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TruSight Oncology Comprehensive (JP)

Enablement Services Guide

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Getting Started

Overview

TruSight™ Oncology Comprehensive (JP) is an *in vitro* diagnostic test that uses targeted next-generation sequencing to detect variants in 517 genes using nucleic acids extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from cancer patients with solid malignant neoplasms using the Illumina® NextSeq™ 550Dx instrument. The test can be used to detect single nucleotide variants, multi-nucleotide variants, insertions, deletions, and gene amplifications from DNA, and gene fusions and splice variants from RNA.

About This Guide

This guide describes the various components of the TruSight Oncology Comprehensive (JP)(TSO Comprehensive [JP]) Enablement Services including:

- Software registration and installation
  - Local Run Manager TruSight Oncology Comprehensive (JP) Analysis Module (TSO Comprehensive (JP) analysis module) software
- Site readiness visit
- Laboratory training certification
- Verification testing guidance

Intended Audience

This guide is intended for laboratory directors, managers, and technicians who are implementing TSO Comprehensive (JP) in laboratories.

Reference Documents

TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)

Software Installation

TSO Comprehensive (JP) requires an installed Knowledge Base (KB) to perform analysis. A Field Applications Scientist (FAS) provides the KB to the customer using a data sharing folder. The KB is downloaded by the customer before the Enablement Service starts. The FAS installs the KB together with the TSO Comprehensive (JP) analysis module.

Knowledge Base Updates

As clinical evidence accumulates for variants in precision oncology, KB updates are made available to reflect the changes. Variants that were initially not reportable due to lack of clinical evidence can be reported later through a KB content update. Likewise, variants can change levels within the report. For example, a variant can move from Genomic Findings with Evidence of Clinical Significance (Level 2) to Genomic Findings with Potential Clinical Significance (Level 3) or vice versa. Detected variants not

meeting the criteria for any level are not reported. Susceptibility or cancer risk associations are excluded from the KB and do not impact leveling. Therapeutic associations used for leveling are limited to targeted cancer therapies and immunotherapies (not including cell-based immunotherapies).

Illumina releases new KB content periodically. In the event of future KB updates, the FAS will provide the KB to the customer using a data sharing folder. The customer will then download and install the KB onto the sequencer.

TSO Comprehensive (JP) Analysis Module Software

Data analysis for the TSO Comprehensive (JP) assay is accomplished via the TSO Comprehensive (JP) analysis module. The software includes the TSO Comprehensive (JP) analysis module and the KB. For part numbers and the current version of software, refer to *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*.

An Illumina FAS or Field Service Engineer (FSE) installs the TSO Comprehensive (JP) software onto the NextSeq 550Dx. The software is installed in the Clinical (Dx) mode.

Equipment and Materials

Additional equipment and materials are required to run TSO Comprehensive (JP). Before scheduling Illumina-provided training, set up the equipment and confirm that it is functioning properly. Refer to the *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)* for the equipment and materials required in the pre- and post-amplification areas.

Site Readiness Visit

Before scheduling training, Illumina performs a site readiness visit to make sure that the lab is set up to run the library prep for TruSight Oncology Comprehensive (JP). Illumina also confirms the NextSeq 550Dx is correctly configured.

The visit can be conducted in person or virtually.

During the visit, Illumina evaluates the following items:

- General lab information
- Pre-amplification lab equipment and consumables
- Post-amplification lab equipment and consumables
- NextSeq 550Dx and software configuration

Coordinate with a local Illumina FAS to schedule a site readiness visit before laboratory training certification.

Laboratory Training Certification

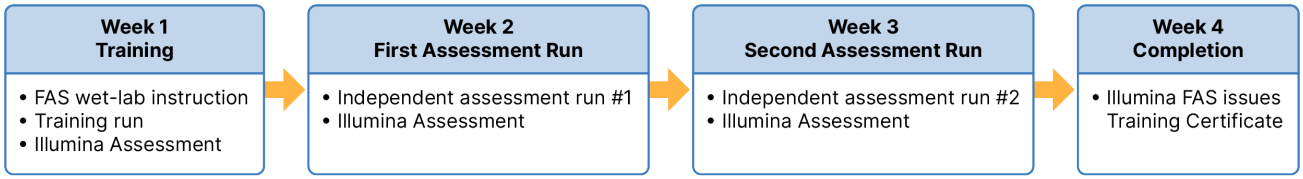
Purpose

A laboratory is responsible for making sure that its lab personnel have sufficient training and competency on a particular *in vitro* diagnostic test before performing the test. Illumina offers laboratory training certification to establish an initial set of trained operators with the laboratory. Following such training, the laboratory is responsible for assessing and ensuring the competence of each person to perform assigned tests, including TSO Comprehensive (JP). These activities must be done in accordance with applicable law, regulations, accreditations, standards, and requirements (for example, ISO 15189 and College of American Pathologists (CAP) requirements). The laboratory training certification does not constitute competency testing or certification under those rules and requirements.

Scope

This section describes the activities that make up the laboratory training certification offered by Illumina. The purpose of the certification is to provide customer site staff with training to perform the TSO Comprehensive (JP) assay in their laboratory per the *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*. Operators who complete the Illumina laboratory training certification, at the discretion of the laboratory, can provide training to additional or future lab personnel.

Training is provided by a certified Illumina FAS. Each operator trained is given a presentation and wet-lab instruction on the TSO Comprehensive (JP) assay. Each operator completes a training run under the supervision of the FAS. After the training run, each operator completes two consecutive assessment runs without FAS supervision. The operator prepares and sequences libraries using instructions provided in the Assay Workflow Guide to customers who purchase the instrument, sequencing reagents, and assay. The FAS reviews the results from each assessment run. Operators with passing results from two consecutive assessment runs are issued a training certificate.



Training Preparation

Illumina provides support to make sure that the equipment, consumables, and all other required materials are on-site and set up before training.

Materials

Materials for laboratory training certification are included as part of the service. An Illumina FAS makes sure that all required materials are available for training. The following materials are required:

Description	Illumina Part Number	Quantities for 1 Trainee	Quantities for 2 Trainees
TSO Comprehensive (JP) Kit	20032573	2	3
NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles)	20028871	3	6
TruSight Oncology DNA Control	20065041	3	5*
TruSight Oncology RNA Control	20065042	3	5

* 6 tubes may be required depending on ultrasonicator configuration.

Plate Layout

The following run setup applies to training and assessment runs:

- Each DNA library preparation includes seven replicates of the TruSight Oncology DNA control and one no template control (NTC). One replicate of the DNA control is designated as the Positive Control (PC) in the TSO Comprehensive (JP) analysis module.
- Each RNA library preparation includes seven replicates of the TruSight Oncology RNA control and one NTC. One replicate of the RNA control is designated as the Positive Control (PC) in the TSO Comprehensive (JP) analysis module.

Table 1 Example Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNA 1*			RNA 1*								
B	DNA 2			RNA 2								
C	DNA 3			RNA 3								
D	DNA 4			RNA 4								
E	DNA 5			RNA 5								
F	DNA 6			RNA 6								
G	DNA 7			RNA 7								
H	NTC			NTC								

* Indicates the sample designated as positive control in TSO Comprehensive (JP) analysis module.

Training

Each operator first completes a supervised training run, and then proceeds to assessment runs. Each operator must successfully complete two consecutive assessment runs without Illumina guidance. A successful assessment run meets the acceptance criteria described in *Acceptance Criteria* on page 7. After the successful completion of two consecutive assessment runs, the operator is issued a training certificate.

Training Run

Each operator completes a training run under the supervision of a certified Illumina FAS. A training run consists of preparing DNA and RNA libraries from the sample panel set and sequencing those libraries on the NextSeq 550Dx. Each operator being trained must complete their own library prep. If multiple operators are being trained and lab equipment is limited, incubation steps can be combined onto a single plate. For run setup examples, refer to *Plate Layout* on page 4.

Operators must follow the instructions from the *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*.

Data generated from the training run are analyzed with the TSO Comprehensive (JP) analysis software. At the completion of the training run, the operator prepares the associated data files for transfer using the instructions provided in *Data Transfer* on page 6.

The Illumina FAS (or certified designee) makes sure that all required output files are transferred and determines if the training run meets acceptance criteria. If libraries fail the sample validity metrics, external control specifications, or both, then the Illumina FAS starts a root cause investigation for the failures and provides feedback.

Assessment Runs

After completing a training run, each operator performs two assessment runs and provides the data to Illumina for review.

Each operator completes each assessment run without Illumina guidance. An assessment run consists of preparing DNA and RNA libraries from the sample panel set and sequencing those libraries on the NextSeq 550Dx. For run setup examples, refer to *Plate Layout* on page 4.

Operators must follow the instructions from *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*.

Data generated from the assessment run are analyzed with the TSO Comprehensive (JP) analysis software. At the completion of each assessment run, the operator prepares the associated data files for transfer using the instructions provided in *Data Transfer* on page 6.

The Illumina FAS (or certified designee) reviews the files to make sure that all required output files are transferred and determines if the run meets acceptance criteria. The Illumina FAS can ask operators to repeat assessment runs beginning from enrichment or pooling based on investigation of failures according to the Troubleshooting section of *TruSight Oncology Comprehensive (JP) Assay Workflow*

Guide (document # 200041566). An independent repeat of the assessment can be performed as needed. Operators are required to pass two consecutive assessment runs (unique library preparation and sequencing event) to receive a training certificate.

Data Transfer

Transfer data files within three business days of completing each run. Use the following instructions to transfer the requisite files.

Locate the Files

1. At the completion of a run, navigate to `D:\Illumina\NextSeqDx Operating Software Temp` and select the run folder.
2. Select the following folders and files, and then copy them to the desktop in a folder labeled <Run ID>:
   - `InterOp`
   - `CopyComplete.txt`
   - `RunCompletionStatus.xml`
   - `RunInfo.xml`
   - `RunParameters.xml`
   - `SampleSheet.csv`
   - `SampleSheet_YYYYMMDD_XXXXXX.csv`
3. Navigate to `D:\Illumina\NextSeqDx Operating Software Temp\RunID\Analysis_X\YYYYMMDD_XXXXXX\IVD\IVD_Reports`.
4. From the IVD_Reports folder, copy the `ControlOutput.tsv` and `MetricsOutput.tsv` files to the folder labeled <Run ID> on the desktop.
5. Navigate to `D:\Illumina\NextSeqDx Operating Software Temp\RunID\Analysis_X\YYYYMMDD_XXXXXX\IVD\Logs_Intermediates`.
6. From the Logs_Intermediates folder, copy the RnaFusionMerge and RnaSpliceVariantCalling folders to the folder labeled <Run ID> on the desktop.

Transferring Run Data to Kiteworks

After compressing the <Run ID> folder on the desktop, transfer the file to Illumina for review via the Illumina Kiteworks sFTP.

1. Navigate to the Illumina Kiteworks website and sign in with an email and password. Register for an account if you do not have one.
2. Select the folder provided by the Illumina FAS.
3. In the folder, select **Upload**, and then select **Upload files**.
4. Navigate to the compressed run folder on the computer being used for the data transfer. Select and upload the folder.

Acceptance Criteria

The Illumina FAS conducts assessments based on the acceptance criteria described in the following sections.

Sequencing Run Validity

Run validity for the assessment run is assessed against the sequencing validity metrics in Table 2. The TSO Comprehensive (JP) software automatically determines the results and reports them in the metrics output file (`MetricsOutput.tsv`). Invalid runs are investigated to determine cause and are repeated after retraining (if deemed necessary).

Table 2 Sequencing Validity Metrics

Run Validity Metric	Acceptance Criteria
PCT_Q30_R1	≥80%
PCT_Q30_R2	≥80%
PCT_PF_READS	≥80%

Control Validity

One DNA and one RNA library are designated as positive and negative controls and are assessed against the control validity metrics in Table 3. The TSO Comprehensive (JP) software determines the results and reports them in the controls output file (`ControlOutput.tsv`). Failed controls result in an investigation to determine root cause and invalidation of the library preparation event.

Table 3 Control Validity Metrics

Library type	Validity Metric	Acceptance Criteria
RNA Positive Control	SENSITIVITY	≥0.92
RNA NTC	GENE_ABOVE_MEDIAN_CUTOFF	≤1
DNA Positive Control	SENSITIVITY	≥0.95
DNA NTC	MEDIAN_EXON_COVERAGE	≤8

Library Validity

Libraries not designated as controls are compared against the library validity metrics in Table 4 by the FAS using the output from the TSO Comprehensive (JP) software in the `MetricsOutput.tsv` file. Invalid libraries are investigated to determine cause and can be repeated if necessary.

NOTE Metrics and Acceptance Criteria in Table 4 are specific to competency testing. Refer to the Local Run Manager TruSight Oncology Comprehensive (JP) Analysis Module Workflow Guide (document # 200049183) for assay specific quality control metrics.

Table 4 Sample Validity Metrics

Sample Type	Library Validity Metrics	Acceptance Criteria[1]
RNA Sample	TOTAL_ON_TARGET_READS	≥9 M
	MEDIAN_INSERT_SIZE	≥ 120 bp
	SENSITIVITY	≥ 0.92
DNA Sample	MEDIAN_INSERT_SIZE	84–145 bp[2]
	PCT_TARGET_100X	≥84.5

[1] The acceptance criteria for each assessment run allow one library validity drop out from six DNA libraries and one library validity drop out from six RNA libraries per sequencing event.
[2] The upper bound acceptance criteria for MEDIAN_INSERT_SIZE is waived for certain ultrasonicator models.

RNA Sensitivity

RNA variant calling results are assessed against expected variants.

Training Certificate

After successful completion of assessment runs, Illumina issues a laboratory training certificate to each passing operator.

Verification Testing Guidance

Purpose

The customer is responsible for performing an independent verification of TSO Comprehensive (JP) within the laboratory before introducing the test into routine use in accordance with applicable law, regulations, accreditations, standards, and requirements. For software version and claims information, to make sure that appropriate verification testing is conducted, refer to the *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*.

This section describes the process recommended by Illumina to verify the TSO Comprehensive (JP) test within the customer's laboratory. This suggested process includes recommendations for the number of runs and testers, sample selection, and data analysis to confirm, through the attainment of objective evidence, that the performance claims in the Assay Workflow Guide have been met.

Illumina's recommendation is not a substitute for the laboratory's autonomous determination of the appropriate process to use and independent performance of its verification of TSO Comprehensive (JP). The customer bears sole responsibility for these independent decisions.

Each customer is solely responsible for performing laboratory testing (including TSO Comprehensive (JP) testing) in accordance with the laws, regulations, accreditations, standards, rules, and requirements of every jurisdiction that apply to the customer.

Laboratories should verify the performance of TSO Comprehensive (JP) as established in *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*. Table 5 and Table 6 show the run and sample performance specifications from the Assay Workflow Guide.

Table 5 Run Performance Specifications

Type	Metric	Specification	Impact of Specification Failure
Run	Reads PF	≥ 80%	Sequencing run invalidated, no results reported
	Q30 (Read 1)	≥ 80%	
	Q30 (Read 2)	≥ 80%	

Table 6 Sample Performance Specifications

Type	Metric	Specification	Impact of Specification Failure
DNA Libraries	CONTAMINATION_ SCORE	≤ 3106 or > 3106 with p-value ≤ 0.049	No DNA results reported
	MEDIAN_ INSERT_SIZE	≥ 70 bp	No TMB and small variant results reported
	MEDIAN_EXON_ COVERAGE	≥ 150	
	PCT_EXON_50	≥ 90.0%	
	USABLE_MSI_SITES	≥ 40	No MSI results reported
	COVERAGE_MAD	≥ 0.000 and ≤ 0.210	No gene amplification (CNV) results reported
	MEDIAN_BIN_COUNT_ CNV_TARGET	≥ 1.0	
RNA Libraries	MEDIAN_GENE_CV_ 500X	≤ 0.93	No fusion or splice variant results reported
	TOTAL_ON_TARGET_ READS	≥ 9,000,000	
	MEDIAN_INSERT_SIZE	≥ 80 bp	
Positive Control	DNA Sensitivity	23 of 24 variants detected	Fail control
	RNA Sensitivity	12 of 13 variants detected	Fail control
No Template Control	DNA Median Exon Coverage	≤ 8	Fail no template control
	RNA Gene Above Median Cutoff	≤ 1	Fail no template control

The following information details the recommended verification process.

NOTE As noted earlier in this section, the customer is responsible for making the independent determination of the scope and makeup of the verification process its laboratory uses, including the decision of whether to use this process.

Scope

Recommended studies to confirm, through attainment of objective evidence, the accuracy performance claims in *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)* are within the scope of this guidance.

Study Design

Accuracy

Accuracy describes how close a test result is to the true value of a sample. Verifying accuracy makes sure that the test gives correct results in your laboratory. Accuracy can be verified by testing samples with known values and comparing the expected results with the obtained results.

To confirm accuracy for qualitative tests, make sure that the test correctly identifies the presence or absence for each of the variant classes claimed for Tumor Profiling. For information on Tumor Profiling claims, refer to *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*.

When designing an accuracy study for the Tumor Profiling claim, consider the following recommendations:

- For tumor profiling, test ≥ 3 unique samples for each of the following variant classes:
  - SNVs
  - MNVs
  - Insertions
  - Deletions
  - Gene amplifications
  - Gene fusions
  - Splice variants
  - TMB
  - MSI
- If more than one variant class is present in the sample, a single sample can satisfy multiple variant classes.
- Select samples that contain mutations of interest to the testing laboratory.
- Under this suggested study design, a mix of synthetic samples, samples derived from cell lines, and deidentified clinical samples can be used for accuracy.

- The customer is responsible for determining the appropriate selection of samples to permit the laboratory to verify the performance specifications.

Samples

To achieve verification, test a mix of commercially available samples listed in Table 7, Table 8, and samples selected at the discretion of the clinical laboratory director or designee. Using the specified samples minimizes the number of sequencing runs needed to demonstrate the ability of a lab to meet the manufacturer specifications for the TSO Comprehensive (JP) assay. The lab director must select additional samples to demonstrate performance adequately.

NOTE Under this or any other design, the customer is solely responsible for characterizing any samples used for TSO Comprehensive (JP) verification and for orthogonal testing. Illumina is not responsible for TSO Comprehensive (JP) performance of samples selected by the customer and cannot guarantee availability or version control of third-party samples.

For any sample used in verification testing, a variant is reported only if it is determined to have clinical utility in the version of the Knowledge Base being used by the TSO Comprehensive (JP) analysis module. Some samples tested during an accuracy study can have a variant that orthogonal testing has confirmed but is not in the final TSO Comprehensive (JP) report. The variants not included in the report do not have clinical utility as determined by the Knowledge Base.

Tips on Sample Selection

Samples with known values, such as external control materials, or previously tested proficiency testing (PT) samples are characterized by the manufacturer package insert (for controls) or PT summary report (for previously tested PT samples).

If using previously tested, deidentified patient samples, establish an orthogonal method to characterize the samples before performing the verification study. Take good laboratory practice and patient privacy into consideration.

Select samples that provide data for multiple variants or variant classes. Commercial samples, such as controls or standards, might be more efficient to use when verifying accuracy in this situation. They have the additional advantage of representing the normal and abnormal values that you would expect to recover when testing patients.

It can be difficult to find suitable samples with sufficient volume that would allow the laboratory to use the same samples throughout the study. The clinical laboratory director is responsible for determining the appropriate selection of samples to verify performance specifications and have confidence in test results while being cognizant of the resources expended.

Table 7 DNA Samples

Sample	Manufacturer Part #	Variant Classes Represented
TruSight Oncology DNA Control	20065041	SNV, insertions, deletions
SeraSeq Tri-level Tumor Mut DNA Mix v2	0710-0097	SNVs, insertions, deletions, amplifications
SeraSeq gDNA TMB Mix (TMB 7)	0710-1326	TMB
SeraSeq gDNA TMB Mix (TMB 26)	0710-1323	TMB
SeraSeq Breast CNV Mix (3 copies)	0710-0411	Amplifications
SeraSeq Breast CNV Mix (12 copies)	0710-0413	Amplifications
SeraSeq MSI Ref Mat (AF 5%)	0710-1675	MSI
SeraSeq MSI Ref Mat (AF 20%)	0710-1676	MSI

Table 8 RNA Samples

Sample	Manufacturer Part #	Fusion
TruSight Oncology RNA Control	20065042	Fusions, splice variants
SeraSeq FFPE NTRK Fusion Ref Mat	0710-1031	Fusions
SeraSeq RNA Fusion Mix v4	0710-0497	Fusions, splice variants
HorizonDx ALK/RET/ROS	HD784	Fusions

Testing

Experimental Setup

After an outline for the study design is achieved, it is beneficial to create a run map. The run map helps organize the details of each run within a study design. Include information such as run ID, operator, samples, date of testing, plate layout, and other relevant details. A customer should be able to look at the run map and understand all the testing that is necessary to complete verification.

A run map can be constructed using an Excel spreadsheet. Start by creating a tab that contains a table of runs. Table 9 shows an example table of runs.

Table 9 Map of Verification Runs

Run #	Study	Variant Classes Tested*	Tech	Instrument
1	Accuracy	SNVs, Insertions, Deletions, TMB, Amplifications, Fusions, Splice Variants	Any	1
2	Accuracy	SNVs, Insertions, Deletions, MSI, Amplifications, Fusions, Splice Variants	Any	1
3	Accuracy	SNVs, Insertions, Deletions, MNVs, Fusions, Splice Variants	Any	1

* Based on sample selection.

Next, create a tab for accuracy. List all the relevant details for the runs contained within the study. The following table shows an example of the run mapping tab for accuracy:

Run ID	ACCU-01	Operator	Tech #
Date	DD/MM/YYYY	Instrument ID	Instrument #
Experiment	Accuracy Run 1	Instrument Run	XXX

Sample ID	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
DNA	TruSight Oncology DNA Control (ACCU-01-D1)	SeraSeq Tri-Level (ACCU-01-D2)	SeraSeq TMB Mix_7 (ACCU-01-D3)	SeraSeq TMB Mix_26 (ACCU-01-D4)	Customer TMB Sample (ACCU-01-D5)	SeraSeq CNV Mix 3 (ACCU-01-D6)	SeraSeq CNV Mix 12 (ACCU-01-D7)	DNA No Template Control (ACCU-01-D8)
RNA	TruSight Oncology RNA Control (ACCU-01-R1)	SeraSeq Fusion RNA Mix v4 (ACCU-01-R2)	SeraSeq Fusion NTRK Mix (ACCU-01-R3)	Customer Sample Fusion (ACCU-01-R4)	Customer Sample Fusion (ACCU-01-R5)	Customer Sample Splice Variant (ACCU-01-R6)	Customer Sample Splice Variant (ACCU-01-R7)	RNA No Template Control (ACCU-01-R8)

Run ID	ACCU-02	Operator	Tech #
Date	DD/MM/YYYY	Instrument ID	Instrument #
Experiment	Accuracy Run 2	Instrument Run	XXX

Sample ID	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
DNA	TruSight Oncology DNA Control (ACCU-02-D1)	SeraSeq MSI AF5 (ACCU-02-D2)	SeraSeq MSI AF20 (ACCU-02-D3)	Customer Sample MSI (ACCU-02-D4)	Customer Sample CNV (ACCU-02-D5)	Customer Sample SNV (ACCU-02-D6)	Customer Sample Insertion (ACCU-02-D7)	DNA No Template Control (ACCU-02-D8)
RNA	TruSight Oncology RNA Control (ACCU-02-R1)	SeraSeq Fusion RNA Mix v4 (ACCU-02-R2)	SeraSeq Fusion NTRK Mix (ACCU-02-R3)	HorizonDx HD784 (ACCU-02-R4)	Customer Sample Fusion (ACCU-02-R5)	Customer Sample Fusion (ACCU-02-R6)	Customer Sample Splice Variant (ACCU-02-R7)	RNA No Template Control (ACCU-02-R8)

Run ID	ACCU-03	Operator	Tech #
Date	DD/MM/YYYY	Instrument ID	Instrument #
Experiment	Accuracy Run 3	Instrument Run	XXX

Sample ID	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
DNA	TruSight Oncology DNA Control (ACCU-03-D1)	SeraSeq MSI AF5 (ACCU-03-D2)	SeraSeq MSI AF20 (ACCU-03-D3)	Customer Sample Deletion (ACCU-03-D4)	Customer Sample MNV (ACCU-03-D5)	Customer Sample MNV (ACCU-03-D6)	Customer Sample MNV (ACCU-03-D7)	DNA No Template Control (ACCU-03-D8)
RNA	TruSight Oncology RNA Control (ACCU-03-R1)	SeraSeq Fusion RNA Mix v4 (ACCU-03-R2)	SeraSeq Fusion NTRK Mix (ACCU-03-R3)	HorizonDx HD784 (ACCU-03-R4)	Customer Sample Fusion (ACCU-03-R5)	Customer Sample Splice Variant (ACCU-03-R6)	Customer Sample Splice Variant (ACCU-03-R7)	RNA No Template Control (ACCU-03-R8)

In the run map example, some samples are named Customer Sample [variant class] for both DNA and RNA. Samples named in this manner must be provided by the customer to achieve the recommended number of samples per variant class.

Based on the number of samples listed by Illumina, customers must supplement with the following number of samples for each variant class: SNV (1), MNV (3), Insertions (1), Deletions (1), Amplifications (1), TMB (1), MSI (1), Fusions (1), and Splice Variants (2). Extra replicates for RNA samples are present due to the limited variant classes represented by RNA in the Tumor Profiling claim.

Procedure

While extraction is not part of the TSO Comprehensive (JP) library preparation, it is an important upstream event to consider carefully. It is recommended to establish a consistent and robust extraction methodology to generate input for the TSO Comprehensive (JP) assay.

Prepare libraries according to the *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*.

Data Analysis

Analysis of Results

Like the study design, the customer is responsible for determining the approach to data analysis. The final study design selected by the customer can influence the approach for data analysis. The following example is an approach to data analysis that might be considered.

A first level of data analysis can evaluate the validity of the data. Illumina recommends evaluating data for both run and sample (library) validity. Use the specifications in *TruSight Oncology Comprehensive (JP) Assay Workflow Guide (document # 200041566)*. Only data that meets these thresholds should be considered in downstream analysis. A customer can choose to repeat testing on replicates that are not considered valid on their first attempt. Invalid results should be summarized in the final study report. Samples that are designated as controls within the software should be evaluated as passing or failing based on the output.

After validity of the data is determined, customers can proceed to analysis of results for accuracy.

Reporting

Customers are responsible for reporting their results. It is recommended to use a report template approved by the quality system for their site.

Revision History

Document	Date	Description of Change
Document # 200051958 v02	June 2025	• Corrected typographical errors. • Clarified table references throughout.

Document # 200051958 v01	March 2025	• Clarified the Knowledge Base process that specifically applies to Japan.
		• Updated kit part number.
		• Removed the following:
		  • Lighthouse Portal registration instructions.
		  • Instructions for creating an MD5 report for data transfer.
		  • Consumables and equipment list entry from list of what is included in this guide.

Document # 200051958 v00	February 2024	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

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