

TruSight Tumor 15

Reference Guide



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Revision History

Document	Date	Description of Change
Document # 1000000001245 v07	November 2020	Updated Index Targets section for clarity.
Document # 1000000001245 v06	March 2018	In Tips and Techniques: <ul style="list-style-type: none"> • Under Sealing the Plate, updated Microseal A • Under Handling Beads updated bead prep (bullets 2 and 3) In Clean Up Libraries, updated the About Reagents section
Document # 1000000001245 v05	June 2016	<ul style="list-style-type: none"> • Added TruSight Tumor 15 MiniSeq Kit catalog number to Kit Contents. • Added steps to apply seals before centrifuging and shaking when appropriate.
Document # 1000000001245 v04	February 2016	<ul style="list-style-type: none"> • Corrected catalog number for Veriti Thermal Cycler.
Document # 1000000001245 v03	January 2016	<ul style="list-style-type: none"> • Added reaction volumes to the TST15 PCR1 and TST15 PCR2 thermal cycler programs. • Added instructions for mixing TAM (TruSight Tumor Amplification Mix) after thawing. • In the Clean Up Libraries section, clarified wording in step 6 to wait until the beads bind to the magnets. • Added multichannel pipettes to the Consumables list. • Removed instrument-specific instructions. Information is now available in the denature and dilute libraries guide and system guide for your sequencing instrument.
Document # 1000000001245 v02	November 2015	<ul style="list-style-type: none"> • Clarified TST15 PCR1 ramp rate requirements. • Corrected TST15 PCR2 cycling description. • Corrected agarose gel example well labels.
Document # 1000000001245 v01	October 2015	Updated the product name to TruSight Tumor 15.
Document # 1000000001245 v00	September 2015	Initial release.

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Introduction

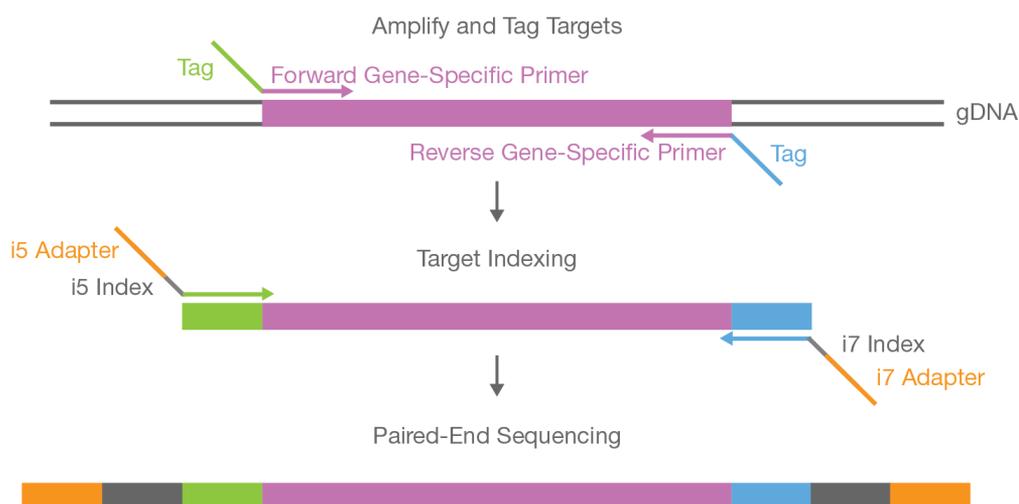
The Illumina® TruSight® Tumor 15 protocol describes a multiplexed PCR-based approach to prepare libraries for sequencing from DNA extracted from formalin-fixed, paraffin embedded (FFPE) tissue samples. The TruSight Tumor 15 kit reagents allow for preparation of up to 48 indexed, paired-end libraries from 24 DNA samples. The kit is optimized to provide amplicon coverage of 15 genes for highly sensitive analysis of low-frequency somatic variants from FFPE solid tumor samples. These genes and gene regions include single nucleotide variants (SNVs), insertions, deletions (indels), and amplifications that have been associated with cancer.

The TruSight Tumor 15 protocol offers:

- ▶ Fast and easy sample preparation
 - ▶ Prepare up to 48 libraries from 24 samples (each sample is prepared with Primer Mix A and Primer Mix B) in approximately 7 hours, including 2.5 hours of hands-on time
- ▶ Low DNA input
 - ▶ Higher data quality and robust performance with low input of 20 ng (10 ng from each sample for mix A and B) at a minimum concentration for input DNA of 2 ng/μl
- ▶ Filtered variant reporting lists relevant variants

How Does the Assay Work?

The assay contains two separate pools of tagged oligonucleotide primers. These pools are used in a multiplex PCR to amplify and target regions of interest from DNA extracted from FFPE samples for a select set of targets. Using index adapters provided in the kit, libraries are indexed and further amplified, and then combined in a single tube in preparation of a paired-end sequencing run. After sequencing is complete, an analysis report provides a specific set of single nucleotide variants (SNV) and small insertions, deletions, and amplifications associated with cancer.



DNA Input Recommendations

The TruSight Tumor 15 kit has been optimized for a specific DNA input amount. Quantify the input DNA before beginning the protocol.

- ▶ Use 20 ng total (10 ng for Mix A and Mix B per sample) human genomic DNA (gDNA) input at a minimum concentration of 2 ng/μl.
- ▶ Use a fluorometric quantification method that uses dsDNA binding dyes such as AccuClear (recommended), Qubit, or PicoGreen.
- ▶ Dilute starting material in RNase/DNase-free water.
- ▶ Use DNA samples that result in a delta Cq value ≤ 5. Using samples with a delta Cq > 5 may result in low product yields (< 20ng/ul), high primer-dimer, and sequencing runs with low %Q>30.
- ▶ DNA sample's delta Cq value can be assessed using the Illumina FFPE QC Kit.

Reference Samples [Optional]

- ▶ Use a characterized reference material when running the library preparation, such as HorizonDx HD701 or HD200 (requires DNA extraction), or AcroMetrix Oncology Hotspot Control.
- ▶ Use 5 μl RNase/DNase-free water as a no template control for PCR amplification. Do not sequence the no template control.



NOTE

Running a reference sample or no template control reduces the total number of unknown samples that can be processed with each library prep kit.

FFPE DNA Extraction

Use the following recommendations to extract DNA from FFPE tissue:

- ▶ Use at least 140 mm² nonmelanoma tissue with at least 30% tumor
- ▶ Use 1 of the following kits to extract DNA from FFPE tissue:
 - ▶ AllPrep DNA/RNA FFPE Kit (QIAGEN) (Recommended)
 - ▶ QIAamp DSP DNA FFPE Tissue Kit (QIAGEN)
 - ▶ ReliaPrep FFPE gDNA Miniprep System (Promega)

Additional Resources

Visit the [TruSight Tumor 15 kit support page](#) on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

The following documentation is available for download from the Illumina website.

Resource	Description
Custom Protocol Selector	support.illumina.com/custom-protocol-selector.html A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
<i>TruSight Tumor 15 Checklist (document # 100000000127)</i>	Provides a checklist of steps for the experienced user.
<i>TruSight Tumor 15 Consumables & Equipment List (document # 1000000005498)</i>	Provides an interactive checklist of user-provided consumables and equipment.

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Protocol Introduction

- ▶ Follow the TruSight Chimerism protocol in the order shown using the specified parameters.
- ▶ Before proceeding, confirm kit contents and make sure that you have the required consumables and equipment. See *Kit Contents on page 1* and *Consumables and Equipment on page 1*.
- ▶ Before beginning library preparation, record information about your samples for later use in data analysis.

Tips and Techniques

Protocol Continuity

- ▶ Follow the protocol in the order described using the specified parameters.
- ▶ Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Avoiding Cross-Contamination

- ▶ When adding or transferring samples, change tips between *each sample*.
- ▶ When adding adapters or primers, change tips between *each row* and *each column*.
- ▶ Remove unused index adapter tubes from the working area.
- ▶ Use a unidirectional workflow when moving from pre-amp to post-amp areas.
- ▶ To prevent amplification product or probe carryover, avoid returning to the pre-amp area after beginning work in the post-amp area.
- ▶ When adding indexing primers, change tips between each well.
- ▶ If gloves contact Mix A or Mix B primers, indexing primers, or samples, change gloves.
- ▶ Clean work surfaces thoroughly before and after the procedure.

Sealing the Plate

- ▶ Always seal the 96-well plate before the following steps in the protocol:
 - ▶ Shaking steps
 - ▶ Vortexing steps
 - ▶ Centrifuge steps
 - ▶ Thermal cycling steps
- ▶ Apply the adhesive seal to cover the plate, and seal with a rubber roller or wedge.
- ▶ Use the appropriate seal:
 - ▶ Use Microseal 'A' nonadhesive film for thermal cycling steps to prevent evaporation.

- ▶ Use Microseal 'B' adhesive seals for thermocycling, shaking, centrifuging, and long-term storage. The seals are effective at -40°C to 110°C and suitable for skirted or semiskirted PCR plates.
- ▶ After each use, discard all seals from plates.

Plate Transfers

- ▶ When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.

Centrifugation

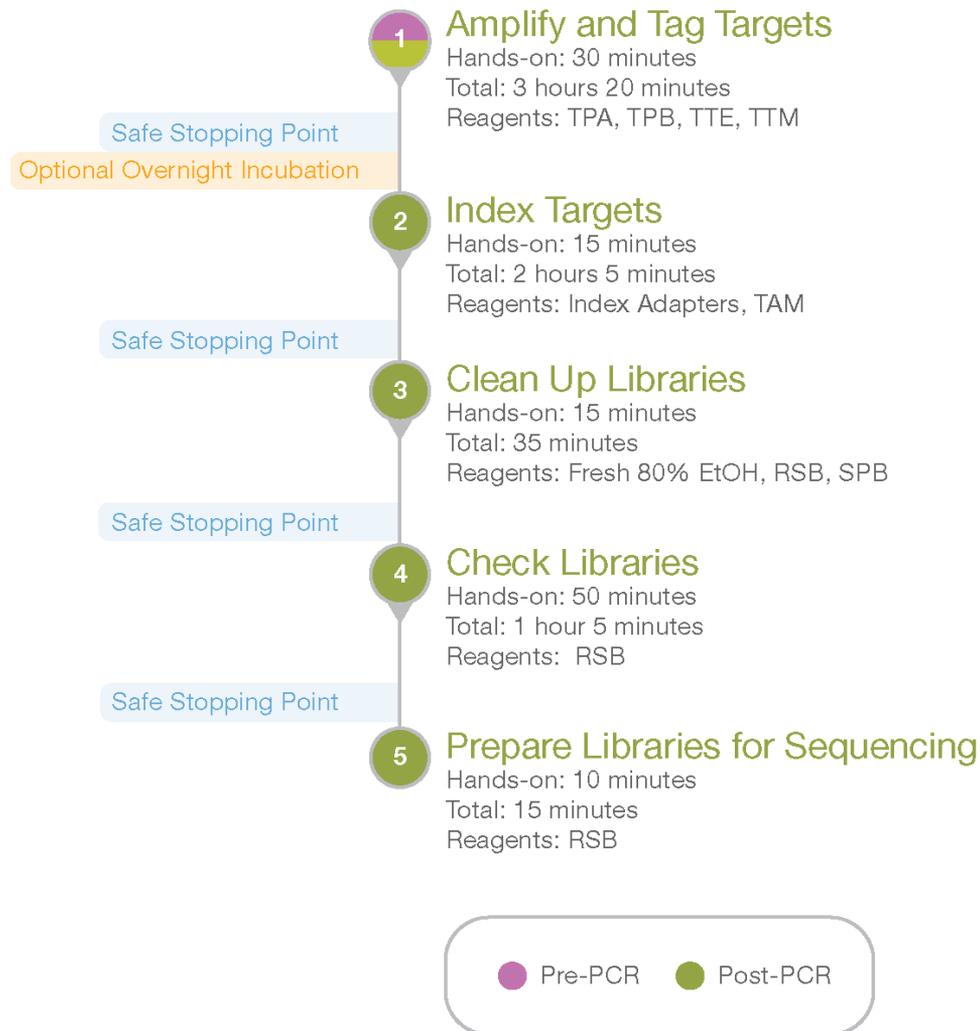
- ▶ Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the well and prevent sample loss.

Handling Beads

- ▶ Pipette bead suspensions slowly.
- ▶ Immediately before use, vortex the beads until they are well dispersed. The color of the liquid must appear homogeneous. Vortex throughout protocol as necessary to keep homogenous.
- ▶ If beads are aspirated into pipette tips, dispense back to the plate on the magnetic stand, and wait until the liquid is clear (~2 minutes).
- ▶ When washing beads:
 - ▶ Use the DynaMag-96 Side Skirted Magnet for PCR plates.
 - ▶ Dispense liquid so that beads on the side of the wells are wetted.
 - ▶ Keep the plate on the magnetic stand until the instructions specify to remove it.
 - ▶ Do not agitate the plate while it is on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

Figure 1 TruSight Tumor 15 Workflow



Amplify and Tag Targets

This process amplifies target regions to create 2 separate libraries per sample using 2 primer mixes, Mix A using Primer Mix A, and Mix B using Primer Mix B.

Consumables

- ▶ TTM (TruSight Tumor Targeting Mix)
- ▶ TPA (TruSight Tumor Primer Mix A)
- ▶ TPB (TruSight Tumor Primer Mix B)
- ▶ TTE (TruSight Tumor Targeting Enzyme)
- ▶ Sample DNA

- ▶ RNase/DNase-free water
- ▶ 1.7 ml microcentrifuge tubes (2)
- ▶ 96-well PCR plate
- ▶ Microseal 'A' film
- ▶ Microseal 'B' adhesive seal



NOTE

Use Microseal 'A' when sealing the plate before placing it on the thermal cycler. Use Microseal 'B' for other steps that require a sealed plate.

Preparation

- 1 Prepare the following consumables:

Item	Storage	Instructions
TTM	-25°C to -15°C	Thaw at room temperature. Vortex each tube to mix. Centrifuge briefly.
TPA	-25°C to -15°C	Thaw at room temperature. Vortex each tube to mix. Centrifuge briefly.
TPB	-25°C to -15°C	Thaw at room temperature. Vortex each tube to mix. Centrifuge briefly.
TTE	-25°C to -15°C	Centrifuge briefly and place on ice or in an enzyme cooler.
Sample DNA	-25°C to -15°C	Thaw at room temperature.

- 2 Label two 1.7 ml microcentrifuge tubes as Master Mix A and Master Mix B.
- 3 Save the following program as TST15 PCR1 on a thermal cycler with a heated lid.
 - ▶ Choose the preheated lid option and set to 102°C
 - ▶ Set the reaction volume to 15 µl
 - ▶ 98°C for 3 minutes
 - ▶ 16 cycles of:
 - ▶ 96°C for 45 seconds
 - ▶ 70°C for 1 minute
 - ▶ 54°C for 3 minutes with the following ramp rate, depending on the thermal cycler:

Thermal Cycler	Ramp Rate	
Bio-Rad C1000 Thermal Cycler	Down	0.1 °C/s
	Up	0.1 °C/s
Bio-Rad S1000 Thermal Cycler	Down	0.1 °C/s
	Up	0.1 °C/s
Applied Biosystems GeneAmp PCR System 9700	Down	7%
	Up	7%
Applied Biosystems Veriti Thermal Cycler	Down	4.5%
	Up	4.0%
Eppendorf Mastercycler ep Gradient	Down	6%
	Up	4%
Eppendorf Mastercycler ep Gradient-S	Down	2%
	Up	2%

- ▶ 72°C for 15 seconds with the following ramp rate, depending on the thermal cycler:

Thermal Cycler	Ramp Rate	
Bio-Rad C1000 Thermal Cycler	Down	0.1 °C/s
	Up	0.1 °C/s
Bio-Rad S1000 Thermal Cycler	Down	0.1 °C/s
	Up	0.1 °C/s
Applied Biosystems GeneAmp PCR System 9700	Down	7%
	Up	7%
Applied Biosystems Veriti Thermal Cycler	Down	4.5%
	Up	4.0%
Eppendorf Mastercycler ep Gradient	Down	6%
	Up	4%
Eppendorf Mastercycler ep Gradient-S	Down	2%
	Up	2%

- ▶ 72°C for 5 minutes
- ▶ Hold at 10°C



CAUTION

Ramp rate is critical for TruSight Tumor 15 performance. Failure to set the ramp rate results in low product yields and high primer-dimer.

Procedure

- 1 Quantify the sample DNA using a fluorometric method specific for double-stranded DNA, such as AccuClear (recommended), Qubit, or PicoGreen.
- 2 Dilute each sample DNA to 2 ng/μl with RNase/DNase-free water in a final volume of 12.5 μl.
- 3 Combine the following reagents in separate microcentrifuge tubes to create PCR master mixes for TPA and TPB.

PCR Component	Per Well	Per 8 Samples	Per 16 Samples	Per 24 Samples
TTM	5.875 μl	47 μl	94 μl	141 μl
TPA or TPB	6.25 μl	50 μl	100 μl	150 μl
TTE	0.375 μl	3 μl	6 μl	9 μl

Prepare a minimum of 4 samples.

- 4 Pipette to mix.
- 5 Add 10 μl of each PCR master mix to each well.
 - ▶ Master Mix A—Rows A and C
 - ▶ Master Mix B—Rows B and D
- 6 Add 5 μl of 2 ng/μl DNA to the corresponding well.
 - ▶ Samples 1–12—Rows A and B
 - ▶ Samples 13–24—Rows C and D
- 7 Pipette to mix.
- 8 Apply the seal and centrifuge at 1000 × g for 1 minute.

- 9 Immediately place on a thermal cycler and run the TST15 PCR1 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days. Alternatively, leave on the thermal cycler overnight.

Index Targets

This process tags amplified DNA and adds Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for cluster formation with each library receiving a unique combination of Index 1 and Index 2 adapters.

Consumables

- ▶ Index 1 (i7) adapters and orange tube caps
- ▶ Index 2 (i5) adapters and white tube caps
- ▶ TAM (TruSight Tumor Amplification Mix)
- ▶ 1.7 ml microcentrifuge tubes (1 per index adapter tube)
- ▶ Microseal 'A' film
- ▶ Microseal 'B' adhesive seal



NOTE

Use Microseal 'A' when sealing the plate before placing it on the thermal cycler. Use Microseal 'B' for other steps that require a sealed plate.

Preparation

- 1 Prepare the following consumables:

Item	Storage	Instructions
Index adapters (i7 and i5)	-25°C to -15°C	Only remove adapters being used. Thaw at room temperature for 20 minutes. Vortex each tube to mix. Centrifuge briefly using a 1.7 ml microcentrifuge tube as a holder/adaptor in the centrifuge.
TAM	-25°C to -15°C	Thaw at room temperature for 20 minutes. Vortex to mix. Centrifuge briefly.

- 2 Save the following program as TST15 PCR2 on a thermal cycler with a heated lid.
 - ▶ Choose the preheated lid option and set to 102°C
 - ▶ Set the reaction volume to 50 µl
 - ▶ 98°C for 30 seconds
 - ▶ 17 cycles of:
 - ▶ 98°C for 20 seconds
 - ▶ 60°C for 30 seconds
 - ▶ 72°C for 45 seconds
 - ▶ 72°C for 5 minutes
 - ▶ Hold at 10°C
- 3 Use the following guidelines when pooling samples:
 - ▶ Use i7 index sets outlined in [Table 1](#) when pooling samples in the same sequencing run. These optimal index sets minimize low-level crosstalk.

- ▶ Do not pool libraries in a single sequencing run with any of the i7 index pairs outlined in Table 2. These sub-optimal pairs of indexes display low-level crosstalk.
- ▶ i7 index combination sets other than those in Table 1 can be used, provided they do not pool libraries with any of the i7 index pairs outlined in Table 2.

Table 1 Recommended Optimal Sets of i7 Indexes for Pooling 8 Samples

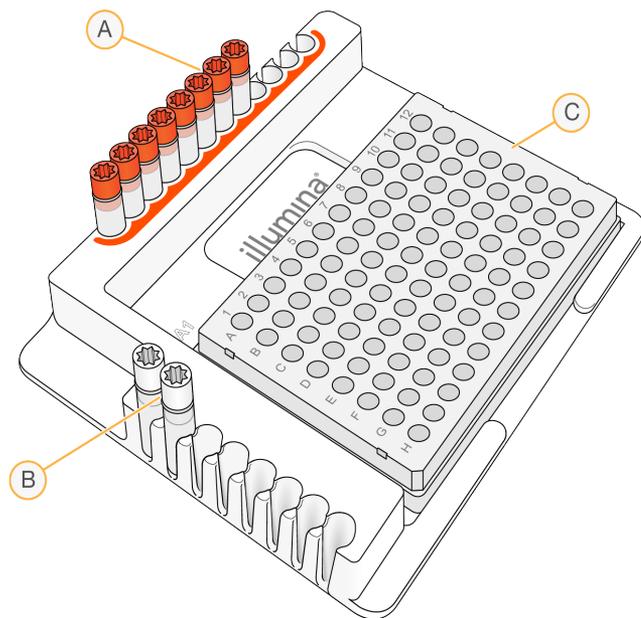
Index Set 1	Index Set 2	Index Set 3
R701	R706	R704
R702	R707	R726
R703	R708	R727
R705	R709	R728
R712	R711	R729
R725	R734	R730
R732	R735	R731
R733	R736	R749

Table 2 Sub-Optimal Pairs of i7 Indexes

R701 and R709
R707 and R731
R708 and R725
R708 and R712
R725 and R731
R726 and R734
R729 and R732
R729 and R736
R733 and R734
R749 and R702
R749 and R734

Procedure

- 1 Arrange Index 1 (i7) adapters in the top row of the TruSeq Index Plate Fixture.
- 2 Arrange Index 2 (i5) adapters in rows A-B of the TruSeq Index Plate Fixture so Mix A and Mix B are given unique i5/i7 index combinations. For example A501 in row A for Mix A, and A502 in row B for Mix B.
- 3 Record index adapters used for each sample for sequencing.
- 4 Place the plate on the TruSeq Index Plate Fixture.

Figure 2 Index Plate Fixture

- A Columns 1–8: Index 1 (i7) adapters (orange caps)
- B Rows A–B: Index 2 (i5) adapters (white caps)
- C 96-well plate

- 5 For samples 1–8, perform the following.
 - a Using a multichannel pipette, add 4 μ l of each Index 2 (i5) adapter (i501 and i502) for rows A and B respectively. If you do not have additional samples, replace the cap on each i5 adapter tube with a new white cap.
 - b Using a multichannel pipette, add 4 μ l of each Index 1 (i7) adapter (R701-R703, R705, R712, R725, R732-R733) to each column of rows A and B. Replace the cap on each i7 adapter tube with a new orange cap.

- 6 For samples 9–16, perform the following.
 - a Move Index 2 (i5) adapters (i501 and i502) to rows C and D respectively (i501 to row C and i502 to row D).
 - b Replace Index 1 (i7) adapters in the top row with the second set (R706-R709, R711, R734-R736).
 - c Using a multichannel pipette, add 4 μ l of each Index 2 (i5) adapter across rows C and D. Replace the cap on each i5 adapter tube with a new white cap.
 - d Using a multichannel pipette, add 4 μ l of each Index 1 (i7) adapter (R706-R709, R711, R734-R736) to each column of rows C and D. Replace the cap on each i7 adapter tube with a new orange cap.

- 7 For samples 17–24, perform the following.
 - a Move Index 2 (i5) adapters (i501 and i502) to rows E and F respectively (i501 to row E and i502 to row F).
 - b Replace Index 1 (i7) adapters in the top row with the second set (R704, R726-R731, R749).
 - c Using a multichannel pipette, add 4 μ l of each Index 2 (i5) adapter across rows E and F. Replace the cap on each i5 adapter tube with a new white cap.

- d Using a multichannel pipette, add 4 μ l of each Index 1 (i7) adapter (R704, R726-R731, R749) to each column of rows E and F. Replace the cap on each i7 adapter tube with a new orange cap.

**NOTE**

Record index arrangement used for preparing the sample sheet.

- 8 Add 27 μ l TAM to each well.
- 9 Pipette to mix.
- 10 Apply the seal and centrifuge at 1000 \times g for 1 minute.
- 11 Immediately place on a thermal cycler and run the TST15 PCR2 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Clean Up Libraries

This step uses SPB (Sample Purification Beads) to purify the PCR products from other reaction components.

Consumables

- ▶ SPB (Sample Purification Beads)
- ▶ RSB (Resuspension Buffer)
- ▶ Freshly prepared 80% ethanol (EtOH)
- ▶ 96-well midi plate
- ▶ 96-well PCR plate
- ▶ Microseal 'B' adhesive seals

About Reagents

- ▶ Prior to use, allow SPB to come to room temperature.
- ▶ Immediately prior to use, vortex SPB until beads are well dispersed. The color of the liquid should appear homogeneous.
- ▶ Aspirate and dispense SPB slowly due to the viscosity of the solution.

Preparation

- 1 Prepare the following consumables:

Reagent	Storage	Instructions
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

- 2 Prepare fresh 80% EtOH.
- 3 Label a new 96-well PCR plate PLP (Purified Library Plate).

Procedure

- 1 Centrifuge at 1000 \times g for 1 minute.

- 2 Add 40 μ l SPB to each well of a new midi plate.
- 3 Transfer 45 μ l supernatant from the PCR plate to the corresponding well of the midi plate.
- 4 Apply the seal and shake at 1800 rpm for 5 minutes.
- 5 Incubate at room temperature for 5 minutes.
- 6 Place on a magnetic stand and wait until the beads bind to the magnet (~2 minutes).
- 7 Remove and discard all supernatant from each well.
- 8 Wash 2 times as follows.
 - a Add 200 μ l fresh 80% EtOH to each well.
 - b Incubate on the magnetic stand for 30 seconds.
 - c Remove and discard all supernatant from each well.
- 9 Using a 20 μ l pipette, remove residual 80% EtOH from each well.
- 10 Air-dry on the magnetic stand for 5 minutes.
- 11 Add 32 μ l RSB to each well.
- 12 Apply the seal and shake at 1800 rpm for 2 minutes. If the beads are not resuspended, pipette to mix or repeat shake at 1800 rpm for 2 minutes.
- 13 Incubate at room temperature for 2 minutes.
- 14 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
- 15 Transfer 30 μ l supernatant from each well to the corresponding well of the PLP plate.
- 16 Apply the seal and centrifuge at 1000 \times g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the PCR plate and store at -25°C to -15°C for up to 2 months.

Check Libraries

Perform the following procedures to quantify libraries and check library quality.

Accurately quantify DNA libraries to ensure optimum cluster densities on the flow cell.

For best results, sequence samples with library Mix A and Mix B yields \geq 20 ng/ μ l. Libraries with lower yield give poor quality and less accurate variant reporting.

Consumables

- ▶ RSB (Resuspension Buffer)
- ▶ 96-well PCR plate
- ▶ 1.7 ml microcentrifuge tubes

Preparation

- 1 Prepare the following consumables:

Item	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

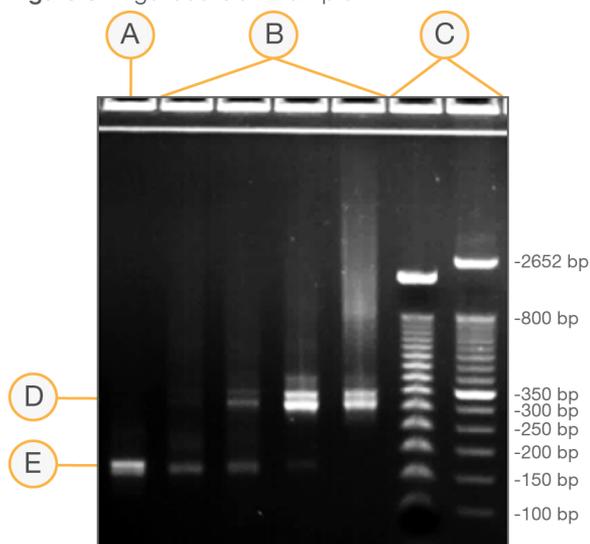
- 2 Label a new 96-well PCR plate NLP (Normalized Library Plate).

Procedure

- 1 Quantify the library using a fluorometric method , such as AccuClear (recommended), PicoGreen, or Qubit.
- 2 Calculate the volume of RSB required to adjust the library concentration to 5 ng/μl as follows.
 - a Use the formula $C_1V_1 = C_2V_2$, where C_1 is the result of library quantification, V_1 is 8 μl, and C_2 is 5 ng/μl to calculate the value for V_2 .
 - b Calculate the amount of RSB ($V_2 - 8 \mu\text{l}$) required to adjust the concentration of each library to 5 ng/μl.
- 3 Add the required volume of RSB to the corresponding well of the NLP plate. If a library is $\geq 100 \text{ ng}/\mu\text{l}$, transfer the RSB to a 1.7 ml tube.
- 4 Transfer 8 μl of each library from the PLP plate to the corresponding well of the NLP plate or 1.7 ml tube.
- 5 Run an aliquot of each normalized library on either of the following methods:
 - ▶ 15 μl on a 2% agarose gel using a 50 bp DNA ladder
 - ▶ 1 μl on a Bioanalyzer using a DNA 1000 chip

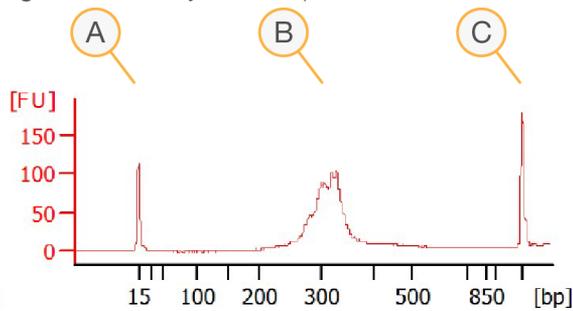
The expected PCR product is ~350 bp, which indicates successful library amplification. A strong primer dimer signal at ~160 bp can indicate library prep failure.

Figure 3 Agarose Gel Example



- A No template control
- B Sample libraries (~350 bp)
- C Size standards
- D Targeted amplicons
- E Primer dimer (~160 bp)

Figure 4 Bioanalyzer Example



- A Marker
- B Sample libraries (~350 bp)
- C Marker

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 14 days.

Pool Libraries

Use the following protocol to pool libraries.

Consumables

- ▶ RSB (Resuspension Buffer)
- ▶ 1.7 ml microcentrifuge tubes (2)

About Reagents

- ▶ The PNL tube can be stored at -25°C to -15°C for up to 7 days.
- ▶ The DNL tube can be stored at -25°C to -15°C for up to 7 days.

Preparation

- 1 Prepare the following consumables.

Item	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

- 2 Label a new 1.7 ml microcentrifuge tube PNL (Pooled Normalized Libraries).
- 3 Label a new 1.7 ml microcentrifuge tube DNL (Diluted Normalized Libraries).
- 4 If the NLP plate was stored frozen, thaw at room temperature and then centrifuge briefly. Pipette to mix.
- 5 To prepare for the sequencing run, begin thawing reagents according to the instructions for your sequencing instrument.

Procedure

- 1 Apply the seal and centrifuge the NLP plate at 1000 × g for 1 minute.
- 2 Transfer 4 µl of each library from the NLP plate to the PNL tube.

- 3 Vortex to mix, and then centrifuge briefly.
- 4 Add 41 μ l RSB to the DNL tube.
- 5 Transfer 9 μ l from the PNL tube to the DNL tube.
- 6 Vortex to mix, and then centrifuge briefly to create a 4 nM pooled library.

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Introduction

The protocol described in this guide assumes that you have reviewed the contents of this section, confirmed protocol contents, and obtained all required consumables and equipment.

Acronyms

Acronym	Definition
DAL	Denatured Amplicon Libraries
DNL	Diluted Normalized Libraries
HP3	2N NaOH
HT1	Hybridization Buffer
NLP	Normalized Library Plate
PLP	Purified Library Plate
PNL	Pooled Normalized Libraries
RSB	Resuspension Buffer
SPB	Sample Purification Beads
TAM	TruSight Tumor Amplification Mix
TPA	TruSight Tumor Primer Mix A
TPB	TruSight Tumor Primer Mix B
TTE	TruSight Tumor Targeting Enzyme
TTM	TruSight Tumor Targeting Mix

Kit Contents

Make sure that you have all the reagents identified in this section before proceeding to the library preparation procedures. Kits are available in the following configurations.

Consumable	Catalog #
TruSight Tumor 15 MiSeq Kit (1 Library Prep, 24 Samples; 3 MiSeq Reagent Kits v3)	OP-101-1001
TruSight Tumor 15 (1 Library Prep, 24 Samples)	OP-101-1002
TruSight Tumor 15 MiniSeq Kit (1 Library Prep, 24 Samples; 3 MiniSeq Reagent Kits)	20005610

Box 1 (Pre-PCR)— Store at -25°C to -15°C

Quantity	Reagent	Description
1	TTM	TruSight Tumor Targeting Mix
1	TTE	TruSight Tumor Targeting Enzyme
1	TPA	TruSight Tumor Primer Mix A
1	TPB	TruSight Tumor Primer Mix B

Box 2 (Post-PCR)— Store at -25°C to -15°C

Quantity	Reagent	Description
2	TAM	TruSight Tumor Amplification Mix
1	HP3	2 N NaOH
2	A501, A502	i5 Index Adapters
24	R701-R709, R711-R712, R749, R725-R736	i7 Index Adapters

Box 3 (Post-PCR)— Store at 2°C to 8°C

Quantity	Reagent	Description
1	SPB	Sample Purification Beads
1	RSB	Resuspension Buffer

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol.

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

Consumables

Consumable	Supplier
1.7 ml microcentrifuge tubes	General lab supplier
20 µl multichannel pipettes	General lab supplier
200 µl multichannel pipettes	General lab supplier
20 µl barrier pipette tips	General lab supplier
200 µl barrier pipette tips	General lab supplier
1000 µl barrier pipette tips	General lab supplier
96-well flat clear bottom black microplates Note: Used when quantifying samples with a SpectraMax M5 spectrofluorometer.	Corning, part # 3904
96-well storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, part # AB-0859

Consumable	Supplier
Conical centrifuge tubes (15 ml or 50 ml)	General lab supplier
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
96-well PCR Plates Note: Use a PCR plate compatible with your thermal cycler (see <i>Thermal Cyclers</i> on page 1)	General lab supplier
Microseal 'A' film	Bio-Rad, part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
PCR-grade water	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
RNase/DNase-free water	General lab supplier
Fluorescence-based quantification reagents such as AccuClear (recommended), Qubit, or PicoGreen. • AccuClear Ultra High Sensitivity dsDNA Quantitation Kit	• Biotium, catalog # 31029
One of the following (for validating libraries): • 2% Agarose gel • DNA 1000 Chip	• General lab supplier • Agilent, part # 5067-1504
[Optional] Screw-cap tubes	General lab supplier

Sequencing Kits [Optional]

Equipment	Catalog #
PhiX Control v3	FC-110-3001
MiSeq Reagent Kit v3, 600 Cycles	MS-102-3003

Equipment

Equipment	Supplier/Description
One of the following magnetic stands: • Magnetic Stand-96 • DynaMag-96 Side Skirted Magnet	• Thermo Fisher, catalog # AM10027 • Thermo Fisher, catalog # 12027
Microcentrifuge	General lab supplier
Microplate centrifuge	General lab supplier
Microplate fluorometer	General lab supplier
Vortexer	General lab supplier
One of the following 96-well thermal cyclers: • C1000 Touch Thermal Cycler • S1000 Thermal Cycler • Veriti Thermal Cycler	• Bio-Rad, part # 1851196 • Bio-Rad, part # 1852196 • Thermo Fisher, catalog # 4375786

Equipment	Supplier/Description
One of the following microplate shakers: <ul style="list-style-type: none"> • BioShake iQ High-Speed Thermal Mixer • BioShake XP High-Speed Mixer • VWR Advanced High-Speed Microplate Shaker • VWR Signature High-Speed Microplate Shaker 	<ul style="list-style-type: none"> • Q Instruments, model # 1808-0506 • Q Instruments, model # 1808-0505 • VWR, catalog # 14216-214 (230 V) • VWR, catalog # 13500-890 (110 V/120 V)
One of the following (for library validation): <ul style="list-style-type: none"> • Gel electrophoresis apparatus • 2100 Bioanalyzer Desktop System 	<ul style="list-style-type: none"> • General lab supplier • Agilent, part # G2940CA and part # 5067-1504

TruSeq Index Plate Fixture Kit [Optional]

Equipment	Supplier
[Optional] TruSeq Index Plate Fixture Kit Note: Recommended for setting up indexed adapters. This part is reusable.	Illumina, catalog # FC-130-1005

Index Sequences

Use the following sequences to set up your run.

Index	Sequence
A501	TGAACCTT
A502	TGCTAAGT
R701	ATCACG
R702	CGATGT
R703	TTAGGC
R704	TGACCA
R705	ACAGTG
R706	GCCAAT
R707	CAGATC
R708	ACTTGA
R709	GATCAG
R749	GATGCT
R711	GGCTAC
R712	CTTGTA
R725	ACTGAT
R726	ATGAGC
R727	ATTCCT
R728	CAAAAG
R729	CAACTA
R730	CACCGG
R731	CACGAT
R732	CACTCA

Index	Sequence
R733	CAGGCG
R734	CATGGC
R735	CATTTT
R736	CCAACA

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.



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