove the Elute Target Buffer 2 and Streptavidin Magnetic Beads from 2°C to 8°C storage and let stand at room temperature.

NOTE
Make sure that you use the Streptavidin Magnetic Beads (2 ml tube) and not the Sample Purification Beads (15 ml tube) for this procedure.

- Pre-heat the microheating system to 50°C.

NOTE
The Enrichment Wash steps are key to ensuring high enrichment specificity. Illumina recommends using a microheating system with a MIDI plate insert for these steps to make sure that samples are maintained at the desired temperature. Too low or too high temperatures can result in lower percent enrichments and decreased yields. If a microheating system is not available, a thermal cycler can be used with modifications by following the directions in *Alternative Thermal Cycler Steps for Successful Enrichment* on page 59. This approach requires additional sample transfers.

- Label a new 96-well MIDI plate **NEW1** (Nextera Enrichment Wash 1) with a smudge resistant pen.
- Label a new 96-well HSP plate **NEH2** (Nextera Enrichment Hyb 2) with a smudge resistant pen.

First Bind

1. Remove the NEH1 plate from the thermal cycler.
2. Centrifuge the NEH1 plate to 280 × g for 1 minute.
3. Remove the adhesive seal from the NEH1 plate. Take care when removing the seal to avoid spilling the contents of the wells.
4. Transfer the entire contents (~100 µl) from each well of the NEH1 plate to the corresponding well of the new 96-well MIDI plate labeled NEW1.

NOTE
If an overnight First Hybridization was performed, it is normal to see a small degree of sample loss. However, if the sample loss is greater than 15%, Illumina does not recommend proceeding with the sample preparation. Poor sealing or not heating the lid can cause this amount of loss.

5. Vortex the Streptavidin Magnetic Beads tube until the beads are well dispersed, then add 250 µl well-mixed Streptavidin Magnetic Beads to the wells of the NEW1 plate.
6. Mix thoroughly as follows:
   a. Seal the NEW1 plate with a Microseal 'B' adhesive seal.
   b. Shake the NEW1 plate on a microplate shaker at 1200 rpm for 5 minutes.
7. Let the NEW1 plate stand at room temperature for 25 minutes.
8. Centrifuge the NEW1 plate to 280 × g for 1 minute.

9 Remove the adhesive seal from the NEW1 plate.

10 Place the NEW1 plate on the magnetic stand for 2 minutes at room temperature or until the liquid appears clear.

11 Carefully remove and discard all of the supernatant from each well of the NEW1 plate without disturbing the beads.

12 Remove the NEW1 plate from the magnetic stand.

First Wash

1 Make sure that the Enrichment Wash Solution tube is at room temperature, then thoroughly vortex the tube.

NOTE
It is normal that the Enrichment Wash Solution can be cloudy after vortexing.

2 Add 200 µl Enrichment Wash Solution to each well of the NEW1 plate.

3 Mix thoroughly and resuspend the bead pellet by repeatedly dispensing the wash solution over the bead pellet until it is immersed in the solution. Then gently pipette the entire volume of each well up and down 10 times to ensure complete resuspension of the sample.

NOTE
Proper resuspension of the Streptavidin Magnetic Beads is needed to ensure efficient removal of non-specific DNA from the reaction, which otherwise results in poor enrichment statistics.

4 Seal the NEW1 plate with a Microseal 'B' adhesive seal.

5 Place the sealed NEW1 plate on the **pre-heated** microheating system. Close the lid and incubate at 50°C for 30 minutes.

NOTE
The Enrichment Wash steps are key to ensuring high enrichment specificity. Illumina recommends using a microheating system with a MIDI plate insert for these steps to make sure that samples are maintained at the desired temperature. Too low or too high temperatures can result in lower percent enrichments and decreased yields. If a microheating system is not available, a thermal cycler can be used with modifications by following the directions in *Alternative Thermal Cycler Steps for Successful Enrichment* on page 59. This approach requires additional sample transfers.

6 Place the magnetic stand next to the microheating system for immediate access.

7 Remove the NEW1 plate from the microheating system and ***immediately*** place it on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Remove the adhesive seal from the NEW1 plate.

9 Immediately remove and discard all of the supernatant from each well of the NEW1 plate.

10 Remove the NEW1 plate from the magnetic stand.

11 Repeat steps 2–10 one time for a total of two Enrichment Wash Solution washes.

First Elution

1 Add the following reagents in the order listed in a new 1.7 ml microcentrifuge tube to create the elution pre-mix. Multiply each volume by the number of pooled samples being prepared. The volumes include an excess amount for processing multiple samples.

Reagent	Volume (µl)
Enrichment Elution Buffer 1	28.5
HP3	1.5
Total Volume per Sample	**30**

2 Vortex the elution pre-mix tube, then add **23.5** µl of the mix to each well of the NEW1 plate.

3 Mix thoroughly as follows:
   a Seal the NEW1 plate with a Microseal 'B' adhesive seal.
   b Shake the NEW1 plate on a microplate shaker at 1800 rpm for 2 minutes.

4 Let the NEW1 plate stand at room temperature for 2 minutes.

5 Centrifuge the NEW1 plate to 280 × g for 1 minute.

6 Carefully remove the adhesive seal from the NEW1 plate to avoid spilling the contents of the wells.

7 Place the NEW1 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Transfer 21 µl of clear supernatant from each well of the NEW1 plate to the corresponding well of the new HSP plate labeled NEH2. Take care not to disturb the beads.

NOTE

Illumina recommends using a 20 µl single channel or multichannel pipette set to 10.5 µl to perform two consecutive transfers of 10.5 µl. This technique reduces sample loss by making sure that all of the liquid is transferred without disturbing the beads.

9 Add 4 µl Elute Target Buffer 2 to each well of the NEH2 plate containing samples to neutralize the elution.

10 Mix thoroughly as follows:

a Seal the NEH2 plate with a Microseal 'B' adhesive seal.

b Shake the NEH2 plate on a microplate shaker at 1200 rpm for 1 minute.

11 Centrifuge the NEH2 plate to 280 × g for 1 minute.

12 Store the remaining reagents as follows:

a Place the Elute Target Buffer 2 and Streptavidin Magnetic Beads tubes in 2°C to 8°C storage.

b Place the HP3, Enrichment Elution Buffer 1, and Enrichment Wash Solution tubes in -15°C to -25°C storage.

c Discard any remaining elution pre-mix.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Second Hybridization* on page 34, you can safely stop the protocol here. If you are stopping, seal the NEH2 plate with a Microseal 'B' adhesive seal and store it at -15°C to -25°C for up to 7 days.

Second Hybridization

This process combines the eluted DNA library from the first enrichment round with additional capture probes to targeted regions of interest. This second hybridization is required to ensure high specificity of the captured regions.

Consumables

Item	Quantity	Storage	Supplied By
TruSight One Oligos (TOO)	1 tube	-15°C to -25°C	Illumina
Enrichment Hybridization Buffer (EHB)	1 tube	-15°C to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
Microseal 'B' adhesive seal	1	15°C to 30°C	User

Preparation

- Remove the following from -15°C to -25°C storage and thaw them at room temperature:
  - Enrichment Hybridization Buffer
  - TruSight One Oligos
- Make sure that the Resuspension Buffer is at room temperature.
- Remove the NEH2 plate from -15°C to -25°C storage, if it was stored at the conclusion of *First Capture* and thaw on ice.
  - Centrifuge the thawed NEH2 plate to 280 × g for 1 minute.

Procedure

1 Thoroughly vortex the Enrichment Hybridization Buffer tube until the solution is completely resuspended. Visually make sure that no crystal structures are present.

NOTE
If crystals and cloudiness are observed, vortex the Enrichment Hybridization Buffer tube until it appears clear.

2 Remove the adhesive seal from the NEH2 plate.

3 Add the following reagents in the order listed to each well of the NEH2 plate:

Reagent	Volume (µl)
Resuspension Buffer	15
Enrichment Hybridization Buffer	50
TruSight One Oligos	10

4 Mix thoroughly as follows:

a Seal the NEH2 plate with a Microseal 'B' adhesive seal. Make sure that the plate is tightly sealed to prevent potential evaporation. Use an adhesive seal roller to apply force to the seal and make sure that the seal is secured.

b Shake the NEH2 plate on a microplate shaker at 1200 rpm for 1 minute

5 Centrifuge the NEH2 plate to 280 × g for 1 minute.

6 Place the sealed NEH2 plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NRC HYB** program.
Incubate the plate at the 58°C holding temperature overnight for at least 14.5 hours and up to a maximum of 24 hours. Do not remove the plate from 58°C incubation until you are ready to proceed to *Second Capture* on page 36.

Second Capture

This process uses streptavidin beads to capture probes hybridized to the targeted regions of interest. Two heated wash procedures remove non-specific binding from the beads. The enriched library is then eluted from the beads and prepared for sequencing. The use of the correct equipment and temperatures is needed to ensure removal of non-specific DNA as well as retention of the target regions.

NOTE
These procedures are similar to the *First Capture* on page 29.

Consumables

Item	Quantity	Storage	Supplied By
2N NaOH (HP3)	1 tube	-15°C to -25°C	Illumina
Elute Target Buffer 2 (ET2)	1 tube	2°C to 8°C	Illumina
Enrichment Elution Buffer 1 (EE1)	1 tube	-15°C to -25°C	Illumina
Enrichment Wash Solution (EWS)	1 tube	-15°C to -25°C	Illumina
Streptavidin Magnetic Beads (SMB)	1 tube	2°C to 8°C	Illumina
1.7 ml microcentrifuge tube	1	15°C to 30°C	User
96-well MIDI plates	2	15°C to 30°C	User
Microseal 'B' adhesive seals	6	15°C to 30°C	User

Preparation

- Remove the HP3, Enrichment Elution Buffer 1, and Enrichment Wash Solution from -15°C to -25°C storage and thaw at room temperature.
- Remove the Elute Target Buffer 2 and Streptavidin Magnetic Beads from 2°C to 8°C storage and let stand at room temperature.

NOTE
Make sure that you use the Streptavidin Magnetic Beads (2 ml tube) and not the Sample Purification Beads (15 ml tube) for this procedure.

- Pre-heat the microheating system to 50°C.

NOTE
The Enrichment Wash steps are key to ensuring high enrichment specificity. Illumina recommends using a microheating system with a MIDI plate insert for these steps to make sure that samples are maintained at the desired temperature. Too low or too high temperatures can result in lower percent enrichments and decreased yields. If a microheating system is not available, a thermal cycler can be used with modifications by following the directions in *Alternative Thermal Cycler Steps for Successful Enrichment* on page 59. This approach requires additional sample transfers.

- Label a new 96-well MIDI plate **NEW2** (Nextera Enrichment Wash 2) with a smudge resistant pen.
- Label a new 96-well MIDI plate **NEC1** (Nextera Enriched Clean Up 1) with a smudge resistant pen.

Second Bind

1. Remove the NEH2 plate from the thermal cycler.
2. Centrifuge the room temperature NEH2 plate to 280 × g for 1 minute.
3. Remove the adhesive seal from the NEH2 plate. Take care when removing the seal to avoid spilling the contents of the wells.
4. Transfer the entire contents (~100 µl) from each well of the NEH2 plate to the corresponding well of the new 96-well MIDI plate labeled NEW2.

NOTE
It is normal to see a small degree of sample loss after overnight hybridization. However, if the sample loss is greater than 15%, Illumina does not recommend proceeding with the sample preparation. Poor sealing or not heating the lid can cause this amount of loss.

5. Vortex the Streptavidin Magnetic Beads tube until the beads are well dispersed, then add 250 µl well-mixed Streptavidin Magnetic Beads to the wells of the NEW2 plate.
6. Mix thoroughly as follows:
   a. Seal the NEW2 plate with a Microseal 'B' adhesive seal.
   b. Shake the NEW2 plate on a microplate shaker at 1200 rpm for 5 minutes
7. Let the NEW2 plate stand at room temperature for 25 minutes.

8 Centrifuge the NEW2 plate to 280 × g for 1 minute.

9 Remove the adhesive seal from the NEW2 plate.

10 Place the NEW2 plate on the magnetic stand for 2 minutes at room temperature or until the liquid appears clear.

11 Carefully remove and discard all of the supernatant from each well of the NEW2 plate without disturbing the beads.

12 Remove the NEW2 plate from the magnetic stand.

Second Wash

1 Make sure that the Enrichment Wash Solution tube is at room temperature, then thoroughly vortex the tube.

NOTE
It is normal that the Enrichment Wash Solution can be cloudy after vortexing.

2 Add 200 µl Enrichment Wash Solution to each well of the NEW2 plate.

3 Mix thoroughly and resuspend the bead pellet by repeatedly dispensing the wash solution over the bead pellet until it is immersed in the solution. Then gently pipette the entire volume of each well up and down 10 times to ensure complete resuspension of the sample.

NOTE
Proper resuspension of the Streptavidin Magnetic Beads is needed to ensure efficient removal of non-specific DNA from the reaction, which otherwise results in poor enrichment statistics.

4 Seal the NEW2 plate with a Microseal 'B' adhesive seal.

5 Incubate the NEW2 plate on the **pre-heated** microheating system, with the lid closed, at 50°C for 30 minutes.

NOTE
The Enrichment Wash steps are key to ensuring high enrichment specificity. Illumina recommends using a microheating system with a MIDI plate insert for these steps to make sure that samples are maintained at the desired temperature. Too low or too high temperatures can result in lower percent enrichments and decreased yields. If a microheating system is not available, a thermal cycler can be used with modifications by following the directions in *Alternative Thermal Cycler Steps for Successful Enrichment* on page 59. This approach requires additional sample transfers.

6 Place the magnetic stand next to the microheating system for immediate access.

7 Remove the NEW2 plate from the microheating system and ***immediately*** place it on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Remove the adhesive seal from the NEW2 plate.

9 Immediately remove and discard all of the supernatant from each well of the NEW2 plate.

10 Remove the NEW2 plate from the magnetic stand.

11 Repeat steps 2–10 one time for a total of two Enrichment Wash Solution washes.

Second Elution

1 Add the following reagents in the order listed in a new 1.7 ml microcentrifuge tube to create the elution pre-mix. Multiply each volume by the number of pooled samples being prepared. The volumes include an excess amount for processing multiple samples.

Reagent	Volume (µl)
Enrichment Elution Buffer 1	28.5
HP3	1.5
Total Volume per Sample	**30**

2 Vortex the elution pre-mix tube, then add **23.5** µl of the mix to each well of the NEW2 plate.

3 Mix thoroughly as follows:
   a Seal the NEW2 plate with a Microseal 'B' adhesive seal.
   b Shake the NEW2 plate on a microplate shaker at 1800 rpm for 2 minutes.

4 Let the NEW2 plate stand at room temperature for 2 minutes.

5 Centrifuge the NEW2 plate to 280 × g for 1 minute.

6 Carefully remove the adhesive seal from the NEW2 plate to avoid spilling the contents of the wells.

7 Place the NEW2 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Transfer 21 µl of clear supernatant from each well of the NEW2 plate to the corresponding well of the new MIDI plate labeled NEC1. Take care not to disturb the beads.

NOTE
Illumina recommends using a 20 µl single channel or multichannel pipette set to 10.5 µl to perform two consecutive transfers of 10.5 µl. This technique reduces sample loss by making sure that all of the liquid is transferred without disturbing the beads.

9 Add 4 µl Elute Target Buffer 2 to each well of the NEC1 plate containing samples to neutralize the elution.

10 Mix thoroughly as follows:
   a Seal the NEC1 plate with a Microseal 'B' adhesive seal.
   b Shake the NEC1 plate on a microplate shaker at 1800 rpm for 1 minute.

11 Centrifuge the NEC1 plate to 280 × g for 1 minute.

12 Store the remaining reagents as follows:
   a Place the Elute Target Buffer 2 and Streptavidin Magnetic Beads tubes in 2°C to 8°C storage.
   b Place the HP3, Enrichment Elution Buffer 1, and Enrichment Wash Solution tubes in -15°C to -25°C storage.
   c Discard any remaining elution pre-mix.

Capture Sample Clean Up

This process uses Sample Purification Beads to purify the captured library before PCR amplification.

Consumables

Item	Quantity	Storage	Supplied By
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
Sample Purification Beads (SPB)	1 tube	2°C to 8°C	Illumina
96-well HSP plate	1	15°C to 30°C	User
Freshly prepared 80% ethanol (EtOH)	400 µl per sample	15°C to 30°C	User
Microseal 'B' adhesive seals	3	15°C to 30°C	User

Preparation

- Review *Best Practices for Handling Magnetic Beads*. See *Additional Resources* on page 5 for information on how to access TruSight One Best Practices on the Illumina website.
- Make sure that the Resuspension Buffer and Sample Purification Beads are at room temperature.
- Label a new 96-well HSP plate **NEA** (Nextera Enrichment Amplification) with a smudge resistant pen.

Procedure

1. Remove the adhesive seal from the NEC1 plate.
2. Vortex the Sample Purification Beads tube until the beads are well dispersed, then add 45 µl well-mixed Sample Purification Beads to each well of the NEC1 plate.
3. Mix thoroughly as follows:
   a. Seal the NEC1 plate with a Microseal 'B' adhesive seal.
   b. Shake the NEC1 plate on a microplate shaker at 1800 rpm for 1 minute.
4. Incubate the NEC1 plate at room temperature for 10 minutes.

5 Centrifuge the NEC1 plate to 280 × g for 1 minute.

6 Remove the adhesive seal from the NEC1 plate.

7 Place the NEC1 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Remove and discard all of the supernatant from each well of the NEC1 plate.

NOTE
Leave the NEC1 plate on the magnetic stand while performing the following 80% EtOH wash steps (9–11).

9 With the NEC1 plate on the magnetic stand, slowly add 200 µl freshly made 80% EtOH to each well without disturbing the beads. Incubate the plate at room temperature for 30 seconds.

10 Remove and discard the 80% EtOH from each well of the NEC1 plate.

11 Repeat steps 9 and 10 one time for a total of two 80% EtOH washes.

12 Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NEC1 plate without disturbing the beads.

13 Let the NEC1 plate stand at room temperature for 10 minutes to dry on the magnetic stand.

14 Remove the NEC1 plate from the magnetic stand.

15 Add 27.5 µl Resuspension Buffer to each well of the NEC1 plate. Do not touch the beads with the pipette tips.

16 Mix thoroughly as follows:
   a Seal the NEC1 plate with a Microseal 'B' adhesive seal.
   b Shake the NEC1 plate on a microplate shaker at 1800 rpm for 1 minute.

17 Incubate the NEC1 plate at room temperature for 2 minutes.

18 Centrifuge the NEC1 plate to 280 × g for 1 minute.

19 Remove the adhesive seal from the NEC1 plate.

20 Place the NEC1 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

21 Transfer 25 µl of clear supernatant from each well of the NEC1 plate to the corresponding well of the new HSP plate labeled NEA. Take care not to disturb the beads.

NOTE

Illumina recommends using a 20 µl single channel or multichannel pipette set to 12.5 µl to perform two consecutive transfers of 12.5 µl. This technique reduces sample loss by making sure that all of the liquid is transferred without disturbing the beads.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Second PCR Amplification* on page 44, you can safely stop the protocol here. If you are stopping, seal the NEA plate with a Microseal 'B' adhesive seal and store it at -15°C to -25°C for up to 7 days.

Second PCR Amplification

This process amplifies the captured library via a 10-cycle PCR program. It is critical to use the full amount of recommended input DNA and not add extra PCR cycles to ensure libraries produce high-quality sequencing results.

Consumables

Item	Quantity	Storage	Supplied By
Nextera Enrichment Amplification Mix (NEM)	1 tube	-15°C to -25°C	Illumina
PCR Primer Cocktail (PPC)	1 tube	-15°C to -25°C	Illumina
Microseal 'A' film	1	15°C to 30°C	User
Microseal 'B' adhesive seal	1	15°C to 30°C	User

Preparation

- Remove the Nextera Enrichment Amplification Mix and PCR Primer Cocktail from -15°C to -25°C storage and thaw on ice.
  - Briefly centrifuge the thawed Nextera Enrichment Amplification Mix and PCR Primer Cocktail tubes for 5 seconds.

NOTE
If you do not intend to consume the Nextera Enrichment Amplification Mix and PCR Primer Cocktail in one use, dispense the reagents into single use aliquots. Freeze the aliquots in order to avoid repeated freeze thaw cycles.

- Remove the NEA plate from -15°C to -25°C storage, if it was stored at the conclusion of *Second Capture* and thaw on ice.
  - Centrifuge the thawed NEA plate to 280 × g for 1 minute.
  - Remove the adhesive seal from the thawed NEA plate.

- Pre-program the thermal cycler with the following program and save as **NEM AMP10**:
  - Choose the pre-heat lid option and set to 100°C
  - 98°C for 30 seconds
  - **10 cycles** of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C for 5 minutes
  - Hold at 10°C

NOTE

Illumina has optimized the number of recommended PCR cycles for enrichment assays based on the level of pre-enrichment sample pooling and the size of the oligonucleotide set. Do not add or reduce the cycles of PCR, as it may compromise data quality.

Procedure

1. Add 5 µl PCR Primer Cocktail to each well of the NEA plate.
2. Add 20 µl Nextera Enrichment Amplification Mix to each well of the NEA plate.
3. Mix thoroughly as follows:
   a. Seal the NEA plate with a Microseal 'A' film. Use an adhesive seal roller to apply force to the film and make sure that the film is secured.
   b. Shake the NEA plate on a microplate shaker at 1200 rpm for 1 minute
4. Centrifuge the NEA plate to 280 × g for 1 minute.
5. Place the sealed NEA plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NEM AMP10** program.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Second PCR Clean Up* on page 46, the NEA plate can remain on the thermocycler overnight. If you are stopping, replace the Microseal 'A' film with a Microseal 'B' adhesive seal and store the NEA plate at 2°C to 8°C for up to two days.

Second PCR Clean Up

This process uses Sample Purification Beads to purify the enriched library and remove unwanted products.

Consumables

Item	Quantity	Storage	Supplied By
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
Sample Purification Beads (SPB)	1 tube	2°C to 8°C	Illumina
96-well HSP plate	1	15°C to 30°C	User
96-well MIDI plate	1	15°C to 30°C	User
Freshly prepared 80% ethanol (EtOH)	400 µl per sample	15°C to 30°C	User
Microseal 'B' adhesive seals	3	15°C to 30°C	User

Preparation

- Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 5 for information on how to access TruSight One Best Practices on the Illumina website.
- Make sure that the Resuspension Buffer and Sample Purification Beads are at room temperature.
- Remove the NEA plate from 2°C to 8°C storage, if it was stored at the conclusion of *Second PCR Amplification* and let stand to bring to room temperature.
- Label a new 96-well MIDI plate **NEC2** (Nextera Enriched Clean Up 2) with a smudge resistant pen.
- Label a new 96-well HSP plate **NES** (Nextera Enrichment Sample) with a smudge resistant pen.

Procedure

1. Centrifuge the NEA plate to 280 × g for 1 minute.
2. Remove the adhesive seal from the NEA plate.
3. Transfer the entire contents from each well of the NEA plate to the corresponding well of the new 96-well MIDI plate labeled NEC2.
4. Vortex the Sample Purification Beads until the beads are well dispersed.
5. Add 90 µl well-mixed Sample Purification Beads to each well of the NEC2 plate containing 50 µl of PCR amplified library.
6. Mix thoroughly as follows:
   a. Seal the NEC2 plate with a Microseal 'B' adhesive seal.
   b. Shake the NEC2 plate on a microplate shaker at 1800 rpm for 1 minute.
7. Incubate the NEC2 plate at room temperature for 10 minutes.
8. Centrifuge the NEC2 plate to 280 × g for 1 minute.
9. Remove the adhesive seal from the NEC2 plate.
10. Place the NEC2 plate on the magnetic stand at room temperature for 2 minutes or until the liquid appears clear.
11. Carefully remove and discard all of the supernatant from each well of the NEC2 plate.

NOTE
Leave the NEC2 plate on the magnetic stand while performing the following 80% EtOH wash steps (12–14).

12. With the NEC2 plate on the magnetic stand, slowly add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads. Incubate the plate at room temperature for 30 seconds.
13. Remove and discard the 80% EtOH from each well of the NEC2 plate.
14. Repeat steps 12–13 one time for a total of two 80% EtOH washes.
15. Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NEC2 plate without disturbing the beads.
16. Let the NEC2 plate stand at room temperature for 10 minutes to dry on the magnetic stand.

17 Remove the NEC2 plate from the magnetic stand.

18 Add 32.5 µl Resuspension Buffer to each well of the NEC2 plate. Do not touch the beads with the pipette tips.

19 Mix thoroughly as follows:
   a Seal the NEC2 plate with a Microseal 'B' adhesive seal.
   b Shake the NEC2 plate on a microplate shaker at 1800 rpm for 1 minute.

20 Incubate the NEC2 plate at room temperature for 2 minutes.

21 Centrifuge the NEC2 plate to 280 × g for 1 minute.

22 Remove the adhesive seal from the NEC2 plate.

23 Place the NEC2 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

24 Transfer 30 µl of clear supernatant from each well of the NEC2 plate to the corresponding well of the new HSP plate labeled NES. Take care not to disturb the beads.

NOTE

Illumina recommends using a 20 µl single channel or multichannel pipette set to 15 µl to perform two consecutive transfers of 15 µl. This technique reduces sample loss by making sure that all of the liquid is transferred without disturbing the beads.

25 Seal the NES plate with a Microseal 'B' adhesive seal.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Validate Library* on page 49, store the sealed NES plate at -15°C to -25°C for up to 7 days. If the plate is stored for more than 7 days, requantify your library to guarantee the accuracy of your enrichment results.

Validate Library

Illumina recommends performing the following procedures for quality control analysis and quantification of your enriched library.

Quantify Libraries

In order to achieve the highest data quality on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. Optimizing cluster densities requires accurate quantitation of DNA library templates.

Illumina recommends a fluorometric dsDNA assay to quantitate dsDNA samples, because it can quantitate small DNA volumes and measure DNA directly. Other techniques can pick up contamination such as RNA and proteins. Illumina recommends using a spectrofluorometer, because fluorometry provides DNA-specific quantification. Spectrophotometry can also measure RNA and yield values that are too high.

If quantifying a 3–12-plex library, before quantification dilute the post-enriched library by adding 2 µl library to 28 µl Resuspension Buffer in a new tube or well. Use this dilution for quantification and quality assessment as well as sequencing. For 1 or 2-plex enrichments a dilution is not needed.

NOTE

Use the following formula to convert from ng/µl to nM. Assume a 650 bp library size or calculate based on the average size of the enriched library:

$$\frac{\text{(concentration in ng/µl)}}{\text{(660 g/mol *average library size)}} \times 10\text{^}6 = \text{concentration in nM}$$

For example:

$$\frac{\text{15 ng/µl)}}{\text{(660 g/mol *650)}} \times 10\text{^}6 = 34.9\ \text{nM}$$

Alternatively, you can quantitate libraries using qPCR according to the *Sequencing Library qPCR Quantification Guide (part # 11322363)*.

NOTE

You can download the *Sequencing Library qPCR Quantification Guide* from the Illumina website at support.illumina.com/sequencing/kits.ilmn.

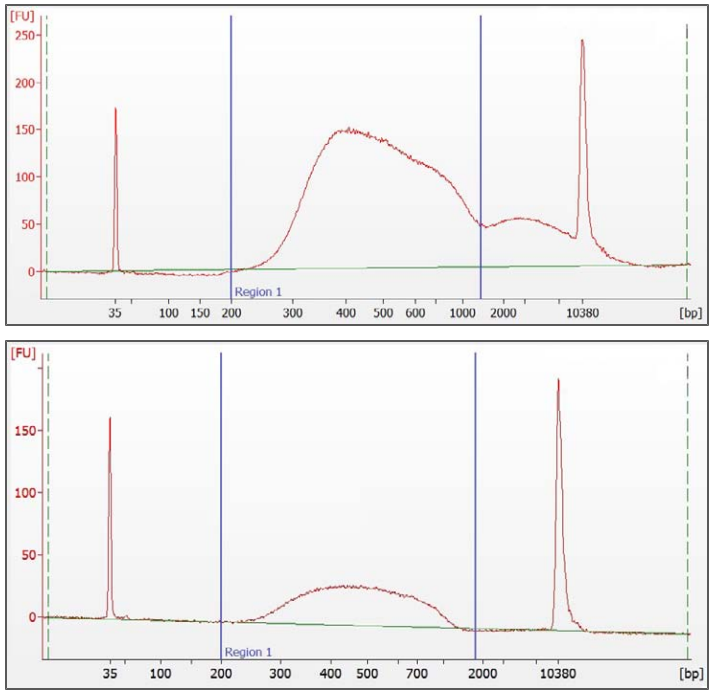
Click **TruSight One Sequencing Panel Support**. Then click **Documentation & Literature** on the **TruSight One Sequencing Panel Support** page.

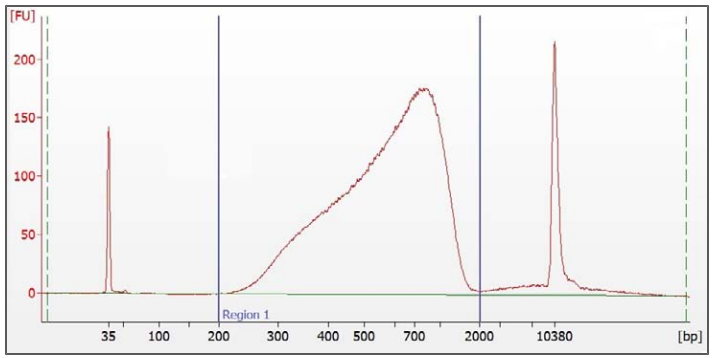
Assess Quality [Optional]

To assess library quality, load 1 µl of diluted post-enriched library on an Agilent Technologies 2100 Bioanalyzer using an Agilent High Sensitivity DNA Chip.

Check the size of the library for a distribution of DNA fragments with a size range from approximately 200 bp–1 kb. Depending on the level of indexing, insert size distribution can vary slightly, however the library peak must not be significantly shifted compared to the examples in Figure 4.

Figure 4 Example TruSight One Post-Enrichment Library Distributions





NOTE

The blue lines indicate the boundaries that were manually created to determine average library size. In the first example, a second minor peak at ~2000 bp is visible. Do not include this in the determination of average library size. The presence of these larger fragments does not affect downstream clustering and sequencing of your enriched library.

Preparing Your Libraries for Sequencing on a MiSeq

Illumina recommends using the following protocol to achieve optimal cluster density when sequencing a TruSight One library on a MiSeq®. Optimization of loading concentrations may be necessary to achieve desired cluster densities.

Consumables

Item	Quantity	Storage	Supplied By
Hybridization Buffer (HT1)	1 tube	-15°C to -30°C	Illumina
2N NaOH (HP3)	1 tube	-15°C to -30°C	User
PCR-grade water		-15°C to -30°C	User
PhiX (Optional)		-15°C to -30°C	User

Prepare a Fresh Dilution of NaOH

1 Dilute the post-enriched dilution that was used for fluorometric quantification to a final concentration of 1.25 nM using RSB buffer. 10 µl of diluted material is needed per MiSeq cartridge. Try to use a minimum of 4 µl of library to ensure aspirating the appropriate volumes.

NOTE
Following this protocol achieves a final concentration of 12.5 pM of library loaded onto the MiSeq by creating a post enriched dilution of 1.25 nM. If an 8 pM final concentration is desired, dilute the post enriched dilution to a final concentration of 0.8 nM. Alternatively, if a 10.0 pM final concentration is desired, dilute the post enriched dilution to a final concentration of 1.0 nM.

2 Prepare a fresh 0.1N NaOH solution using HP3 by taking 10 µl HP3 + 190 µl water.

3 Combine 10 µl of 1.25 nM library + 10 µl 0.1N NaOH and mix well.

4 Incubate the sample at room temp for 5 minutes.

5 Following incubation, add 980 µl HT1 and mix well by vortexing.

6 Place the denatured DNA on ice until you are ready to load into the MiSeq cartridge.

7 Load the entire sample onto the MiSeq cartridge and move forward with sequencing.

Sequence Library

Proceed to cluster generation. For more information, see the cluster generation section of the user guide for your Illumina platform.

- When quantifying a TruSight One post-enriched library using a fluorometric method, clustering at 12.5 pM generates cluster densities in the range of 1,200 k–1,400 k clusters/mm2 using the MiSeq V3 software and reagents. Results may vary based on your method of quantification. Illumina recommends that you determine the library concentration to cluster density relationship based on your lab instrumentation.
- Review the procedures, in the IEM quick reference card, on how to create a sample sheet for the TruSight One Sequencing Panel. See *Additional Resources* on page 5 for information on how to download IEM software and TruSight One appropriate IEM documentation from the Illumina website.
- A short sequencing run on a MiSeq can also be performed to optimize cluster density prior to performing a high density sequencing run.
- TruSight One prepared libraries contain dual 8 bp indexes. Depending on the combination of indexes used in your library pool, configure the sequencing run for either single or dual 8 bp index reads.
- When clustering TruSight One libraries on the cBot™ and sequencing on the HiSeq 1000/2000, GAIIx, or in High Output mode on the HiSeq 1500/2500, new primers are required whether performing a non-indexed, single-indexed, or dual-indexed run. Use the TruSeq Dual Index Sequencing Primer Kit for Paired End runs (catalog # PE-121-1003), which is good for a single run and contains the required primers for sequencing (HP10, HP11, HP12). These primers are included with MiSeq and HiSeq 1500/2500 rapid run reagent kits.
- For sequencing TruSight One libraries, Illumina recommends a paired-end 151-cycle sequencing run. Due to the library sizes generated in TruSight One, sequencing at longer read lengths can lead to an increase in the likelihood of sequencing into the flanking adapter sequence.

Supporting Information

Appendix A

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Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all of the requisite consumables and equipment.

Acronyms

Table 2 TruSight One Sequencing Panel Acronyms

Acronym	Definition
dsDNA	Double-stranded DNA
EE1	Enrichment Elution Buffer 1
EHB	Enrichment Hybridization Buffer
ET2	Elute Target Buffer 2
EWS	Enrichment Wash Solution
gDNA	Genomic DNA
HP3	2N NaOH
HSP	Hard Shell Plate
NEA	Nextera Enrichment Amplification Plate
NEC1	Nextera Enriched Clean Up Plate 1
NEC2	Nextera Enriched Clean Up Plate 2
NEH1	Nextera Enrichment Hyb Plate 1
NEH2	Nextera Enrichment Hyb Plate 2
NEM	Nextera Enrichment Amplification Mix
NES	Nextera Enrichment Sample Plate
NEW1	Nextera Enrichment Wash Plate 1
NEW2	Nextera Enrichment Wash Plate 2
NLA	Nextera Library Amplification Plate
NLC	Nextera Library Clean Up Plate

Acronym	Definition
NLM	Nextera Library Amplification Mix
NLS	Nextera Library Sample Plate
NLT	Nextera Library Tagment Plate
PCR	Polymerase Chain Reaction
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
ST	Stop Tagment Buffer
TD	Tagment DNA Buffer
TDE1	Tagment DNA Enzyme 1
TOO	TruSight One Oligos

Alternative Thermal Cycler Steps for Successful Enrichment

The Enrichment Wash steps remove non-specific DNA bound to the Streptavidin Magnetic Beads and their success require that samples are maintained at a specific temperature. Too low or too high temperatures can result in lower percent enrichments due to non-specific binding, and decreased yields from loss of targeted regions. Illumina recommends using a microheating system for these steps. If a microheating system is not available, a thermal cycler can be used. Follow these steps when using a thermal cycler.

1. Transfer the sample and beads, resuspended in Enrichment Wash Solution, to a PCR plate (approximate 200 µl in volume).
2. Seal the PCR plate with a Microseal 'B' adhesive seal. Use an adhesive seal roller to apply force to the seal and make sure that the seal is secured.
3. Incubate the PCR plate on the thermal cycler at 42°C for 30 minutes with a heated lid set to 100°C.

   NOTE
   For optimal results, it is important that the thermal cycler lid is heated to 100°C.
4. Place the magnetic stand next to the thermal cycler for immediate access.
5. Remove the PCR plate from the thermal cycler and immediately place it on the magnetic stand for 2 minutes until the liquid appears clear.
6. Remove the adhesive seal from the PCR plate.
7. Immediately remove and discard all of the supernatant from each well.
8. Remove the PCR plate from the magnetic stand.
9. Add 200 µl Enrichment Wash Solution to each sample well of the PCR plate. Gently pipette the entire volume up and down 10–20 times. Mix thoroughly and avoid excessive bubbling or foaming. Make sure that the beads are fully resuspended.
10. Repeat steps 2–8 one time.
11. Continue to the elution step.

Kit Contents

Check to make sure that you have all of the reagents identified in this section before proceeding to the library preparation procedures. TruSight One Sequencing Panel kits are available in the following configurations.

Table 3 TruSight One Sequencing Panel Kits

Kit Name	Catalog #	*TG Catalog #
TruSight One Sequencing Panel (9 Samples)	FC-141-1006	TG-141-1006
TruSight One Sequencing Panel (36 Samples)	FC-141-1007	TG-141-1007

NOTE

*TG-labeled consumables include features intended to help reduce the frequency of revalidation. They are available only under supply agreement and require you to provide a binding forecast. Please contact your account manager for more information.

Note regarding biomarker patents and other patents unique to specific uses of products.

Some genomic variants, including some nucleic acid sequences, and their use in specific applications may be protected by patents. Customers are advised to determine whether they are required to obtain licenses from the party that owns or controls such patents in order to use the product in customer's specific application.

TruSight One Sequencing Panel Contents (9 Samples) (FC-141-1006, TG-141-1006)

Box 1 - Rapid Capture Reagents

This box is shipped at room temperature. As soon as you receive your kit, store the components as specified.

Quantity	Acronym	Reagent Name	Storage Temperature
1	SPB	Sample Purification Beads	2°C to 8°C
2	SMB	Streptavidin Magnetic Beads	2°C to 8°C
1	ET2	Elute Target Buffer 2	2°C to 8°C
1	ST	Stop Tagment Buffer	15°C to 30°C

Box 2 - Rapid Capture Reagents

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Acronym	Reagent Name	Storage Temperature
1	TDE1	Tagment DNA Enzyme	-15°C to -25°C
1	EE1	Enrichment Elution Buffer 1	-15°C to -25°C
1	TD	Tagment DNA Buffer	-15°C to -25°C
1	RSB	Resuspension Buffer	-15°C to -25°C
1	NLM	Nextera Library Amplification Mix	-15°C to -25°C
1	EHB	Enrichment Hybridization Buffer	-15°C to -25°C
1	EWS	Enrichment Wash Solution	-15°C to -25°C
1	HP3	2N NaOH	-15°C to -25°C
1	PPC	PCR Primer Cocktail	-15°C to -25°C
2	NEM	Nextera Enrichment Amplification Mix	-15°C to -25°C

Box 3 - Indices

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Reagent Name	Storage Temperature
1 tube	Index Primer, E503 to E504	-15°C to -25°C
1 tubes	Index Primer, N701, N705, and N709	-15°C to -25°C

Box 4 - Oligos

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Reagent Name	Storage Temperature
1 tube	TruSight One Content Set	-15°C to -25°C

MiSeq Reagent Kit v3 Box 1

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Reagent Name	Storage Temperature
3	Hybridization Buffer	-15°C to -25°C
3	MiSeq V3 Cartridge	-15°C to -25°C

MiSeq Reagent Kit v3 Box 2

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Reagent Name	Storage Temperature
3	MiSeq Flowcell	-15°C to -25°C
3	PR2 Bottle	-15°C to -25°C

TruSight One Sequencing Panel Contents (36 Samples) (FC-141-1007, TG-141-1007)

Box 1 - Rapid Capture Reagents

This box is shipped at room temperature. As soon as you receive your kit, store the components as specified.

Quantity	Acronym	Reagent Name	Storage Temperature
2	SPB	Sample Purification Beads	2°C to 8°C
2	SMB	Streptavidin Magnetic Beads	2°C to 8°C
1	ET2	Elute Target Buffer 2	2°C to 8°C
1	ST	Stop Tagment Buffer	15°C to 30°C

Box 2 - Rapid Capture Reagents

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Acronym	Reagent Name	Storage Temperature
2	TDE1	Tagment DNA Enzyme	-15°C to -25°C
1	EE1	Enrichment Elution Buffer 1	-15°C to -25°C
1	TD	Tagment DNA Buffer	-15°C to -25°C
1	RSB	Resuspension Buffer	-15°C to -25°C
2	NLM	Nextera Library Amplification Mix	-15°C to -25°C
1	EHB	Enrichment Hybridization Buffer	-15°C to -25°C
1	EWS	Enrichment Wash Solution	-15°C to -25°C
1	HP3	2N NaOH	-15°C to -25°C
1	PPC	PCR Primer Cocktail	-15°C to -25°C
2	NEM	Nextera Enrichment Amplification Mix	-15°C to -25°C

Box 3 - Indices

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Reagent Name	Storage Temperature
1 tube	Index Primer, E502 to E505	-15°C to -25°C
2 tubes	Index Primer, N701 to N712	-15°C to -25°C

Box 4 - Oligos

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15°C to -25°C.

Quantity	Reagent Name	Storage Temperature
1 tube	TruSight One Content Set	-15°C to -25°C

Consumables and Equipment

Check to make sure that you have all of the necessary user-supplied consumables and equipment before proceeding to the library preparation and enrichment procedures.

NOTE

The TruSight One protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Table 4 User-Supplied Consumables

Consumable	Supplier
1.7 ml microcentrifuge tubes	General lab supplier
20 µl barrier pipette tips	General lab supplier
20 µl multichannel pipettes	General lab supplier
20 µl single channel pipettes	General lab supplier
200 µl barrier pipette tips	General lab supplier
200 µl multichannel pipettes	General lab supplier
200 µl single channel pipettes	General lab supplier
1000 µl barrier pipette tips	General lab supplier
1000 µl multichannel pipettes	General lab supplier
1000 µl single channel pipettes	General lab supplier
Adhesive seal roller	General lab supplier
96-well storage plates, round well, 0.8 ml (“MIDI” plate)	Fisher Scientific, part # AB-0859
Hard-Shell 96-well PCR Plates (“HSP” plate)	Bio-Rad, part # HSP-9601

Consumable	Supplier
[Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) Note: Used to concentrate a pooled library. Another option is to use a vacuum concentrator.	Millipore, part # UFC503008
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
Microseal 'A' film	Bio-Rad, part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
RNase/DNase-free eight-tube strips and caps	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
Tris-HCl 10 mM, pH 8.5	General lab supplier
[Optional] TruSeq Index Plate Fixture Kit Note: Recommended for setting up indexed PCR primers. This part is reusable.	Illumina, catalog # FC-130-1005
PCR-grade water	General lab supplier

Table 5 User-Supplied Equipment

Equipment	Supplier
[Optional] 2100 Bioanalyzer Desktop System	Agilent, part # G2940CA
[Optional] DNA 1000 Chip	Agilent, part # 5067-1504
[Optional] High Sensitivity DNA Chip	Agilent, part # 4067-4626

Equipment	Supplier
DNA Engine Multi-Bay Thermal Cycler See *Thermal Cyclers* on page 68.	Bio-Rad, part # PTC-0240G or PTC-0220G, with Alpha Unit, part # ALS-1296GC
High-Speed Microplate Shaker	VWR, catalog # • 13500-890 (110 V/120 V) or • 14216-214 (230 V)
Magnetic stand-96	Life Technologies, part # AM10027
Microcentrifuge	General lab supplier
Microheating System-SciGene TruTemp Heating System	Illumina, catalog # • SC-60-503 (115 V) or • SC-60-504 (220 V)
Microplate centrifuge	General lab supplier
MIDI plate insert for microheating system	Illumina, catalog # BD-60-601
Fluorometric quantitation with dsDNA binding dye reagents	General lab supplier
[Optional] Vacuum concentrator Note: Used to concentrate a pooled library. Another option is to use Amicon Ultra-0.5 centrifugal filter units.	General lab supplier
Vortexer	General lab supplier

Thermal Cyclers

The following table lists the recommended settings for the Illumina recommended thermal cycler, as well as other comparable models. If your lab has a thermal cycler that is not listed, validate the thermal cycler before performing the TruSight One protocol.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, Constant at 100°C	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate

Index Sequences

The following list of index sequences is provided for generating sample sheets to demultiplex the samples. A dual indexing strategy uses two 8 base indices, Index 1 (i7) next to the P7 sequence and Index 2 (i5) next to the P5 sequence. Dual indexing is enabled by adding a unique Index 1 (i7) and Index 2 (i5) to each sample.

- N refers to Nextera
- E refers to Enrichment
- 7 refers to Index 1 (i7)
- 5 refers to Index 2 (i5)
- 01–12 refers to the Index number

Table 6 TruSight One Index Adapter Sequences

Index 1 (i7)	Sequence		Index 2 (i5)	Sequence
N701	TAAGGCGA		E502*	CTCTCTAT
N702*	CGTACTAG		E503	TATCCTCT
N703*	AGGCAGAA		E504	AGAGTAGA
N704*	TCCTGAGC		E505*	GTAAGGAG
N705	GGACTCCT			
N706*	TAGGCATG			
N707*	CTCTCTAC			
N708*	CAGAGAGG			
N709	GCTACGCT			
N710*	CGAGGCTG			
N711*	AAGAGGCA			
N712*	GTAGAGGA			

* Only available in the TruSight One Sequencing Panel Kit (36 Samples).

NOTE

The E500 series Index 2 (i5) sequences in the TruSight One kits are identical to S500 series Index 2 (i5) sequences in other kits. However, the Index 2 (i5) adapters are not interchangeable across kits.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 7 Illumina General Contact Information

Illumina Website	www.illumina.com
Email	techsupport@illumina.com

Table 8 Illumina Customer Support Telephone Numbers

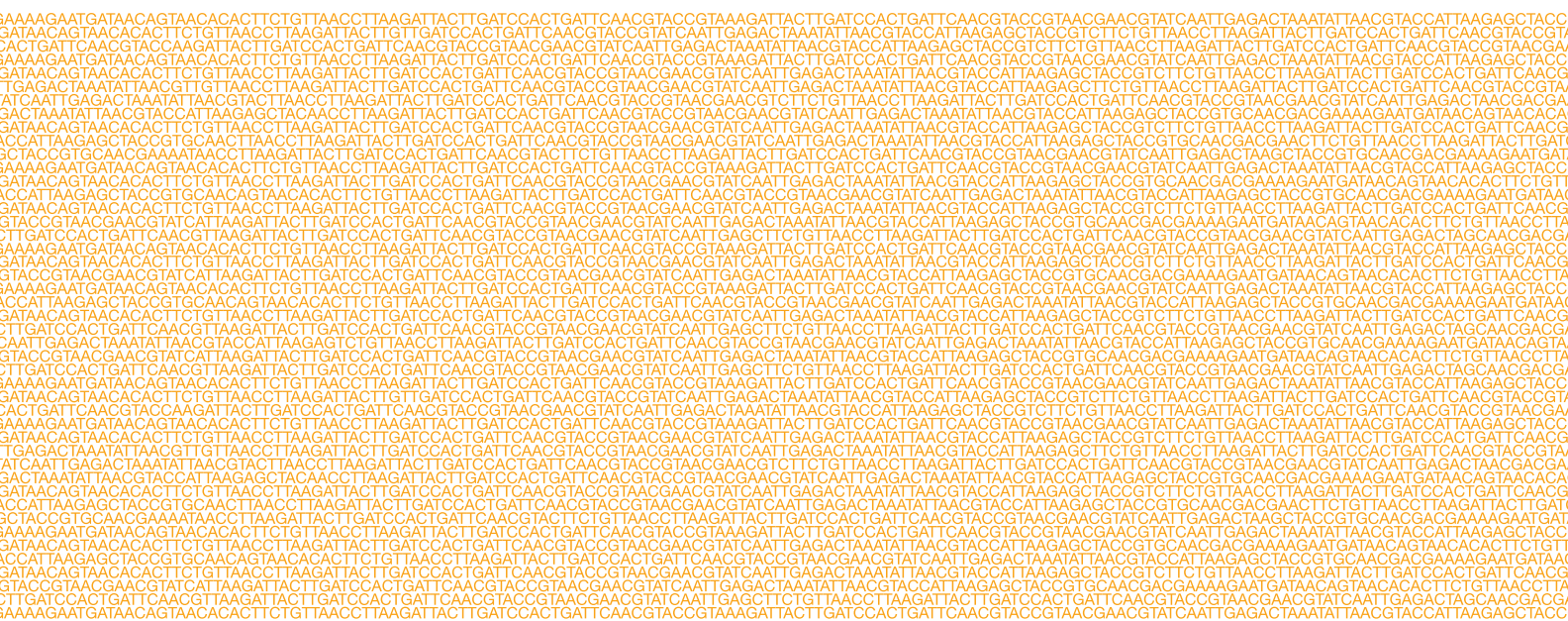
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at www.illumina.com/msds.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support, select a product, then click **Documentation & Literature**.



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