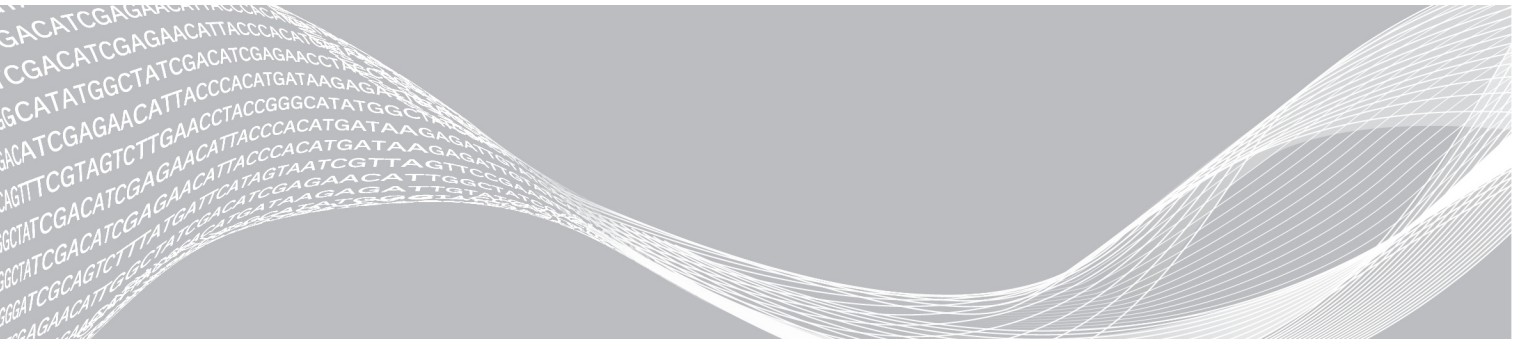
illumina®

TruSight Oncology UMI Reagents Workflow

Integration Guide



Document # 1000000038330 v00

ILLUMINA PROPRIETARY

April 2018

Introduction

The TruSight Oncology UMI Reagents (PN 20024586) contain the TruSight UMI Adapters v1 (PN 20018492) and the TruSight Oncology DNA library prep and enrichment assay components (PN 20006937, 20007002, 20007003, and 20010187). Combined with the UMI Error Correction software, these reagents enable researchers to distinguish low frequency somatic variants present in DNA from inherent sequencing noise. The TruSight Oncology UMI Reagents are sufficient to create 48 libraries labeled with unique molecular identifiers (UMIs) and include 16 Unique Index Primer (UP) pairs to enable multiplexing. You can process sequencing data using either a locally installed Docker containing the UMI Error Correction Local App or the UMI Error Correction BaseSpace Sequence Hub App to enable the collapsing of unique reads and the subsequent reduction in error rates to ≤ 0.007%.

TruSight Oncology UMI Reagents do not include the TruSight Tumor 170 Content Set (PN 20010188). The TruSight Tumor 170 Content Set must be ordered separately and only DNA content used. Error correction with RNA content has not been evaluated.

For Research Use Only (RUO). Not for use in diagnostic procedures.

Use of TruSight Oncology UMI Reagents with TruSight Tumor 170 DNA Content

Follow the TruSight Tumor 170 workflow (see the *TruSight Tumor 170 Reference Guide*, 1000000024091), with the following changes.

1 **Nucleic Acid Input:** Illumina recommends using 30 ng of cfDNA as measured using a nonfluorometric quantification method. Quantification of cfDNA between 75–250 bp in size using a Fragment Analyzer or similar quantification instrument is highly recommended. Shearing cfDNA before starting the assay is not recommended.

NOTE

Use of sheared DNA with the TruSight UMI Reagents and UMI Error Correction App is not recommended. However, if sheared gDNA is used, the input amount should be increased to 75 ng to most closely mimic cfDNA performance. See the *Options for Control Materials for NGS Analysis of Circulating Cell-Free DNA* tech note for example data with sheared gDNA inputs.

CAUTION

Insufficient DNA input amounts can result in reduced target coverage and QC flags.

2 **Assay Starting Point:** Omit all steps related to RNA. Begin assay workflow with cfDNA input in 50 µl volume at End-Repair and A-Tailing Step.

3 **Ligate Adapters step:** 10 µl of UMI Adapters v1 replaces 10 µl of SUA1 reagent in a final volume of 135 µl per sample well.

4 **Index PCR step**: Add 5 µl of UPXX (Unique Index Primer Mixes) to each sample well for a final well volume of 50 µl.

5 **First and Second Hybridization steps:** Add 2.5 µl of OPD1 and 2.5 µl of RSB to each sample well.

   a If you are preparing 4 samples or fewer, add 2.5 µl of RSB and 2.5 µl of OPD1.
   b If you are preparing more than 4 samples, you can make a master mix of equal volumes of OPD1 to RSB and add 5 µl of the master mix to your samples. Illumina recommends making 20% overage of the mix.

NOTE

If using a patterned flow cell for sequencing, manual library normalization should be performed (see *Normalize Libraries and Prepare for Sequencing* on page 4).

Use of TruSight Oncology UMI Reagents with Custom Enrichment Content

Follow the TruSight Tumor 170 workflow (see the *TruSight Tumor 170 Reference Guide*, 1000000024091), with the following changes.

1 **Nucleic Acid Input:** Illumina recommends using 30 ng of cfDNA as measured using a nonfluorometric quantification method. Quantification of cfDNA between 75–250 bp in size using a Fragment Analyzer or similar quantification instrument is highly recommended. Shearing cfDNA before starting the assay is not recommended.

NOTE

Use of sheared DNA with the TruSight UMI Reagents and UMI Error Correction App is not recommended. However, if sheared gDNA is used, the input amount should be increased to 75 ng to most closely mimic cfDNA performance. See the *Options for Control Materials for NGS Analysis of Circulating Cell-Free DNA* tech note for example data with sheared gDNA inputs.

CAUTION

Insufficient DNA input amounts can result in reduced target coverage and QC flags.

2 **Assay Starting Point:** Omit all steps related to RNA. Begin assay workflow with cfDNA input in 50 µl volume at End-Repair and A-Tailing Step.

3 **Ligate Adapters step:** 10 µl of UMI Adapters v1 replaces 10 µl of SUA1 reagent in a final volume of 135 µl per sample well.

4 **Index PCR step**: Add 5 µl of UPXX (Unique Index Primer Mixes) to each sample well for a final well volume of 50 µl.

5 **First and Second Hybridization steps:** Add 5 µl of custom enrichment content panel. The working concentration of the content panel should be determined empirically.

NOTE

If using a patterned flow cell for sequencing, manual library normalization should be performed (see *Normalize Libraries and Prepare for Sequencing* on page 4).

Normalize Libraries and Prepare for Sequencing

The TruSight Oncology UMI Reagents are compatible with sequencing on the HiSeq 2500/4000. Compatibility with the NovaSeq 6000 was demonstrated but not extensively tested.

NOTE

For use on the HiSeq 2500, follow the bead-based normalization (BBN) steps and dilution scheme outlined in the *TruSight Tumor 170 Reference Guide* (1000000024091). Omit the RNA library pooling steps and make sure that you have a minimum of 25 µl of pooled DNA library (PDL).

NOTE

For patterned flow cells, replace bead-based normalization (BBN) steps with manual library normalization.

For use on the HiSeq 4000 using TruSight Tumor 170, follow these steps for manual library normalization and flow cell clustering.

1 Normalize libraries to 2 nM using RSB.

2 Prepare clustered flow cell with the pooled, normalized libraries according to the procedure outlined in the *cBot System Guide* (15006165). Final recommended loading concentration for the HiSeq 4000 is 200–300 pM.

3 Load and start the sequencer according to the appropriate HiSeq sequencing protocols. Refer to the *HiSeq 4000 System Guide* (15066496).

For use on the NovaSeq 6000, refer to the normalization, pooling, and denaturation recommendations in the *NovaSeq 6000 Sequencing System Guide* (1000000019358).

Post-sequencing error correction can be performed using the UMI Error Correction Local App (installed via a Docker image) or the UMI Error Correction BaseSpace Sequence Hub App. For more information, refer to the *UMI Error Correction Local App User Guide* (1000000037270) or the *UMI Error Correction BaseSpace Sequence Hub App Online Help* (1000000035906).

Prepare Sample Sheet

To start secondary analysis, you must create a sample sheet prior to initiating a sequencing run.

- Two sample sheet templates are provided on the Illumina Support website, as follows.
  - For sequencers that use standard i5 primer notation
  - For sequencers using reverse complemented i5 primer notation

  For more information, see the *Indexed Sequencing Overview Guide* (15057455).
- The sample sheet templates contain the following parameters that are required for processing by the UMI Error Correction Software.
  - Read1UMILength 7
  - Read2UMILength 7
  - Read1StartFromCycle 9
  - Read2StartFromCycle 9
- If you are using a sample sheet within the run folder, the sample sheet must be named SampleSheet.csv.
- The Reads section must be defined in the sample sheet.
  - Maximum number of samples per analysis run is 48 for the UMI Error Correction BaseSpace Sequence Hub App and 96 for the UMI Error Correction Local App.
  - Data section—required columns and column headers are as follows.
    - Sample_ID
    - Sample_Name
    - Index
    - Index2

NOTE

The HiSeq 4000 uses reverse complements of the second index (i5).

- The Sample ID is used in output file names instead of the sample name. For an easier transition between the UMI Error Correction BaseSpace Sequence Hub App and the UMI Error Correction Local App (that does not require changing the file name), do not use underscores in the Sample ID and leave the Sample Name blank.

- Sample_ID naming conventions are as follows.
  - Alphanumeric characters, length fewer than 100 characters
  - Cannot contain nonalphanumeric special characters (* . " / \ [ ] : ; | = ,) or spaces
  - Prefix Sample_IDs with a keyword
  - Unique combinations of Sample_ID and Lane Number (required)

CAUTION

If the files will be transferred to BaseSpace Sequence Hub, do not use underscores (_) in the Sample_ID.

- When uploading data to BaseSpace Sequence Hub, do not use the 1+2+3+4 naming convention to specify multiple lane usages. Instead specify sample and lanes individually or leave the lane column blank to indicate all lanes.
- Any of the following conditions may cause an error message.
  - Missing sample name column header
  - Missing IEMFIleVersion
  - Missing Header section header
  - Missing Settings section header
  - Missing Reads section header

Tips and Techniques

Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves, protective eye wear, and laboratory coats when handling specimens and assay reagents. Wash hands thoroughly after handling specimens and assay reagents.

Use caution when handling UPs and UMIs to avoid contamination of stock vials. To prevent contamination, it is important to establish lab procedures and follow best practices. Clean lab areas daily and weekly using 0.5% sodium hypochlorite (10% bleach).

CAUTION

To prevent sample or reagent degradation, make sure that all vapors from the cleaning solution have fully dissipated before beginning any processes.

The PCR process is commonly used in the laboratory to amplify specific DNA sequences. Unless proper laboratory hygiene is used, PCR products can contaminate reagents, instrumentation, and DNA samples, causing inaccurate and unreliable results. PCR product contamination can shut down lab processes and significantly delay normal operations. Make sure that the lab is set up appropriately to reduce the risk of PCR product contamination.

- Physically separate pre- and post-PCR areas
- Use dedicated equipment and supplies

To avoid index misassignment on multiplexed samples and downstream analysis (also known as index hopping), follow best practices to minimize index hopping, as described in the white paper *Effects of Index Misassignment on Multiplexing and Downstream Analysis*. Best practices include the following.

- Remove free adapters from library preps
- Store libraries individually at -20°C
- Pool libraries to be sequenced immediately prior to clustering/sequencing

Consumables

The TruSight Oncology UMI Reagents (PN 20024586) are configured for 48 samples, and consist of the following.

Product number	Component	Number of Boxes	Storage
20018492	UMI Adapter Box containing UMI Adapters v1	1	-25°C to -15°C
20006937	TruSight UP Index Box containing (UP01-UP16)	1	-25°C to -15°C
20007002	TruSight Oncology DNA Library Prep (Pre-PCR, -20°C)	1	-25°C to -15°C
20007003	TruSight Oncology DNA Library Prep (Pre-PCR, 4°C)	1	2°C to 8°C
20010187	TruSight Oncology Enrichment	2	-25°C to -15°C 2°C to 8°C
20010188	[Optional] TruSight Tumor 170 Content Set	1	-25°C to -15°C

Referenced Documents

Document Name
TruSight Tumor 170 Reference Guide (1000000024091)
White Paper: *Effects of Index Misassignment on Multiplexing and Downstream Analysis* (770-2017-004)
White Paper: *Detection of Low-Frequency Variants in Cell-Free DNA Using TruSight™ Tumor 170 With the TruSight Oncology UMI Reagents* (1000000050427)
Tech Note: *Options for Control Materials for NGS Analysis of Circulating Cell-Free DNA*
UMI Error Correction Local App User Guide (1000000037270)
UMI Error Correction BaseSpace Sequence Hub App Online Help (1000000035906)
cBot System Guide (15006165)
HiSeq 4000 System Guide (15066496)
HiSeq 2500 System Guide (15035786)
NovaSeq 6000 Sequencing System Guide (1000000019358)

Revision History

Document	Date	Description of Change
Document # 1000000038330 v00	April 2018	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

Document # 1000000038330 v00

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

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

illumina®