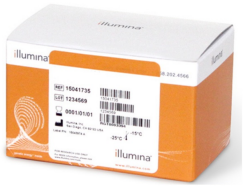
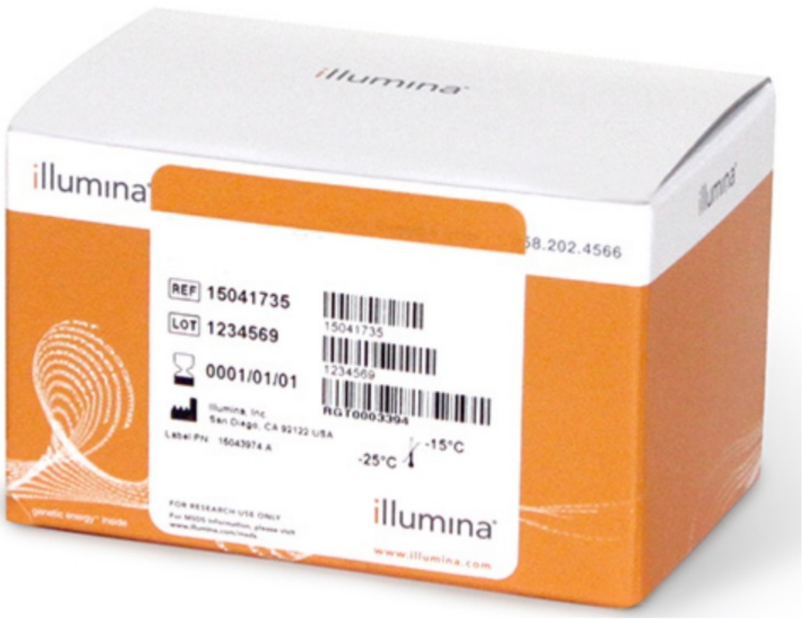


illumina TruSight Oncology Comprehensive Lab Tracking Form Instruction Manual

Contents

illumina

Illumina TruSight Oncology Comprehensive Lab Tracking Form

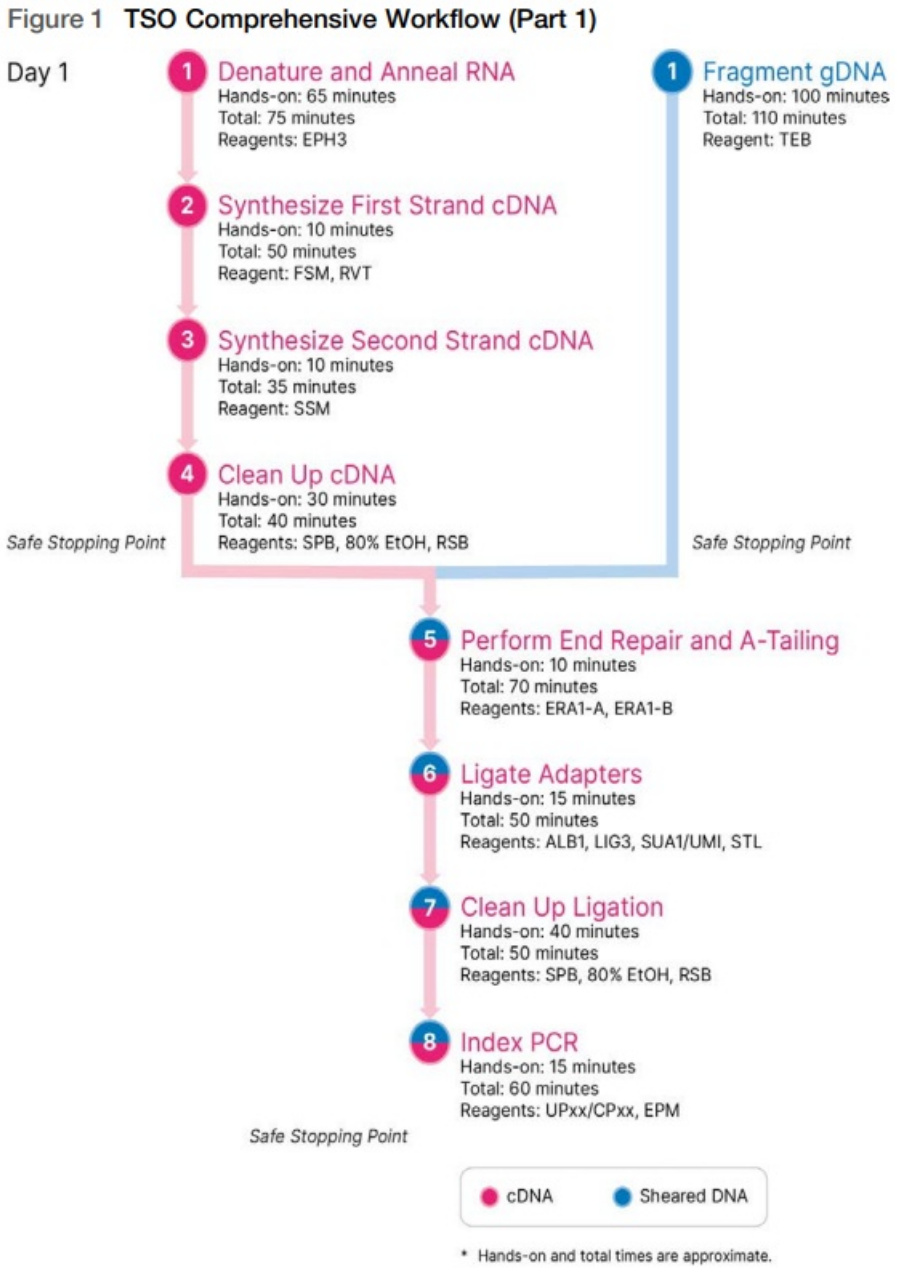


TruSight™ Oncology Comprehensive (EU) Lab Tracking Form
FOR IN VITRO DIAGNOSTIC USE FOR EXPORT ONLY

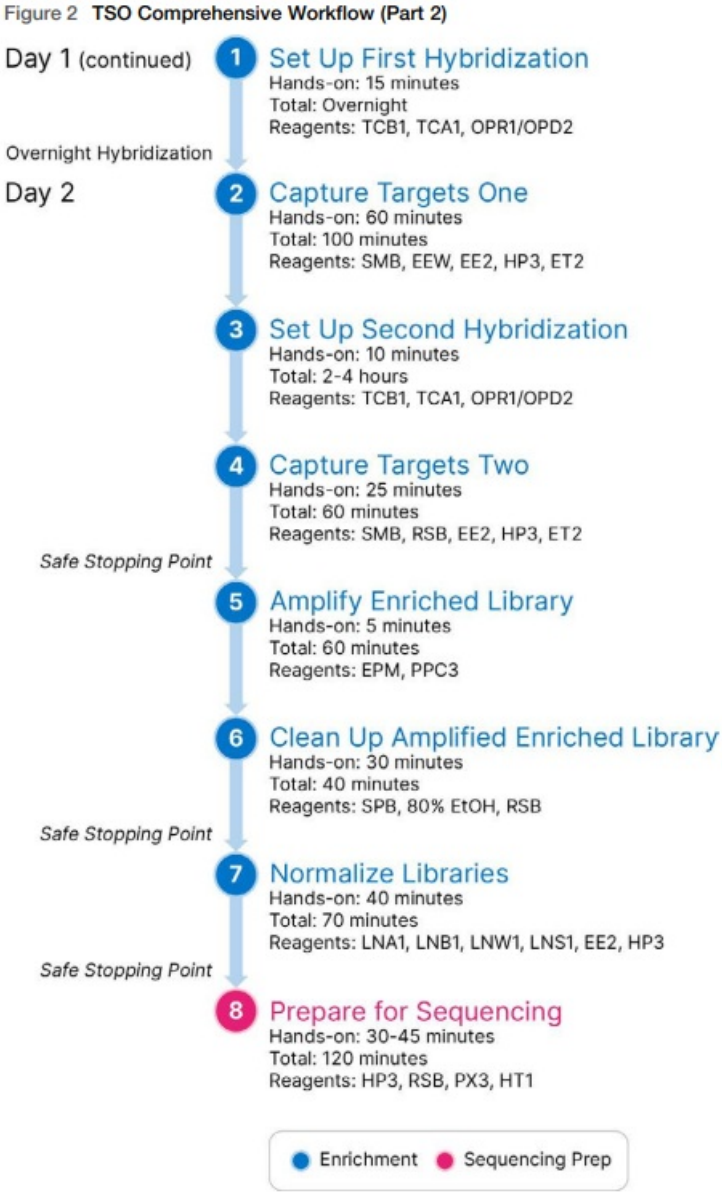
Instructions for Use

An overview of the TruSight Oncology Comprehensive (TSO Comprehensive) workflow is shown in Figure 1 and Figure 2. Before you begin the protocol, review the warnings and precautions in the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789).

Library Prep Workflow



Enrichment Workflow



Program Thermal Cyclers

1. Before starting the assay, save the following programs on pre- and post-amplification thermal cyclers.

Table 1 Pre-Amplification Thermal Cycler Programs

Procedural Step	Program Name	Lid Temperature	Reaction Volume	Thermal Cycler Parameters
Denature and Anneal RNA	LQ-RNA	100°C	17 µl	• 65°C for 5 minutes • 4°C for 1 minute • 4°C Hold
Synthesize First Strand cDNA	1stSS	100°C	25 µl	• 25°C for 10 minutes • 42°C for 15 minutes • 70°C for 15 minutes • 4°C for 1 minute • 4°C Hold
Synthesize Second Strand cDNA	2ndSS	30°C	50 µl	• 16°C for 25 minutes • 4°C for 1 minute • 4°C Hold

If the lid temperature for 2ndSS cannot be set to 30°C, turn off the preheated lid heat option.

Table 2 Post-amplification Thermal Cycler Programs

Procedural Step	Program Name	Lid Temperature	Reaction Volume	Thermal Cycler Parameters

Index PCR	I-PCR	100°C	50 µl	• 98°C for 30 seconds
				• 15 cycles of:
				  • 98°C for 10 seconds
				  • 60°C for 30 seconds
				  • 72°C for 30 seconds
				• 72°C for 5 minutes
				• 10°C Hold

Perform First Hybridization	HYB1	100°C	50 µl	• 95°C for 10 minutes • 85°C for 2 min 30 seconds • 75°C for 2 min 30 seconds • 65°C for 2 min 30 secpmds • 57°C Hold for 8 to 24 hours
Perform Second Hybridization	HYB2	100°C	50 µl	• 95°C for 10 minutes • 85°C for 2 min 30 seconds • 75°C for 2 min 30 seconds • 65°C for 2 min 30 seconds • 57°C Hold for 1.5 to 4 hours

Amplify Enriched Library	EL-PCR	100°C	50 µl	• 98°C for 30 s
				• 18 cycles of:
				  • 98°C for 10 s
				  • 60°C for 30 s
				  • 72°C for 30 s
				• 72°C for 5 min
				• 10°C Hold

Enter Run Information

- NextSeq 550Dx instrument Local Run Manager is the software used to set up a TSO Comprehensive run. For more information, see the Local Run Manager TruSight
- Oncology Comprehensive (EU) Analysis Module Workflow Guide (document # 200008661).
- Enter run and sample setup information directly into the TruSight Oncology Comprehensive analysis module.

Set Run Parameters

1. Log in to Local Run Manager on the instrument or from a networked computer.
2. Select Create Run, and then select TSO Comp (EU).
3. Enter a run name that identifies the run from sequencing through analysis with the following criteria.
4. 1–40 characters.
5. Only alphanumeric characters, underscores, or dashes.
6. Underscores and dashes must be preceded and followed by an alphanumeric character.
7. Unique across all runs on the instrument.
8. [Optional] Enter a run description to help identify the run with the following criteria.
9. 1–150 characters.

10. Only alphanumeric characters or spaces.
11. Spaces must be preceded and followed by an alphanumeric character.

Specify Samples for the Run

- Specify samples for the run using one of the following options.
- **Enter samples manually—**Use the blank table on the Create Run screen.
- **Import samples—**Navigate to an external file in a comma-separated values (*.csv) format. A template is available for download on the Create Run screen.

CAUTION

- Mismatches between the samples and index primers cause incorrect result reporting due to loss of positive sample identification. Enter sample IDs and assign indexes in Local Run Manager before beginning library preparation. Record sample IDs, indexes, and plate well orientation for reference during library preparation.

CAUTION

- To avoid data loss, make sure KB installation is not in progress before saving a run.

Enter Samples Manually

- Enter a unique sample ID in the Sample ID field with the following criteria. All control samples should be added first. See Control Samples on page 6 for more information.
- 1–25 characters.
- Only alphanumeric characters, underscores, or dashes.
- Underscores and dashes must be preceded and followed by an alphanumeric character.
- [Optional] Enter a sample description in the Sample Description field with the following criteria.
- 1–50 characters.
- Only alphanumeric characters, dashes, underscores, or spaces.
- Spaces underscores, and dashes must be preceded and followed by an alphanumeric character.
- Select an index for the DNA library and/or RNA library prepared from the sample.
- Make sure that RNA and DNA samples are in separate columns.
- The DNA i7+i5 Sequence field auto-populates after selecting a DNA Index ID. The RNA i7+i5 Sequence field auto-populates after selecting an RNA Index ID.
- In addition to the summary here, see the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) for index ID selection.
- For a DNA sample library, select a unique index ID (UPxx or CPxx indexes) from the DNA Index ID drop-down list.
- For an RNA sample library, select a unique index ID (UPxx only) from the RNA index ID drop-down list.
- If there are three libraries in total in the run, follow the index selection guidelines in the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789).
- Use the Tumor Type field to assign a tumor type for each sample, selecting the most specific tumor type available. See Select a Tumor Type on page 7.

- Use the Tumor Type field to assign one of the following control types for each control. See Control Samples on page 6.
- DNA External Control
- RNA External Control
- DNA No-Template Control
- RNA No-Template Control
- If using the Consumable Prefix DNA Control, the control type is DNA External Control. If using the Consumable Prefix RNA Control, the control type is RNA External Control.
- Assign sex.
- [Optional] Select Export to CSV to export sample information to an external file.
- Review the information on the Create Run Screen. Incorrect information can impact results.
- Select Save Run.

Import Samples

- Select Import CSV and browse to the location of the sample information file. There are two types of files you can import.
- Select Download CSV on the Create Run screen to download a new sample information template. The CSV file contains the required column headings and format for import. Enter sample information in each column for the samples in the run. For the Tumor Type column, enter the tumor type term or associated code (see Download
- Tumor Types on page 1). The Tumor Type field is also used to designate samples as controls (see Control Samples on page 6).
- Use a file of sample information that was exported from the TSO Comprehensive analysis module using the Export to CSV feature.
- On the Create Run screen, review the imported information.
- Incorrect information can impact results.
- [Optional] Select Export to CSV to export sample information to an external file.
- Select Save Run.

Control Samples

- TSO Comprehensive requires the use of Panel Control. Designating a sample as a control automatically sets the Sex of the sample to Unknown. To designate a sample as a control, select one of four control types from the Tumor Type field: DNA External Control (positive DNA control), DNA No-Template Control, RNA External Control (positive RNA control), or RNA No-Template Control. See Select a Tumor Type on page 7 for more information on setting tumor types for all types of samples during run setup.
- Only one of each control type may be specified within a run. Only a DNA library may be specified for a DNA External Control or a DNA No-Template Control. Only an
- RNA library may be specified for an RNA External Control or an RNA No-Template Control. Libraries designated as DNA or RNA No-Template controls are not counted against the maximum number of libraries in a run.

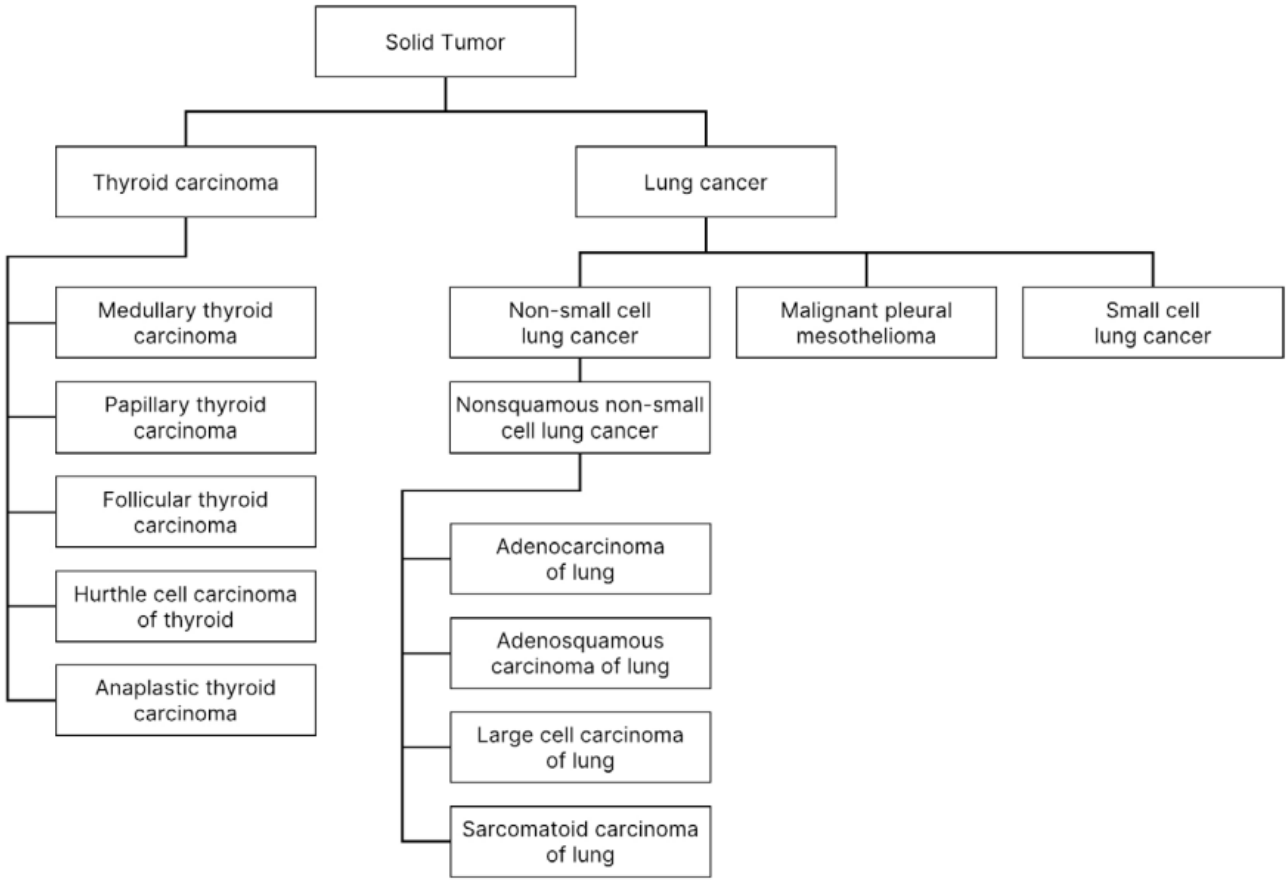
Select a Tumor Type

- A tumor type must be specified for each sample. Except for control types, the available tumor types are derived from the installed Knowledge Base (KB) and might change with updated versions of the KB.

CAUTION

- Incorrect selection of tumor type can cause incorrect results. Resolve any warnings that appear when specifying tumor types to avoid analysis failure.
- The tumor type terms are part of a hierarchical disease ontology in the KB, which is constructed as a set of parent-child relationships. For example, the term non-small cell lung cancer is a child of lung cancer since non-small cell lung cancer is a type of lung cancer. Figure 3 depicts a subset of an example disease ontology, showing solid tumor as the root term, and the terms associated with lung cancer and thyroid cancer (other tumor types are not shown). A term that is connected through parent-child relationships to lower-level terms is called an ancestor. The connected lower-level terms are descendants of the ancestor term. For example, lung cancer is an ancestor of adenocarcinoma of lung and small cell lung cancer, and medullary thyroid carcinoma is a descendant of both thyroid carcinoma and solid tumor.

Figure 3 **Subset of an Example Disease Ontology**



- **The selected tumor type for a patient sample impacts:**
- u Which companion diagnostic intended use(s) are evaluated for the sample? Only patient samples with a tumor type that is an exact match or a descendent of the tumor type for a companion diagnostic intended use will be evaluated for that claim.
- u Which tumor profiling variants are included in the TSO Comprehensive report?
- The following instructions describe the process for selecting a tumor type through the Create Run screen. The tumor type can also be set by importing a CSV file containing a tumor type (see Import Samples on page 6).
- Display the available tumor types by double-clicking within the Tumor Type cell in the row for the sample.
- Available tumor types are displayed in a hierarchical list organized alphabetically.

The Tumor Type field is also used to designate a control type for control samples (see Control Samples on page 6).

- Locate and select the desired tumor type by interacting with the list or by using the search bar at the top of the Tumor Type window.

Prepare for Protocol Steps

CAUTION
All procedures in the workflow require an RNase/DNase-free environment.

1. Set pre-amplification thermal cycler programs. See Program Thermal Cyclers on page 4.
2. Follow manufacturer instructions to set up the ultrasonicator.
3. If processing DNA samples only, proceed directly to Fragment gDNA on page 12.
4. Remove RNA controls from storage.
5. Remove the reagent tubes from the box and follow thaw instructions.

Table 3 TruSight Oncology Comp RNA Library Prep (PN 20031127)

Reagent	Storage	Thaw Instructions	Protocol Step
EPH3	-25°C to -15°C	Thaw to room temperature.	Denature and Anneal RNA
FSM	-25°C to -15°C	Thaw to room temperature.	Synthesize First Strand cDNA
RVT	-25°C to -15°C	Keep on ice.	Synthesize First Strand cDNA
SSM	-25°C to -15°C	Thaw to room temperature.	Synthesize Second Strand cDNA

Table 4 TruSight Oncology Comp Library Prep (Refrigerate) (PN 20031119)

Reagent	Storage	Thaw Instructions	Protocol Step
SPB (light green label)	2°C to 8°C	Bring to room temperature for 30 minutes.	Clean Up cDNA
RSB	2°C to 8°C	Bring to room temperature.	Clean Up cDNA

Denature and Anneal RNA

Preparation

- Prepare the following reagents.
- **EPH3—**Set aside.
- **FSM—**Vortex to mix. Centrifuge briefly, and then pipette to mix. Inspect for precipitates. If present, pipette to mix until precipitates dissolve.
- **RVT—**Centrifuge briefly, and then pipette to mix. Keep on ice.

- **NOTE** RVT is a viscous solution. Always pipette slowly to avoid creating bubbles.
- In a microcentrifuge tube, combine the following volumes to prepare an FSM+RVT Master Mix.

Table 5 FSM+RVT Master Mix

Master Mix Component	3 RNA Samples (µl)	8 RNA Samples (µl)	16 RNA Samples (µl)	24 RNA Samples (µl)
FSM	27	72	144	216
RVT	3	8	16	24

This table includes volume overage. See the Handling Reagents section of the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) for calculations.

- Pipette ten times to mix.
- Place the FSM+RVT Master Mix on ice until Synthesize First Strand cDNA on page 9. Procedure
- Thaw extracted RNA samples and RNA controls on ice.
- Process RNA controls as samples for the remainder of the protocol.
- See the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) to quantify samples.
- Pipette each RNA sample 10 times to mix.
- Use RNase/DNase-free water to prepare 40 ng of each RNA sample in a final volume of 8.5 µl (4.7 ng/µl).
- Pipette each RNA sample 10 times to mix.
- Use RNase/DNase-free water to prepare 40 ng of each RNA sample in a final volume of 8.5 µl (4.7 ng/µl).
- For RNA controls, use the concentration provided on the tube label.
- Label a new 96-well PCR plate CF (cDNA Fragments).
- Add 8.5 µl of each RNA sample to a unique well of the CF PCR plate.
- Make sure that sample plate layout and indexes for each sample match the run planned in Local Run Manager during run setup.
- Vortex EPH3 to mix, and then centrifuge briefly.
- Add 8.5 µl EPH3 to each sample well.
- Apply adhesive plate seal to the CF PCR plate.

CAUTION

- Make sure to seal edges and wells completely to prevent evaporation.
- Shake at 1200 rpm for 1 minute.
- Centrifuge at 280 × g for 1 minute.
- Place on the thermal cycler and run the LQ-RNA program. See Program Thermal Cyclers on page 4.
- When the samples reach 4°C, hold for one minute and then proceed immediately to the next step.

Synthesize First Strand cDNA

Procedure

- Start Date and Time _____

1. Remove the CF PCR plate from the thermal cycler.
2. Pipette 5 times to mix FSM+RVT master mix.
3. Add 8 µl FSM+RVT master mix to each sample well.
4. Pipette to mix 5 times.
5. Discard remaining FSM+RVT master mix.
6. Apply adhesive plate seal to the CF PCR plate.
7. Seal edges and wells completely to prevent evaporation.
8. Shake at 1200 rpm for 1 minute.
9. Centrifuge at 280 × g for 1 minute.
10. Place on a thermal cycler and run the 1stSS program.
11. When the samples reach 4°C, proceed immediately to the next step. First strand samples can be held at 4°C for up to 5 minutes.

Synthesize Second Strand cDNA

Preparation

Start Date and Time _____

1. Prepare the following reagent.
   SSM—Invert 10 times to mix. Centrifuge briefly.

Procedure

1. Remove the CF PCR plate from the thermal cycler.
2. Add 25 µl SSM to each sample well.
3. Apply adhesive plate seal to the CF PCR plate. Seal edges and wells completely to prevent evaporation.
4. Shake at 1200 rpm for 1 minute.
5. Centrifuge at 280 × g for 1 minute.
6. Place on a thermal cycler and run the 2ndSS program.
7. When the samples reach 4°C, hold for one minute and then proceed immediately to the next step.

Clean Up cDNA

1. Prepare the following reagents.
2. SPB—Make sure that beads are at room temperature for 30 minutes.
3. RSB—Set aside for use in the procedure.
4. Prepare fresh 80% EtOH in a 15 ml or 50 ml conical tube.

Reagent	3 Samples	8 Samples	16 Samples	24 Samples
100% Ethanol alcohol, pure	2 ml	4 ml	8 ml	12 ml
RNase/DNase-free wa ter	500 µl	1 ml	2 ml	3 ml

- Vortex fresh 80% EtOH to mix.
- Label a new 96-well MIDI plate BIND1 (cDNA Binding).
- Cover and set aside.
- Set out the magnet.

Procedure Bind

1. Remove the CF PCR plate from the thermal cycler.
2. Vortex SPB for 1 minute to resuspend beads.
3. Immediately add 90 µl SPB to each sample well of the BIND1 MIDI plate.
4. If using a trough to dispense SPB, include a 1.05 overage factor when aliquoting sufficient material per sample.
5. Discard any remaining material once SPB has been added to each sample well.
6. Transfer the entire volume (50 µl) of each sample from the CF PCR plate to the corresponding well of the BIND1 MIDI plate.
7. Discard the empty CF PCR plate.
8. Apply adhesive plate seal to the BIND1 MIDI plate. Seal edges and wells completely.
9. Shake at 1800 rpm for 2 minutes.
10. Incubate at room temperature for 5 minutes.
11. Place the BIND1 MIDI plate on a magnetic stand for 5 minutes.
12. Use a P200 pipette set to 200 µl to remove and discard all supernatant from each sample well without disturbing the bead pellet.

Wash

1. Wash beads as follows.
2. **a** Keep on the magnetic stand and add 200 µl fresh 80% EtOH to each well.
3. **b,** Wait 30 seconds.
4. **c,** Remove and discard all supernatant from each well.
5. Wash beads a second time.
6. Remove residual EtOH from each well.
7. Use a P20 pipette with fine tips.
8. Discard unused 80% EtOH.

Elute

1. Remove the BIND1 MIDI plate from the magnetic stand.
2. Invert or vortex RSB to mix.

3. Add 22 µl RSB to each sample well.
4. Apply adhesive plate seal to the BIND1 MIDI plate. Seal edges and wells completely.
5. Shake at 1800 rpm for 2 minutes.
6. Incubate at room temperature for 2 minutes.
7. Place on a magnetic stand for 2 minutes.
8. Label a new 96-well MIDI plate PCF (Purified cDNA Fragments).
9. If you are stopping at the SAFE STOPPING POINT on page 11, use a PCR plate.
10. Transfer 20 µl eluate from each sample well of the BIND1 MIDI plate to the corresponding well of the PCF plate.
11. Discard empty BIND1 MIDI plate.
12. Add 30 µl RSB to each sample well of the PCF plate.
13. Pipette to mix 10 times.
14. Apply adhesive plate seal to the PCF plate and keep it on ice.
15. Return EPH3, FSM, RVT, and SSM to storage.
16. If you are processing samples derived from RNA (cDNA) only, and not stopping at the safe stopping point,
17. proceed to Perform End Repair and A-Tailing on page 14.

SAFE STOPPING POINT

1. If you are stopping, centrifuge the PCF PCR plate at 280 × g for 1 minute, and store at -25°C to -15°C for up to 7 days.
2. **Stop Date and Time** _____

Prepare for Protocol Steps

1. Remove DNA controls from storage.
2. Remove the reagent tube from the box and follow the thaw instructions.

Table 6 TruSight Oncology Comp Library Prep (Refrigerate) (PN 20031119)

Reagent	Storage	Thaw Instructions	Protocol Step
WEB	2°C to 8°C	Bring to room temperature.	Fragment gDNA

Fragment gDNA

Preparation
Start Date and Time _____

1. Make sure to follow recommendations in the TruSight Oncology Comprehensive (EU) Package Insert

(document # 200007789) to quantify samples.

2. Prepare the following reagent.
3. TEB—Invert or vortex to mix.

Procedure

- **Prepare the Plate**
- Select one of the following three options to prepare the plate.
- **Option #1:** Process gDNA samples simultaneously with cDNA samples in the PCF MIDI plate.
- **a** Label the PCF MIDI plate LP (Library Preparation).
- **b** Place on ice and set aside for use in Transfer Fragmented DNA on page 13.
- Option #2: Process gDNA samples simultaneously with cDNA samples and the PCF PCR plate is frozen.
- **a** Thaw the PCF PCR plate to room temperature.
- **b** Centrifuge at 280 × g for 1 minute.
- **c** Pipette 10 times to mix.
- **d** Label a new 96-well MIDI plate LP (Library Preparation).
- **e** Transfer the entire 50 µl of each sample from the PCF PCR plate to the corresponding well of the LP MIDI plate.
- **f** Discard PCF PCR plate.
- **g** Apply adhesive plate seal and place on ice until Transfer Fragmented DNA on page 13.
- **Option #3:** Process gDNA-only samples.
- **a** Label a new 96-well MIDI plate LP (Library Preparation).
- **b** If you are stopping at the SAFE STOPPING POINT on page 13, use a PCR plate.
- **c** Cover and set aside for use in Transfer Fragmented DNA on page 13. Dilute gDNA
- Thaw gDNA samples and DNA controls at room temperature.
- Process DNA controls as samples for the remainder of the protocol.
- Pipette each gDNA sample 10 times to mix.
- Centrifuge tube briefly to collect droplets.
- Invert or vortex TEB to mix.
- Use TEB to prepare 40 ng of each gDNA sample in a final volume of 52 µl (0.77 ng/µl).
- The assay requires a minimum extraction concentration of 3.33 ng/µl, to allow for at least 40 µl TEB of the 52 µl volume. For DNA controls, use the concentration provided on the tube label. To prevent sample loss, do not pipette less than 2 µl of sample into this dilution.

Fragment

- Add 52 µl of each gDNA sample into a separate well of the ultrasonicator tube.

Record the orientation of the strip.

- Fragment gDNA into fragments with an ultrasonicator.

Transfer Fragmented DNA

- Make sure that sample plate layout and indexes for each sample match the run planned in Local Run Manager during run setup.
- Follow ultrasonicator manufacturer instructions to recover the sample.
- For some ultrasonicator tube types, centrifugation can be necessary to consolidate the sample in the tube.
- For each fragmented gDNA sample, use a p20 pipette with fine tips to perform 3 transfers of 16.7 µl into an empty well of the LP MIDI plate.
- Apply adhesive plate seal to the LP MIDI plate.

SAFE STOPPING POINT

- If you are stopping, apply adhesive plate seal to the LP PCR plate, and centrifuge at 280 × g for 1 minute.
- Store at -25°C to -15°C for up to 7 days.

Stop Date and Time _____

Prepare for Protocol Steps

- Prepare an ice bucket.
- Remove the reagent tube from the box and follow thaw instructions.

Table 7 TruSight Oncology Comp Library Prep (Freeze) Box (PN 20031118)

Reagent	Storage	Thaw Instructions	Protocol Step
ERA1-A	-25°C to -15°C	Keep on ice.	Perform End Repair and A-Tailing
ERA1-B	-25°C to -15°C	Thaw to room temperature.	Perform End Repair and A-Tailing
ALB1	-25°C to -15°C	Thaw to room temperature.	Ligate Adapters
LIG3	-25°C to -15°C	Keep on ice.	Ligate Adapters
SUA1 (blue cap)	-25°C to -15°C	Thaw to room temperature.	Ligate Adapters
UMI (white cap)	-25°C to -15°C	Thaw to room temperature.	Ligate Adapters
STL	-25°C to -15°C	Thaw to room temperature.	Ligate Adapters
EPM	-25°C to -15°C	Keep on ice.	Index PCR

Table 8 TruSight Oncology Comp Library Prep (Refrigerate) Box (PN 20031119)

Reagent	Storage	Thaw Instructions	Protocol Step
SPB (light green label)	2°C to 8°C	Bring to room temperature for 30 minutes.	Clean Up Ligation
RSB	2°C to 8°C	Bring to room temperature.	Clean Up Ligation

Table 9 TruSight Oncology Comp UP Index Primers Box (PN 20031120)

Reagent	Storage	Thaw Instructions	Protocol Step
UPxx	-25°C to -15°C	Thaw the appropriate index primer tubes to room temperature.	Index PCR

Table 10 TruSight Oncology Comp CP Index Primers Box (PN 20031126)

Reagent	Storage	Thaw Instructions	Protocol Step
CPxx	-25°C to -15°C	Thaw the appropriate index primer tubes to room temperature.	Index PCR

Perform End Repair and A-Tailing

Preparation
Start Date and Time _____

- Preheat 2 microsample incubators with MIDI heat block inserts as follows.
- Preheat a microsample incubator to 30°C.
- Preheat a microsample incubator to 72°C.
- Prepare the following reagents.
- ERA1-A—Centrifuge briefly, and then pipette to mix. Keep on ice.
- ERA1-B—Vortex to mix, and then centrifuge briefly. Inspect for precipitates. If present, warm the tube to 37°C, and then pipette to mix until precipitates dissolve.
- Prepare ERA1 master mix in a microcentrifuge tube.

Table 11 ERA1 Master Mix

Master Mix Component	3 Libraries	8 Libraries	16 Libraries	24 Libraries	48 Libraries
ERA1-B	26 µl	69 µl	138 µl	207 µl	415 µl
ERA1-A	10 µl	27 µl	54 µl	81 µl	161 µl

- This table includes volume overage. See the Handling Reagents section of the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) for calculations.
  Pipette slowly 10 times to mix, centrifuge briefly, and then place ERA1 master mix on ice.
- Select the appropriate option of the two following options to prepare the plate.
- Option #1: If samples are in a MIDI plate.
- **a** Relabel the MIDI plate LP2 (Library Preparation 2).
- If some samples are in separate MIDI plates, move all samples to separate wells of the same MIDI plate according to the plate layout.
- Option #2: If the plate is frozen.
- **a** Thaw the PCF PCR plate or the LP PCR plate to room temperature.
- **b** Centrifuge the plate at 280 × g for 1 minute.
- **c** Pipette 10 times to mix.
- **d** Label a new 96-well MIDI plate LP2 (Library Preparation 2).
- **e** Transfer the entire 50 µl of each sample from the PCF PCR plate or the LP PCR plate to the corresponding well of the LP2 MIDI plate.
  f Discard PCF PCR or LP PCR plate.

Procedure

1. Add 10 µl ERA1 master mix to each sample well in the LP2 MIDI plate.
2. Discard remaining ERA1 master mix.
3. Apply adhesive plate seal to the LP2 MIDI plate.
4. Seal edges and wells completely to prevent evaporation.
5. Shake at 1800 rpm for 2 minutes.
6. Incubate in the preheated microsample incubator at 30°C for 30 minutes.
7. Immediately transfer to a second, preheated microsample incubator, and incubate at 72°C for 20 minutes.
8. Place the LP2 MIDI plate on ice for 5 minutes.

Ligate Adapters

- This process ligates adapters to the ends of the cDNA and/or gDNA fragments.
- The TSO Comprehensive assay includes SUA1 adapters and UMI adapters.
- Use SUA1 adapters with RNA samples.
- Use UMI adapters with DNA samples.
- Prepare the following reagents.
- ALB1—Vortex to mix for a minimum of 10 seconds, and then centrifuge briefly.
- LIG3—Centrifuge briefly, and then pipette to mix. Keep on ice.
- SUA1—Vortex to mix for a minimum of 10 seconds, and then centrifuge briefly.
- UMI—Vortex to mix for a minimum of 10 seconds, and then centrifuge briefly.
- STL—Set aside for use in the procedure.

Procedure

1. Remove the LP2 MIDI plate from ice.
2. Add 60 µl ALB1 to each sample well of the LP2 MIDI plate, making sure to pipette slowly.
3. Add 5 µl LIG3 to each sample well.
4. Add adapters.
5. Do not combine different types of adapters together.

- **RNA sample wells—**10 µl SUA1 (blue cap) to each sample derived from RNA.
- **DNA sample wells—**10 µl UMI (white cap) to each sample derived from DNA.

1. Apply adhesive plate seal to the LP2 MIDI plate. Seal edges and wells completely.
2. Shake at 1800 rpm for 2 minutes.
3. Incubate at room temperature for 30 minutes.
4. Vortex STL to mix, and then centrifuge briefly.
5. Add 5 µl STL to each sample well of the LP2 MIDI plate.
6. Apply adhesive plate seal to the LP2 MIDI plate.
7. Seal edges and wells completely to prevent evaporation.
8. Shake at 1800 rpm for 2 minutes.

Clean Up Ligation

- Prepare the following reagents.
- **SPB**—Make sure beads are at room temperature for 30 minutes.
- **RSB—**Set aside for use in the procedure.
- Prepare fresh 80% EtOH in a 15 ml or 50 ml conical tube.

Reagent	3 Libraries	8 Libraries	16 Libraries	24 Libraries	48 Libraries
100% Ethanol alcohol, pure	2 ml	4 ml	8 ml	12 ml	24 ml
RNase/DNase-free water	500 µl	1 ml	2 ml	3 ml	6 ml

- Vortex fresh 80% EtOH to mix.
- Set out the magnet.

1. Vortex SPB for 1 minute to resuspend beads.
2. Immediately add 112 µl SPB to each sample well in the LP2 MIDI plate. If using a trough to dispense SPB, include a 1.05 overage factor when aliquoting sufficient material per sample. Discard any remaining material once SPB has been added to each sample well.
3. Apply adhesive plate seal to the LP2 MIDI plate. Seal edges and wells completely.
4. Shake at 1800 rpm for 2 minutes.
5. Incubate at room temperature for 5 minutes.
6. Place the LP2 MIDI plate on the magnetic stand for 10 minutes.
7. Use a P200 pipette set at 200 µl to remove and discard all supernatant from each sample well without disturbing the bead pellet.

Wash

1. Wash beads as follows.
2. **a** Keep on the magnetic stand and add 200 µl fresh 80% EtOH to each sample well.
3. **b** Wait 30 seconds.
4. **c** Remove and discard all supernatant from each well without disturbing the bead pellet.
5. Wash beads a second time.
6. Remove residual EtOH from each well. Use a P20 pipette with fine tips.
7. Discard unused 80% EtOH.

Elute

1. Remove the LP2 MIDI plate from the magnetic stand.
2. Invert or vortex RSB to mix.
3. Add 27.5 µl RSB to each sample well.
4. Apply adhesive plate seal to the LP2 MIDI plate. Seal edges and wells completely.
5. Shake at 1800 rpm for 2 minutes.
6. Incubate at room temperature for 2 minutes.
7. Place on a magnetic stand for 2 minutes.
8. Label a new 96-well PCR plate LS (Library Samples).
9. Transfer 25 µl of each eluate from the LP2 MIDI plate to the corresponding well of the LS PCR plate.
10. Discard the empty LP2 MIDI plate.
11. Apply adhesive plate seal to LS PCR plate.

Index PCR

Preparation Start Date and Time _____

1. Prepare the following reagents.
2. EPM—Keep on ice.
3. UPxx—Vortex to mix and centrifuge briefly. UPxx is the index primer selected on the Create Run screen in the Local Run Manager software during run setup.
4. CPxx—Vortex to mix and centrifuge briefly. CPxx is the index primer selected on the Create Run screen in the Local Run Manager software during run setup.
5. Make sure that indexes for each sample match the run planned in Local Run Manager during run setup. Make sure to follow instructions regarding index selection in the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789).

CAUTION
Mismatches between the samples and indexing primers cause incorrect result reporting due to loss of positive sample identification.

Procedure

1. Add 5 µl of the appropriate index primer (UPxx or CPxx) to the corresponding sample well in the LS PCR plate according to the indexes selected on the Create Run screen in the Local Run Manager software during run setup.

CAUTION
Handle and open only one index primer tube at a time. Recap each index tube immediately after use. Do not combine index primers together.

1. Vortex EPM to mix for 5 seconds, and then centrifuge briefly.
2. Add 20 µl EPM to each sample well.
3. Apply adhesive plate seal to the LS PCR plate. Seal edges and wells completely to prevent evaporation.
4. Shake at 1200 rpm for 1 minute.
5. Return pre-amplification reagents to storage.

   CAUTION

   Perform all subsequent steps in a post-amplification area to prevent amplification product carryover.
6. Centrifuge the LS PCR plate at 280 × g for 1 minute.
7. Place on the preprogrammed post-amplification thermal cycler and run the I-PCR program.

NOTE If continuing with Set Up First Hybridization on page 18, follow the thaw instructions for reagents in the Prepare Protocol Steps.

1. After the I-PCR program completes, centrifuge the LS PCR plate at 280 × g for 1 minute.
2. Relabel the plate ALS (Amplified Library Samples).

SAFE STOPPING POINT

- If you are stopping, store ALS PCR plate at -25°C to -15°C for up to 30 days.
- Stop Date and Time _____

Prepare for Protocol Steps

1. Make sure that post-amplification thermal cycler programs are set. See Program Thermal Cyclers on page 4.
2. Remove the reagent tube from the box and follow thaw instructions.

Table 12 TruSight Oncology Comp Enrichment (Refrigerate) Box (PN 20031123)

Reagent	Storage	Thaw Instructions	Protocol Step
TCB1	2°C to 8°C	Bring to room temperature.	Set Up First Hybridization

Reagent	Storage	Thaw Instructions	Protocol Step
TCA1	-25°C to -15°C	Thaw to room temperature.	Set Up First Hybridization

Table 14 TruSight Oncology Comp Content Set Box (PN 20031122)

Reagent	Storage	Thaw Instructions	Protocol Step
OPR1 (red cap)	-25°C to -15°C	Thaw to room temperature.	Set Up First Hybridization
OPD2 (white cap)	-25°C to -15°C	Thaw to room temperature.	Set Up First Hybridization

Set Up First Hybridization

1. Prepare the following reagents.
2. TCB1—Warm the tube at 37°C for 5 minutes. Vortex to mix for 10 seconds, and then centrifuge briefly.
3. TCA1—Vortex to mix, and then centrifuge briefly.
4. OPR1—Vortex to mix, and then centrifuge briefly.
5. OPD2—Vortex to mix, and then centrifuge briefly.
6. If the ALS PCR plate was stored, thaw to room temperature and centrifuge at 280 × g for 1 minute. Then pipette to mix.
7. Label a new 96-well PCR plate HYB1 (Hybridization 1).

Procedure

1. Transfer 20 µl of each cDNA and/or gDNA library from the ALS PCR plate to the corresponding well in the HYB1 PCR plate.
2. Apply adhesive plate seal to the ALS PCR plate and set aside. Seal edges and wells completely.
3. Inspect TCB1 for precipitates. If present, warm the tube again and vortex the tube until the crystals dissolve.
4. Add 15 µl TCB1 to each library well in the HYB1 PCR plate.
5. Add 10 µl TCA1 to each library well in the HYB1 PCR plate.
6. Add probes.

- Do not combine different types of probes together.
- **RNA library wells—** 5 µl OPR1 to each library derived from RNA.
- **DNA library wells—** 5 µl OPD2 to each library derived from DNA.

1. Apply adhesive plate seal to the HYB1 PCR plate.

CAUTION
Make sure to seal edges and wells completely to prevent evaporation.

1. Shake at 1200 rpm for 2 minutes.
2. Place on the thermal cycler and run the HYB1 program.
3. Hybridize at 57°C for a minimum of 8 hours to a maximum of 24 hours.
4. Return hybridization reagents to storage.
5. Store the ALS PCR plate at -25°C to -15°C for up to 30 days.

Prepare for Protocol Steps

1. At the beginning of day 2, remove the reagent tube from the box and follow thaw instructions.

Table 15 TruSight Oncology Comp Enrichment (Refrigerate) Box (PN 20031123)

Reagent	Storage	Thaw Instructions	Protocol Step
SMB (dark blue label)	2°C to 8°C	Bring to room temperature for 30 minutes.	Capture Targets One Capture Targets Two
ET2	2°C to 8°C	Bring to room temperature.	Capture Targets One Capture Targets Two
HP3	2°C to 8°C	Bring to room temperature.	Capture Targets One Capture Targets Two Normalize Libraries
TCB1	2°C to 8°C	Bring to room temperature.	Set Up Second Hybridization
RSB	2°C to 8°C	Bring to room temperature.	Capture Targets Two Clean Up Amplified Enriched Library

Table 16 TruSight Oncology Comp Enrichment (Freeze) Box (PN 20031121)

Reagent	Storage	Thaw Instructions	Protocol Step
EE2	-25°C to -15°C	Thaw to room temperature.	Capture Targets One Capture Targets Two Normalize Libraries
EEW	-25°C to -15°C	Thaw to room temperature.	Capture Targets One
TCA1	-25°C to -15°C	Thaw to room temperature.	Set Up Second Hybridization

Table 17 TruSight Oncology Comp Content Set Box (PN 20031122)

Reagent	Storage	Thaw Instructions	Protocol Step
OPR1 (red cap)	-25°C to -15°C	Thaw to room temperature.	Set Up Second Hybridization
OPD2 (white cap)	-25°C to -15°C	Thaw to room temperature.	Set Up Second Hybridization

Capture Targets One

Preparation

Start Date and Time _____

1. Preheat a microsample incubator with a MIDI heat block insert to 57°C.
2. Prepare the following reagents.
3. **EEW—**Vortex to mix for 1 minute.
4. **EE2—**Vortex to mix, and then centrifuge briefly.
5. **HP3—**Vortex to mix, and then centrifuge briefly.
6. **SMB—**Make sure that beads are at room temperature for 30 minutes.
7. Make sure to use SMB, not SPB for this procedure.
8. **ET2—**Set aside for use in the procedure.
9. Prepare fresh EE2+HP3 elution mix in a microcentrifuge tube.

Table 18 EE2+HP3 Elution Mix for Capture Targets One

Elution Mix Component	3 Libraries	8 Libraries	16 Libraries	24 Libraries	48 Libraries
EE2	85.5 µl	228 µl	456 µl	684 µl	1368 µl
HP3	4.5 µl	12 µl	24 µl	36 µl	72 µl

- This table includes volume overage. See the Handling Reagents section of the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) for calculations.
- Vortex EE2+HP3 elution mix, and then centrifuge briefly. Set aside for the Elute step.
- Label a new 96-well MIDI plate CAP1 (Capture 1).
- Set out the magnet.

Procedure Bind

- Remove the HYB1 PCR plate from the thermal cycler.
- Centrifuge the HYB1 PCR plate at 280 × g for 1 minute.
- Vortex SMB for 1 minute to resuspend beads.
- Immediately add 150 µl SMB to each library well of the CAP1 MIDI plate.
- If using a trough to dispense SMB, include a 1.15 overage factor when aliquoting sufficient material per sample.
- Discard any remaining material once SMB has been added to each sample well.
- Set pipette to 50 µl and transfer entire volume of each library from the HYB1 PCR plate to the corresponding well in the CAP1 MIDI plate.
- Discard the empty HYB1 PCR plate.
- Apply adhesive plate seal to the CAP1 MIDI plate.
- Seal edges and wells completely to prevent evaporation.
- Shake at 1800 rpm for 2 minutes.

- Incubate in the preheated microsample incubator at 57°C for 25 minutes.
- Place on a magnetic stand for 2 minutes.
- While keeping the CAP1 MIDI plate on the magnetic stand, use a P200 µl pipette set to 200 µl to remove and discard all supernatant without disturbing the bead pellet.

CAUTION

- Proceed immediately to the next step (Wash). Do not allow the bead pellet to sit for an extended amount of time without liquid present.

Wash

- Wash beads as follows.
- **a** Remove the CAP1 MIDI plate from the magnetic stand.
- **b** Add 200 µl EEW to each well.
- **c** Set pipette volume to 150 µl, and pipette to mix a minimum of 10 times. Make sure all beads are resuspended.

CAUTION

- Make sure that no bead pellets are present by carefully aspirating total bead solution of well into the tip. Then look at bottom of each well for a pellet. Angle pipette tip towards bead pellet during wash steps to dislodge pellet. Make sure that the bead pellet is fully in solution. The solution should look dark brown and have a homogenous consistency.
- **d** Apply adhesive plate seal to the CAP1 MIDI plate.
- **e** Seal edges and wells completely to prevent evaporation.
- **f** Shake at 1800 rpm for 4 minutes.
- **g** Incubate in a microsample incubator at 57°C for 5 minutes.
- **h** Place on a magnetic stand for 2 minutes.
- **i** Keep on the magnetic stand and remove and discard all supernatant from each well without disturbing the bead pellet.
- Wash beads a second time.
- Wash beads a third time.
- Remove residual supernatant from each well.
- Use a P20 pipette with fine tips.

Elute

1. Remove the CAP1 MIDI plate from the magnetic stand.
2. Vortex fresh EE2+HP3 Elution Mix, and then centrifuge briefly.
3. Carefully add 17 µl EE2+HP3 Elution Mix to each library well in the CAP1 MIDI plate.
4. Discard remaining EE2+HP3 Elution Mix.
5. Apply adhesive plate seal to the CAP1 MIDI plate.
6. Seal edges and wells completely.

7. Shake at 1800 rpm for 2 minutes.
8. Place on a magnetic stand for 2 minutes.
9. Label a new 96-well PCR plate ELU1 (Elution 1).
10. Vortex ET2 to mix, and then centrifuge briefly.
11. Add 5 µl ET2 to each corresponding library well in the new ELU1 PCR plate.
12. Carefully transfer 15 µl eluate from each library well of the CAP1 MIDI plate to the corresponding well in the ELU1 PCR plate.
13. Discard empty CAP1 MIDI plate.
14. Apply adhesive plate seal to the ELU1 PCR plate.
15. Seal edges and wells completely to prevent evaporation.
16. Shake at 1200 rpm for 2 minutes.
17. Return EEW to storage.

Set Up Second Hybridization

Preparation
Start Date and Time _____

- Prepare the following reagents.
- TCB1—Warm the tube at 37°C for 5 minutes. Vortex to mix for 10 seconds, and then centrifuge briefly.
- TCA1—Vortex to mix, and then centrifuge briefly.
- OPR1—Vortex to mix, and then centrifuge briefly.
- OPD2—Vortex to mix, and then centrifuge briefly.

Procedure

1. Inspect TCB1 for precipitates. If present, warm the tube again and vortex until crystals dissolve.
2. Add 15 µl TCB1 to each library well in the ELU1 PCR plate.
3. Add 10 µl TCA1 to each library well.
4. Add probes.
5. Do not combine different types of probes together.
6. RNA library wells— 5 µl OPR1 to each library derived from RNA.
7. DNA library wells— 5 µl OPD2 to each library derived from DNA.
8. Apply adhesive plate seal to the ELU1 PCR plate.
9. Seal edges and wells completely to prevent evaporation.
10. Shake at 1200 rpm for 2 minutes.
11. Place on a thermal cycler and run the HYB2 program.
12. See Program Thermal Cyclers on page 4.
13. Hybridize at 57°C for a minimum of 1.5 hours to a maximum of 4 hours.
14. Return TCA1, TCB1, OPR1, and OPD2 to storage.

Capture Targets Two

Preparation
Start Date and Time _____

1. Preheat a microsample incubator with MIDI heat block insert to 57°C.
2. Prepare the following reagents.
3. **EE2—**Vortex to mix, and then centrifuge briefly.
4. **HP3—**Vortex to mix, and then centrifuge briefly.
5. **SMB—**Make sure that beads are at room temperature for 30 minutes.
6. Make sure to use SMB, not SPB for this procedure.
7. **RSB**—Set aside for use in the procedure.
8. **ET2**—Set aside for use in the procedure.
9. Prepare fresh EE2+HP3 elution mix in a microcentrifuge tube.

Table 19 EE2+HP3 Elution Mix for Capture Targets Two

Elution Mix Component	3 Libraries	8 Libraries	16 Libraries	24 Libraries	48 Libraries
EE2	85.5 µl	228 µl	456 µl	684 µl	1368 µl
HP3	4.5 µl	12 µl	24 µl	36 µl	72 µl

1. This table includes volume overage. See the Handling Reagents section of the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) for calculations.
2. Vortex to mix, and then centrifuge briefly. Set aside for the Elute step.
3. Label a new 96-well MIDI plate CAP2 (Capture 2).
4. Set out the magnet.

Procedure Bind

1. Remove the ELU1 PCR plate from the thermal cycler.
2. Centrifuge ELU1 PCR plate at 280 × g for 1 minute.
3. Vortex SMB for 1 minute to resuspend beads.
4. Immediately add 150 µl SMB to each library well of the CAP2 MIDI plate.
5. If using a trough to dispense SMB, include a 1.15 overage factor when aliquoting sufficient material per sample.
6. Discard any remaining material once SMB has been added to each sample well.
7. Set pipette to 50 µl and transfer entire volume of each library from the ELU1 PCR plate to the corresponding well of the CAP2 MIDI plate.
8. Discard the empty ELU1 PCR plate.
9. Apply adhesive plate seal to the CAP2 MIDI plate.
   Seal edges and wells completely to prevent evaporation.
10. Shake at 1800 rpm for 2 minutes.
11. Incubate in a microsample incubator at 57°C for 25 minutes.

NOTE If continuing with Amplify Enriched Library on page 24, follow thaw instructions for reagents in the Prepare for Protocol Steps section.

1. Place on a magnetic stand for 2 minutes.
2. Keep the CAP2 MIDI plate on the magnetic stand, and use a P200 pipette set to 200 µl to remove and discard all supernatant from each library well without disturbing the bead pellet.

CAUTION
Proceed immediately to the next step (Wash). Do not allow the bead pellet to sit for an extended amount of time without liquid present.

Wash

1. Remove the CAP2 MIDI plate from the magnetic stand.
2. Invert or vortex RSB to mix.
3. Add 200 µl RSB to each well.
4. Apply adhesive plate seal to the CAP2 MIDI plate. Seal edges and wells completely.
5. Shake at 1800 rpm for 4 minutes.
6. Place on the magnetic stand for 2 minutes.
7. Keep the CAP2 MIDI plate on the magnetic stand and remove and discard all supernatant without disturbing the bead pellet.
8. Remove residual supernatant from each well.
9. Use a P20 pipette with fine tips.

Elute

1. Remove the CAP2 MIDI plate from the magnetic stand.
2. Vortex fresh EE2+HP3 Elution Mix, and then centrifuge briefly.
3. Add 22 µl EE2+HP3 Elution Mix to each library well in the CAP2 MIDI plate.
4. Discard remaining EE2+HP3 Elution Mix.
5. Apply adhesive plate seal to the CAP2 MIDI plate. Seal edges and wells completely.
6. Shake at 1800 rpm for 2 minutes.
7. Place on a magnetic stand for 2 minutes.
8. Label a new 96-well PCR plate ELU2 (Elution 2).
9. Vortex ET2 to mix, and then centrifuge briefly.
10. Add 5 µl ET2 to each corresponding library well in the new ELU2 PCR plate.
11. Carefully transfer 20 µl eluate from each library well of the CAP2 MIDI plate to the corresponding well in the ELU2 PCR plate.
12. Discard empty CAP2 MIDI plate.
13. Apply adhesive plate seal to the ELU2 PCR plate. Seal edges and wells completely to prevent evaporation.
14. Shake at 1200 rpm for 2 minutes.
15. Return SMB, EE2, HP3, and ET2 to storage.

SAFE STOPPING POINT

- If you are stopping, centrifuge ELU2 PCR plate at 280 × g for 1 minute and store at -25°C to -15°C for up to 7 days. Return RSB to storage.

  Stop Date and Time _____

Prepare for Protocol Steps

- Prepare an ice bucket.
- Remove the reagent tube from the box and follow thaw instructions.

Table 20 TruSight Oncology Comp Enrichment (Freeze) Box (PN 20031121)			
Reagent	**Storage**	**Thaw Instructions**	**Protocol Step**
PPC3	-25°C to -15°C	Thaw to room temperature.	Amplify Enriched Library
EPM	-25°C to -15°C	Keep on ice.	Amplify Enriched Library

Table 21 TruSight Oncology Comp Enrichment (Refrigerate) Box (PN 20031123			
Reagent	**Storage**	**Thaw Instructions**	**Protocol Step**
SPB (light green label)	2°C to 8°C	Bring to room temperature for 30 minutes.	Clean Up Amplify Enriched
			Library
RSB	2°C to 8°C	Bring to room temperature.	Clean Up Amplify Enriched
			Library Prepare for Sequencing

Amplify Enriched Library

Preparation
Start Date and Time _____

1. If the ELU2 plate was stored, thaw to room temperature, and then centrifuge at 280 × g for 1 minute.

Procedure

1. Vortex PPC3 to mix, and then centrifuge briefly.
2. Add 5 µl PPC3 to each library well of the ELU2 PCR plate.
3. Vortex EPM to mix for 5 seconds, and then centrifuge briefly.
4. Add 20 µl EPM to each library well.
5. Apply adhesive plate seal to the ELU2 PCR plate.
   Seal edges and wells completely to prevent evaporation.
6. Shake at 1200 rpm for 2 minutes.
7. Place on a thermal cycler and run the EL-PCR program.
   See Program Thermal Cyclers on page 4.
   NOTE If continuing with Normalize Libraries on page 26, follow the thaw instructions in the Prepare for Protocol Steps section.

8. Return PPC3 and EPM to storage.

Clean Up Amplified Enriched Library

Preparation
Start Date and Time _____

1. Prepare the following reagents.
2. SPB—Make sure that beads are at room temperature for 30 minutes.
3. Make sure to use SPB, not SMB for this procedure.
4. RSB—Set aside for use in the procedure.
5. Prepare fresh 80% ethanol in a 15 ml or 50 ml conical tube.

Reagent	3 Libraries	8 Libraries	16 Libraries	24 Libraries	48 Libraries
100% Ethanol alcohol, pure	2 ml	4 ml	8 ml	12 ml	24 ml
RNase/DNase-free water	500 µl	1 ml	2 ml	3 ml	6 ml

- Vortex fresh 80% EtOH to mix.
- Label a new 96-well MIDI plate BIND2 (Clean Up Binding).
- Set out the magnet.

Procedure

Bind

- Remove the ELU2 PCR plate from the thermal cycler.
- Centrifuge the ELU2 PCR plate at 280 × g for 1 minute.
- Vortex SPB for 1 minute to resuspend the beads.
- Immediately add 110 µl SPB to each library well of the BIND2 MIDI plate.
- Transfer 50 µl of each library from the ELU2 PCR plate to the corresponding well of the BIND2 MIDI plate.
- Discard empty ELU2 PCR plate.
- Apply adhesive plate seal to the BIND2 MIDI plate.
  Seal edges and wells completely.
- Shake at 1800 rpm for 2 minutes.
- Incubate at room temperature for 5 minutes.
- Place plate on magnetic stand for 5 minutes.
- Use a P200 pipette set at 200 µl to remove and discard all supernatant from each library well without disturbing the bead pellet.

Wash

- Wash beads as follows.

    - a Keep on magnetic stand and add 200 µl fresh 80% EtOH to each well.
    - b Wait 30 seconds.
    - c Remove and discard all supernatant from each sample well without disturbing the bead pellet.
- Wash beads a second time.
- Remove residual EtOH from each well.
  Use a P20 pipette with fine tips.
- Discard unused 80% EtoH.

Elute

- Remove the BIND2 MIDI plate from the magnetic stand.
- Invert or vortex to mix RSB.
- Add 32 µl RSB to each library well.
- Apply adhesive plate seal to the BIND2 MIDI plate.
  Seal edges and wells completely.
- Shake at 1800 rpm for 2 minutes.
- Incubate at room temperature for 2 minutes.
- Place on a magnetic stand for 2 minutes.
- Label a new 96-well PCR plate PL (Purified Libraries).
- Transfer 30 µl of each eluate from the BIND2 MIDI plate to the corresponding well of the PL PCR plate.
- Discard the empty BIND2 MIDI plate.
- Apply adhesive plate seal to the PL PCR plate.
- Return SPB to storage.

SAFE STOPPING POINT

If you are stopping, centrifuge the PL PCR plate at 280 × g for 1 minute and store at -25°C to -15°C for up to 30 days. Return RSB to storage.
Stop Date and Time _____

Prepare for Protocol Steps

- Remove the reagent tube from the box and follow thaw instructions.
  Table 22 TruSight Oncology Comp Enrichment (Freeze) Box (PN 20031121

Reagent	Storage	Thaw Instructions	Protocol Step
LNA1	-25°C to -15°C	Thaw to room temperature.	Normalize Libraries
EE2	-25°C to -15°C	Thaw to room temperature.	Normalize Libraries

  Table 23 TruSight Oncology Comp Enrichment (Refrigerate) Box (PN 200311

Reagent	Storage	Thaw Instructions	Protocol Step
LNB1	2°C to 8°C	Bring to room temperature for 30 minutes.	Normalize Libraries
HP3	2°C to 8°C	Bring to room temperature.	Normalize Libraries Prepare for Sequencing
LNW1	2°C to 8°C	Bring to room temperature.	Normalize Libraries
LNS1	2°C to 8°C	Bring to room temperature.	Normalize Libraries

- If you are continuing on the same day with Prepare for Sequencing on page 29, follow the thaw instructions in the Prepare for Protocol Steps section.

Normalize Libraries

Preparation
Start Date and Time _____

1. Prepare the following reagents.
    1. LNB1—Make sure the beads are at room temperature for 30 minutes.
    2. LNA1—Vortex to mix.
    3. EE2—Vortex to mix, and then centrifuge briefly.
    4. HP3—Vortex to mix, and then centrifuge briefly.
    5. LNW1—Vortex to mix. Set aside for use in procedure.
    6. LNS1—Vortex to mix. Set aside for use in the procedure.
2. Vortex LNB1 for 1 minute to resuspend beads.
   Invert LNB1 tube to make sure that all beads are resuspended.
3. Using a P1000 set at 800 µl, pipette LNB1 up and down 10 times to ensure resuspension.
4. Immediately prepare fresh LNA1+LNB1 Master Mix in a conical tube
   CAUTION
   Completely resuspend the LNB1 bead pellet at the bottom of the tube to prevent inconsistent cluster density..
   Table 24 LNA1+LNB1 Master Mix

Master Mix Component	3 Libraries	8 Libraries	16 Libraries	24 Libraries	48 Libraries
LNA1	229 µl	610 µl	1219 µl	1829 µl	3658 µl
LNB1	41 µl	110 µl	221 µl	331 µl	662 µl

   This table includes volume overage. See the Handling Reagents section of the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) for calculations.

5. Vortex LNA1+LNB1 master mix. Set aside for Bind step.

6. Prepare fresh EE2+HP3 Elution Mix in a microcentrifuge tube.

Elution Mix Component	3 Libraries	8 Libraries	16 Libraries	24 Libraries	48 Libraries
EE2	114 µl	304 µl	608 µl	912 µl	1824 µl
HP3	6 µl	16 µl	32 µl	48 µl	96 µl

   This table includes volume overage. See the Handling Reagents section of the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789) for calculations.

7. Vortex fresh elution mix, and then centrifuge briefly. Set aside for the Elute step.
8. If the PL PCR plate was stored, thaw to room temperature, centrifuge at 280 × g for 1 minute, and then pipette to mix.
9. Label a new 96-well MIDI plate BBN (Bead Based Normalization).
10. Set out the magne

Procedure

- **Bind**
  - Vortex LNA1+LNB1 master mix.
  - Immediately add 45 µl LNA1+LNB1 Master Mix to each library well of the BBN MIDI plate.
  - Discard remaining LNA1+LNB1 master mix.
  - Add 20 µl of each library from the PL PCR plate to the corresponding well of the BBN MIDI plate.
  - Apply adhesive plate seal to the BBN MIDI plate.
  - Seal edges and wells completely.
  - Shake at 1800 rpm for 30 minutes.
  - Apply adhesive plate seal to the PL PCR plate and return to storage.
  - Place plate on a magnetic stand for 2 minutes.
  - Keep on a magnetic stand and use a P200 pipette to remove and discard all supernatant from each well without disturbing the bead pellet.
- **Wash**
  - Wash beads as follows.
    - Remove the BBN MIDI plate from the magnetic stand.
    - Add 45 µl LNW1 to each library well.
    - Apply adhesive plate seal to the BBN MIDI plate.
    - Seal edges and wells completely.
    - Shake at 1800 rpm for 5 minutes.
    - Place on a magnetic stand for 2 minutes.
    - Remove and discard all supernatant from each well without disturbing the bead pellet.
  - Wash beads a second time.
  - Remove residual supernatant from each well.
    Use a P20 pipette with fine tips.
- **Elute**

- Remove the BBN MIDI plate from the magnetic stand.
- Vortex fresh EE2+HP3 Elution Mix, and then centrifuge briefly.
- Add 32 µl EE2+HP3 solution to each library well of the BBN MIDI plate
- Discard remaining elution mix.
- Apply adhesive plate seal to the BBN MIDI plate.
  Seal edges and wells completely.
- Shake at 1800 rpm for 2 minutes.
- Place on a magnetic stand for 2 minutes.
- Label a new 96-well PCR plate NL (Normalized Libraries).
- Carefully transfer 30 µl eluate from each library well of the BBN MIDI plate to the corresponding well of the NL PCR plate.
  CAUTION
  If beads are aspirated into the pipette tips, dispense the beads back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes) before proceeding to the next step of the procedure.
- Discard the empty BBN MIDI plate.
- Vortex LNS1 to mix.
- Add 30 µl LNS1 to each library well in the new NL PCR plate.
- Pipette to mix 5 times.
- Apply adhesive plate seal to the NL PCR plate. Seal edges and wells completely.
- Return LNB1, LNA1, EE2, LNW1, and LNS1 to storage

SAFE STOPPING POINT

If you are stopping, centrifuge NL PCR plate at 280 × g for 1 minute and store at -25°C to -15°C for up to 30 days.
Stop Date and Time _____

Prepare for Protocol Steps

Start the preparation of sequencing consumables from the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) (PN 20028871) at least an hour before use.

- Remove Library Dilution Buffer (HT1) from -25°C to -15°C storage, thaw to room temperature, and then place on ice.
- Follow preparation instructions in the NextSeq 550Dx Instrument Reference Guide (document # 1000000009513) for other consumables in the kit.
  - NextSeq 550Dx High Output Reagent Cartridge v2 (300 cycles)
  - NextSeq 550Dx Buffer Cartridge v2 (300 cycles)
  - NextSeq 550Dx High Output Flow Cell Cartridge v2.5 (300 cycles)
- Remove the reagent tube from the box and follow thaw instructions.
  Table 26 TruSight Oncology Comp Enrichment (Freeze) Box (PN 20031121
  Table 27 TruSight Oncology Comp Enrichment (Refrigerate) Box (PN 2003112

Reagent	Storage	Thaw Instructions	Protocol Step
HP3	2°C to 8°C	Bring to room temperature.	Prepare for Sequencing
RSB (pink label)	2°C to 8°C	Bring to room temperature.	Prepare for Sequencing

Prepare for Sequencing

Preparation
Start Date and Time _____

1. Review the guidelines for the number of libraries and selecting indexes in the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789).
2. Label a microcentrifuge tube dHP3 (diluted HP3).
3. Label a microcentrifuge tube dPhiX (diluted PhiX).
4. Preheat a heat block to 96°C for microcentrifuge tubes.
5. Prepare an ice bucket.

Dilute and Denature PhiX Control

1. Vortex HP3 to mix, and then centrifuge briefly.
2. Combine the following volumes in the dHP3 microcentrifuge tube.
    1. 10 µl HP3
    2. 190 µl RNase/DNase-free water
3. Vortex dHP3 to mix, and then centrifuge briefly.
4. Invert or vortex RSB to mix.
5. Vortex PhiX control to mix, and then centrifuge briefly.
6. Combine the following volumes in the dPhiX microcentrifuge tube.
7. µl RSB
8. µl PhiX control
9. Add 10 µl dHP3 to the dPhiX tube.
10. Discard the dHP3 tube.
11. Vortex dPhiX tube to mix, and then centrifuge briefly.
12. Incubate dPhiX at room temperature for 5 minutes to denature.
13. Vortex HT1 to mix.
14. Immediately add 980 µl of prechilled HT1 to dPhiX.
15. Vortex to mix, and then centrifuge briefly.
16. Place dPhiX on ice until use in the preparation for the second dilution.
    The final concentration is 20 pM dPhiX.
17. Return PhiX, HP3, and RSB to storage.

Pool and Denature Libraries

1. If the NL PCR plate was stored, thaw to room temperature, and then centrifuge the plate at 280 × g for 1

minute.

2. Using a multichannel pipette set at 30 µl, gently pipette-mix the libraries in the NL PCR plate 5 times.
   Use fresh tips for each library.
   CAUTION
   Make sure to mix libraries well for optimal performance.
3. Select one of the following options to pool, denature, and dilute the libraries.
   1. Option #1: Sequence libraries derived from RNA samples and DNA samples simultaneously. See Option #1: DNA and RNA Libraries Together
   2. Option #2: Sequence libraries derived from DNA samples only. See Option #2: DNA Only Libraries
   3. Option #3: Sequence libraries derived from RNA samples only. See Option #3: RNA Only Libraries

Option #1: DNA and RNA Libraries Together

1. Label a microcentrifuge tube PRL (Pooled RNA Libraries).
2. Label a microcentrifuge tube PDL (Pooled DNA Libraries).
3. Transfer 10 µl of each normalized RNA (cDNA) library from the NL plate to the PRL tube.
   Do not pool two libraries with the same index primer.
4. Transfer 10 µl of each normalized DNA library from the NL plate to the PDL tube.
   Do not pool two libraries with the same index primer.
5. Apply adhesive plate seal to the NL PCR plate.
   Seal edges and wells completely.
6. Vortex each PRL and PDL tube to mix.
7. Centrifuge PRL and PDL tubes briefly.
8. Incubate PRL and PDL tubes in a heat block at 96°C for 2 minutes.
9. Place PRL and PDL on ice for 5 minutes.
10. Vortex PRL and PDL tubes to mix, and then centrifuge briefly.
11. Return PRL and PDL tubes to ice.

Prepare First Dilution

- Label a 1.7 ml microcentrifuge tube DIL1 (Dilution 1).
- Transfer 20 µl PDL to the empty DIL1 tube.
- Add 5 µl PRL to DIL1.
- Discard the PDL and PRL tubes.
- Add 475 µl prechilled HT1 to the DIL1 tube (1:20 dilution).
- Vortex DIL1 tube to mix, and then centrifuge briefly.

Prepare Second Dilution

- Label a 2.0 mL microcentrifuge tube DIL2 (Dilution 2).
- Transfer 40 µl DIL1 to the empty DIL2 tube.
- Discard the DIL1 tube.
- Add 1660 µl prechilled HT1 to the DIL2 tube (1:850 dilution).
- Vortex prepared 20 pM dPhiX to mix, and then centrifuge briefly.

- Add 2.5 µl prepared 20 pM dPhiX to the DIL2 tube.
- Vortex to mix, and then centrifuge briefly.
- Load 1300 µl DIL2 to the thawed NextSeq 550Dx High Output Reagent Cartridge v2 (300 cycles)
  For more information, see NextSeq 550Dx Instrument Reference Guide (document # 1000000009513).
- Discard the DIL2 tube.
- Centrifuge NL PCR plate at 280 × g for 1 minute, and store at -25°C to -15°C for up to 30 days.
- Proceed to sequencing.
  For more information, see NextSeq 550Dx Instrument Reference Guide (document # 1000000009513).

Option #2: DNA Only Libraries

1. Label a microcentrifuge tube PDL (Pooled DNA Libraries).
2. Transfer 10 µl of each normalized DNA library from the NL plate to the PDL tube.
   Do not pool two libraries with the same index primer.
3. Apply adhesive plate seal to the NL PCR plate.
   Seal edges and wells completely
4. Vortex PDL tube to mix.
5. Centrifuge PDL tube briefly.
6. Incubate PDL tube in a heat block at 96°C for 2 minutes.
7. Place PDL on ice for 5 minutes.
8. Vortex PDL tube to mix, and then centrifuge briefly.
9. Return PDL tube to ice.

Prepare First Dilution

1. Label a 1.7 ml microcentrifuge tube DIL1 (Dilution 1).
2. Transfer 10 µl PDL to the empty DIL1 tube.
3. Discard the PDL tube.
4. Add 190 µl prechilled HT1 to the DIL1 tube (1:20 dilution).
5. Vortex DIL1 to mix, and then centrifuge briefly.

Prepare Second Dilution

1. Label a 2.0 mL microcentrifuge tube DIL2 (Dilution 2).
2. Transfer 40 µl DIL1 to the empty DIL2 tube.
3. Discard the DIL1 tube.
4. Add 1660 µl prechilled HT1 to the DIL2 tube (1:850 dilution).
5. Vortex prepared 20 pM dPhiX, and then centrifuge briefly.
6. Add 2.5 µl prepared 20 pM dPhiX to the DIL2 tube.
7. Vortex to mix, and then centrifuge briefly.
8. Load 1300 µl DIL2 to the thawed NextSeq 550Dx High Output Reagent Cartridge v2 (300 cycles).
   For more information, see NextSeq 550Dx Instrument Reference Guide (document # 1000000009513).
9. Discard the DIL2 tube.
10. Centrifuge NL PCR plate at 280 × g for 1 minute, and then store at -25°C to -15°C for up to 30 days.

11. Proceed to sequencing.
    For more information, see NextSeq 550Dx Instrument Reference Guide (document # 1000000009513).

Option #3: RNA Only Libraries

1. Label a microcentrifuge tube PRL (Pooled RNA Libraries).
2. Transfer 10 µl of each normalized RNA (cDNA) library from the NL plate to the PRL tube.
   Do not pool two libraries with the same index primer.
3. Apply adhesive plate seal to the NL PCR plate.
   Seal edges and wells completely.
4. Vortex PRL tube to mix.
5. Centrifuge PRL tube briefly.
6. Incubate PRL tube in a heat block at 96°C for 2 minutes.
7. Place PRL on ice for 5 minutes.
8. Vortex PRL tube to mix, and then centrifuge briefly.
9. Return PRL tube to ice.

Prepare First Dilution

1. Label a 1.7 ml microcentrifuge tube DIL1 (Dilution 1).
2. Transfer 10 µl PRL to the empty DIL1 tube.
3. Discard the PRL tube.
4. Add 190 µl prechilled HT1 to the DIL1 tube (1:20 dilution).
5. Vortex DIL1 to mix, and then centrifuge briefly.

Prepare Second Dilution

1. Label a 2.0 mL microcentrifuge tube DIL2 (Dilution 2).
2. Transfer 40 µl DIL1 to the empty DIL2 tube.
3. Discard the DIL1 tube.
4. Add 1646 µl prechilled HT1 to the DIL2 tube (1:843 dilution).
5. Vortex prepared 20 pM dPhiX, and then centrifuge briefly.
6. Add 16.7 µl prepared 20 pM dPhiX to the DIL2 tube.
7. Vortex to mix, and then centrifuge briefly.
8. Load 1300 µl DIL2 into the thawed NextSeq 550Dx High Output Reagent Cartridge v2 (300 cycles).
   For more information, see NextSeq 550Dx Instrument Reference Guide (document # 1000000009513).
9. Discard the DIL2 tube.
10. Centrifuge NL PCR plate at 280 × g for 1 minute, and store at -25°C to -15°C for up to 30 days.
11. Proceed to sequencing.
    For more information, see NextSeq 550Dx Instrument Reference Guide (document # 1000000009513).

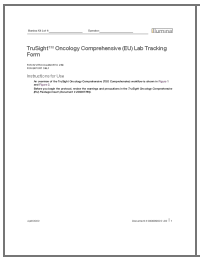
Patents and Trademarks

Contact Information

Illumina 5200 Illumina Way San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com
Illumina Netherlands B.V. Steenoven 19 5626 DK Eindhoven The Netherlands

Product Labeling
For a complete reference to symbols that may appear on product packaging and labeling, refer to the symbol key for your kit at **support.illumina.com**.

Documents / Resources

	**illumina TruSight Oncology Comprehensive Lab Tracking Form** [pdf] Instruction Manual TruSight Oncology Comprehensive Lab Tracking Form, TruSight, Oncology Comprehensive Lab Tracking Form, Comprehensive Lab Tracking Form, Lab Tracking Form, Tracking Form

References

- **Support Center**
- **Illumina | Sequencing and array-based solutions for genetic research**
- **Legal | Legal Information for this website**
- **Support Center**