INSTRUCTIONS FOR USE

32 SAMPLES

SOPHiA DDM™ Dx RNAtarget Oncology Solution

For In Vitro Diagnostic (IVD) Use
Not for self-testing

SOPHiA GENETICS™

SUMMARY INFORMATION

Product Name	SOPHiA DDM™ Dx RNAtarget Oncology Solution
Product Type	Bundle Solution
Product Family	Molecular kit + analytics
Algorithm ID	ILL1XGR1S2_FFPE_NextSeq
Sequencer	Illumina® - NextSeq® 550
Gene Panel ID	ROS_v1
Product Version	v1.0
Sample Type	RNA isolated from formalin-fixed, paraffin embedded (FFPE) tissue specimens
Release Version	1.0.0
Document ID	SG-00662
Document Version	v8.0
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This Instructions For Use (IFU) is applicable for all SOPHiA DDM™ versions.
Please read the IFU thoroughly before using this product.

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REF BS0119ILLCSRY07-32

PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	LIBRARY PREPARATION KIT
REF	BS0119ILLCSRY07-32	B1.L1.0019.C-32	B2.0019.C-48	6C0233

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REVISION HISTORY

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
SG-00662 (v8.0)	January 2026	• Change to version numbering system, no additional versions between version 4.4 and version 8.0. • Section 2: Removal of description of CDS components. • Section 3: Removal of description of CDS components. • Figure 1: Revision of platform name and IVD Scope. • Warnings and Precautions: Addition of CAS identification number, and concentration for each identified hazardous component. • Section 9.0 – removal of warnings and limitations and precautions which are out of the scope of the IVD product. • Section 9.3 merged with Section 9.3.1, as General Limitations. • Removal of Annex referring to CDS Analysis Description and Parameters.
SG-00662 (v4.4)	March 2025	• Section *5.1.1. Kit Content – BOX 1:* : Increased content volume of 2x Hybridization Buffer from 50 µl to 75 µl; increased content volume of Hybridization Buffer Enhancer from 20 µl to 30 µl
SG-00662 (v4.3)	August 2024	• "SOPHiA DDM™ Web App" changed to "SOPHiA DDM™ Dx mode" • Minor rephrasings related to the change above
SG-00662 (v4.2)	July 2024	• Updated the EC REP address • Removed third-party provider's intellectual property from sections *5.1.1 Kit Content*, *5.4.1 Library Pooling for Hybridization and Capture*, and *5.4.2 Hybridization* • Reduced content volume of SOPHiA GENETICS hybridization probes from 20 µl to 18 µl (see section *5.1.1 Kit Content – BOX 1*)
SG-00662 (v4.1)	March 2023	• Sections 2, 3, 4, 5.1.1, 5.2.6, 5.2.8, 5.2.9, 5.2.10, 6.1, 6.3, 7.1, 9.1 and 12 - harmonization of text. • SOPHiA GENETICS™ changed to SOPHiA GENETICS
SG-00662 (v4.0)	December 2022	• Section 5.2.2 - Volume unit corrected in Table RT Pre-Mix.
SG-00662 (v3.0)	September 2022	• Inserted TM symbol throughout the document wherever applicable. • Change of Library Prep Kit name to SOPHiA GENETICS™ RNA Library Prep Kit.
SG-00662 (v2.0)	May 2022	• Change of Library Prep Kit name • Change of wording in Warnings (Section 9)
SG-00610 (v1.0)	May 2022	• Initial Release

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1. INTENDED PURPOSE

SOPHiA DDM™ Dx RNAtarget Oncology Solution (ROS, “the Product”) is intended to be used to identify clinically relevant gene fusions involving the genes *ALK*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *NRG1*, *NTRK1*, *NTRK2*, *NTRK3*, *RET* and *ROS1*, and exon-skipping events in *EGFR* and *MET* in RNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples of patients with diagnosed lung cancer by targeting specific transcripts. The function of the product is to provide an aid to healthcare professionals to make clinical decision(s) related to gene fusions / exon skipping events and to provide molecular rationale for the appropriate disease management strategy. The product is a semi-automated, qualitative device intended to be used as an in-vitro diagnostic for professional use only.

2. GENERAL STATEMENT OF THE TEST PRINCIPLES AND PROCEDURE

Fusion genes are important clinical biomarkers in oncology that guide diagnosis, inform prognosis, and support treatment decisions[1]. For example, up to 17% of solid tumors are characterized by gene fusions. Gene fusions are among the most successful targets of precision cancer medicine with many therapeutics targeting fusion proteins in routine clinical use. The most comprehensive way to detect fusions is using RNA-based Next Generation Sequencing (NGS) assays, which can detect novel and known fusion events.

The SOPHiA DDM™ Dx RNAtarget Oncology Solution (ROS) is an NGS-based test developed by SOPHiA GENETICS intended for the qualitative detection, annotation, and pre-classification of gene fusions or exon skipping events and incorporates a multi-gene panel appropriate for different solid tumor types. The product is intended for processing and analyzing RNA samples extracted from patient FFPE biopsy or surgical resection Lung Cancer tissue specimens. The NGS kit and protocol is designed for users to process 10 ng – 200 ng (recommended 50 ng) of RNA for NGS library construction, hybridization-based capture enrichment of 13 genes (as defined in the Intended Purpose), and sequencing using the Illumina® NextSeq® 550 platform.

The secure cloud-based SOPHiA DDM™ Dx mode, hosting a customized bioinformatics pipeline, allows the users to upload the NGS data and obtain a SOPHiA DDM™ Dx ROS Solution Report as a PDF. The report describes the detected fusion and exon skipping events in a sample, the sample quality score and status. Only the fusions events involved in the 11 target genes and the exon skipping events (MET ex14, EGFRvIII) in the 2 target genes defined in the Intended Purpose will be reported in the IVD Report, with the list of transcripts shown in Table 1.

Table 1. List of transcripts involved in clinically relevant fusions on IVD Report

Gene	RefSeq ID	Gene	RefSeq ID
ALK	NM_004304	NRG1	NM_013964
EGFR	NM_005228	NTRK1	NM_002529
FGFR1	NM_023110	NTRK2	NM_006180
FGFR2	NM_000141	NTRK3	NM_001012338
FGFR3	NM_000142	RET	NM_020975
FGFR4	NM_213647	ROS1	NM_002944
MET	NM_000245		

This table is based on COSMIC[2] or ChimerDB databases[3], for which annotation is provided in the report. For any fusion involving a transcript outside of this list only genomic coordinates will be provided.

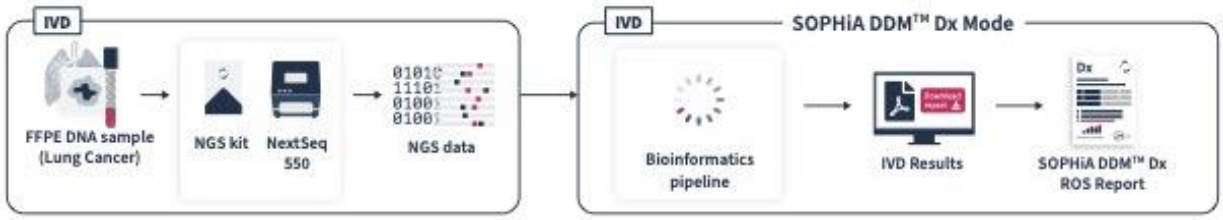
Note that the results of a genetic analysis should only be interpreted by a qualified expert in molecular genetics such as European registered Clinical Laboratory Geneticist (ErCLG) certified by the European Board of Medical Genetics (EBMG).

3. PRODUCT COMPONENTS

SOPHiA DDM™ Dx ROS is a complete solution that bundles the target enrichment kit with the analytical power of AI and is used in combination with an IVD accessory, the cloud-based SOPHiA DDM™ Dx mode. The product consists of two components: The library preparation and capture kit (SOPHiA GENETICS™ Dx RNA Library Prep Kit IV – 32 reactions) and the analytical bioinformatics pipeline used in combination with SOPHiA DDM™ Dx mode.

- The kit is composed of reagents and protocols to support the preparation and enrichment of targeted, Illumina®-compatible libraries from RNA extracted from Lung Cancer FFPE samples, suitable for enrichment and sequencing on an Illumina® NextSeq® 550 sequencer.
- The main purpose of the bioinformatics pipeline is to perform read filtering, alignment and fusion and exon skipping detection (secondary analysis) and annotation (tertiary analysis), to establish SOPHiA DDM™ Dx ROS fusion and exon skipping detection.
- SOPHiA DDM™ Dx mode hosts the bioinformatics pipeline and serves as the graphical user interface for the upload of Next-Generation Sequencing (NGS) sequencing data and the generation and download of the SOPHiA DDM™ Dx ROS Report. Refer to the SOPHiA DDM™ Dx mode User Manual for operational instructions.

Figure 1. Product Components Overview



4. SOPHIA DDM™ DX MODE

SOPHiA DDM™ Dx mode is a web application that provides a software solution to clinicians and researchers to aid in making informed decisions and diagnosis in oncology and inherited disease, including difficult to diagnose conditions like rare diseases. It does this through the analysis of Next-Generation Sequencing data produced from whole genome libraries, DNA or RNA capture-kit libraries, and amplicon kits for germline and somatic applications. SOPHiA DDM™ Dx mode is intended for use by trained lab professionals, clinical geneticists, and molecular pathologists.

To access the platform for the first time, the user will need to sign-up. The user will be requested to:

- Select the "*Sign-up now*" option.
- Enter your email address. You must use the same email address that was used to create your account upon account setup. In case of any doubt please contact the administrator of your account.
- Select "*Send verification code*".
- The verification code will be sent to your email. Enter the code you receive by email and complete the process by entering your name and creating a new password.

The detailed instructions for accessing the platform and performing a genomic analysis request is available in the SOPHiA DDM™ Dx mode User Manual.

5. KIT MATERIALS AND METHODS

5.1. Initial Considerations

Please ensure that all tubes are physically intact and stored at recommended temperatures, upon receipt, for optimum performance of the kit. Inappropriate handling and storage of the kit components at other conditions may adversely affect the performance of the kit.

5.1.1. Kit Content (32 Samples)

Always briefly spin the tubes before use to collect all liquid.

BOX 1 (STORE AT -25°C TO -15°C)

- Universal Blockers - TS Mix (12 µl)
- Human Cot DNA (25 µl)
- ROS_v1 probes by SOPHiA GENETICS (18 µl)
- 2x Hybridization Buffer (75 µl)
- Hybridization Buffer Enhancer (30 µl)
- 2x Bead Wash Buffer (1250 µl)
- 10x Stringent Wash Buffer (200 µl)
- 10x Wash Buffer I (160 µl)
- 10x Wash Buffer II (110 µl)
- 10x Wash Buffer III (110 µl)
- 32 Illumina®-compatible Unique Dual Index Primers V2 in a 96-well plate format (7 µl each): see Appendix II for primers display and sequences.
- Post Capture Illumina® Primers Mix (20 µl)
- PCR Enhancer (20 µl)
- Post Capture PCR Master Mix 2x (122 µl)

BOX 2 (STORE AT +2°C TO +8°C)

- Dynabeads® M-270 Streptavidin (440 µl)
- Agencourt® AMPure® XP (11.6 ml)
- IDTE Low TE Buffer (10 ml)
- Nuclease-free water (20 ml)

SOPHiA GENETICS™ Dx RNA Library Prep Kit IV* (STORE AT -25°C TO -15°C)

- RT Primers (85 µl)
- RT Reaction Buffer (170 µl)
- RT Enzyme Mix (86 µl)
- Second Strand Reaction Buffer (340 µl)
- Second Strand Enzyme Mix (170 µl)n
- End Prep Reaction Buffer (296 µl)
- End Prep Enzyme Mix (128 µl)
- Ligation Master Mix (1268 µl)
- Ligation Enhancer (44 µl)
- Stubby Universal Adapter (220 µl)
- PCR Master Mix (2 x 520 µl)
- Nuclease-free water (2 x 1.8 ml)

* For 32 samples, one 32-sample kit is provided.

* SOPHiA GENETICS is the exclusive distributor of this Library Prep Kit.

IMPORTANT: Refer to Warnings and Precautions below for additional details

WARNINGS AND PRECAUTIONS

Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
2X Hybridization Buffer		• H300 Fatal if swallowed. • H311 Toxic in contact with skin. • H315 Causes skin irritation. • H370 Causes damage to organs. • H370 Causes damage to organs (Central nervous system). • H411 Toxic to aquatic life with long lasting effects. • P260 Do not breathe vapor/ spray. • P264 Wash contaminated skin thoroughly after handling. • P270 Do not eat, drink or smoke when using this product. • P273 Avoid release to the environment. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P301+P310 If swallowed: Immediately call a poison center/ doctor. • P302+P352 If on skin: Wash with plenty of water. • P308+P311 If exposed or concerned: Call a poison center or doctor. • P321 Specific treatment (see medical advice on this label). • P330 Rinse mouth. • P332+P313 If skin irritation occurs: Get medical advice/ attention. • P362+P364 Take off contaminated clothing and wash it before reuse. • P391 Collect spillage. • P405 Store locked up. • P501 Dispose of contents/ container in accordance with national regulations.	Danger	Tetramethyl- ammonium chloride Concentration: 49% CAS: 75-57-0
Hybridization Buffer Enhancer		• H351 Suspected of causing cancer. • H360 May damage fertility or the unborn child.	Danger	Formamide Concentration: 100% CAS: 75-12-7

Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
		• H373 May cause damage to organs through prolonged or repeated exposure. • P201 Obtain special instructions before use. • P202 Do not handle until all safety precautions have been read and understood. • P260 Do not breathe vapour/ spray. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P308+P313 IF exposed or concerned: Get medical advice/ attention. • P314 Get medical advice/ attention if you feel unwell. • P405 Store locked up. • P501 Dispose of contents/ container in accordance with national regulations.		
10x Stringent Wash Buffer		• H302 Harmful if swallowed. • H315 Causes skin irritation. • H319 Causes serious eye irritation	Danger	Ethylenediaminetetraacetic acid disodium salt Concentration: 2.5% CAS: 6381-92-6
10x Wash Buffer I		• H228 Flammable solid. • H302 Harmful if swallowed. • H315 Causes skin irritation. • H318 Causes serious eye damage. • H332 Harmful if inhaled. • H401 Toxic to aquatic life. • H402 Harmful to aquatic life. • H412 Harmful to aquatic life with long lasting effects. • P273 Avoid release to the environment. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. • P310 Immediately call a poison center/ doctor.	Danger	Sodium dodecyl sulfate Concentration: 4.9% CAS: 151-21-3

Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
		• P501 Dispose of contents/ container in accordance with national regulations.		

Please use  and  as personal protective equipment.

5.1.2. Material Required (Not Provided)

USER-SUPPLIED MATERIALS (TO BE PURCHASED SEPARATELY)

- RNase/DNase-free 0.2 ml 8-tube strips
- DNA low binding 1.5 ml tubes
- 1.5 ml tubes
- 50 ml conical tubes
- Filter tips
- Ethanol (molecular biology grade)
- Illumina® sequencing reagents

  RNA isolated from Seraseq® FFPE Tumor Fusion RNA v4 Reference Material (Seracare, product no. 0710-0496) can be used as a positive control. Processing of 50 ng of RNA with SOPHiA DDM™ Dx ROS should allow for detection of all targeted rearrangements present in this material.

LABORATORY EQUIPMENT

To avoid sample contamination:

- Pre-PCR zone
  - Fluorometric quantitation equipment and reagents
  - Magnetic separation rack (96-well type)
  - Multichannel pipettes (P10 or P20; P100; P200)
  - Tabletop microcentrifuge (8-tube strips compatible)
  - Thermal cycler (programmable heated lid)
  - Vortex mixer

- Post-PCR zone
    - Capillary electrophoresis system
    - DNA vacuum concentrator
    - Fluorometric quantitation equipment and reagent
    - Magnetic separation rack (1.5 ml tube compatible)
    - Magnetic separation rack (96-well type)
    - Multichannel pipettes (P10 or P20; P100; P200)
    - Tabletop microcentrifuge (8-tube strips compatible)
    - Thermal cycler (programmable heated lid)
    - Thermoblock or water bath (1.5 ml tube compatible)
    - *Vortex* mixer

5.2. Library Preparation

5.2.1. Total RNA Preparation

MATERIALS

- Formalin-fixed paraffin-embedded tissue extracted RNA (FFPE RNA) (*See Note below)
- IDTE
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

Note: Input FFPE tissue samples should undergo standard histological deparaffinization followed by RNA purification. The chosen RNA purification method should ensure efficient preservation of RNA and removal of other components, such as hemoglobin, EDTA, salts, detergents, proteinases, organic solvents, and other compounds that have a potentially negative impact on the subsequent enzymatic reactions. We recommend storing purified RNA in buffered solution composed of 10 mM Tris pH 7.5 or 8.0, and 0.1 mM EDTA.

IMPORTANT:The SOPHiA DDM™ Dx ROS is intended for samples with a tumor content of at least 20 percent. Processing RNA from samples with a tumor content below 20 percent is not supported by the solution.

RNA Handling

Ribonucleases (RNases) are highly prevalent and stable enzymes that can rapidly degrade RNA molecules. Hence, when working with RNA, certain precautions must be taken to avoid RNase contamination:

- Decontaminate your workplace, use gloves, pipettes, and nuclease cleaning products before working with RNA.
- Keep purified RNA on ice while working, and store it at -80°C.
- Wear a lab coat and change gloves frequently.
- Use disposable nuclease-free plasticware, aerosol-barrier pipette-tips, and nuclease-free reagents.

RNA Integrity

The quality of FFPE extracted RNA is variable and might impact sequencing data. Exposure to formalin damages integrity of the molecules by generating nucleic acid fragmentation and chemical modifications. It also induces sequencing artifacts due to deamination events.

RNA integrity of FFPE RNA samples must be assessed prior to the experiment using high resolution capillary electrophoresis (e.g., Agilent Fragment Analyzer™ systems, Agilent Bioanalyzer™, Agilent TapeStation™).

Important: Processing a sample without measuring DV200 (percentage of RNA fragments with a size of at least 200 bp) or a DV200 lower than 20% might lead to suboptimal library preparation yield and insufficient NGS data quality possibly resulting in inconclusive results (see Warnings and Limitations section).

RNA Quantification

To accurately pipette the correct amount of input RNA solution, we recommend performing an initial dilution to obtain a concentration in the range of 10 to 50 ng/µl. The RNA concentration should be confirmed by a fluorometric quantitation (e.g., Qubit™, Thermo Fisher) and the obtained value used to calculate the final dilution.

Input amount requirements and PCR cycles

The workflow has been optimized for FFPE RNA samples. The recommended input amount is 50 ng of total RNA. Library preparation is possible with input amounts ranging from 10 ng to 200 ng; however, the performance characteristics of quantities other than 50 ng have not been systematically established.

Depending on the DV200 metric for each sample, adjust the number of PCR cycles for library amplification (Section 5.2.9) according to the following table:

Table 2. Number of PCR cycles required as a function of the quality of starting material.

FFPE DV200 < 50%	FFPE DV200 ≥ 50%
12	10

NOTE:

- Using RNA amount lower than 50 ng might require performing additional PCR cycle(s) during library amplification to ensure sufficient yield of library.
- We do not recommend using samples with DV200 < ~20% as this may result in decreased assay performance.

PROCEDURE

1. Thaw the RNA samples on ice.
2. Prepare the following PCR strips according to the number of reactions:

   When arranging the samples, it is recommended to place the samples undergoing cDNA synthesis, which will later be amplified with the same number of PCR cycles, in adjacent tubes. This will facilitate the separation of tubes for the PCR step.

NUMBER OF REACTIONS	8	16	24	32
PCR strip	4-tube	8-tube	8-tube	8-tube
Number of strips	2	2	3	4

3. Prepare a dilution for each FFPE extracted RNA (FFPE RNA) sample into the appropriate number of PCR strips, in the following manner:

FFPE RNA DILUTION	
1-12 µl FFPE RNA	FFPE RNA (cf. Table 2 in the previous page Section 5.2)
IDTE	Complete to 12 µl

- Mix briefly by gently pipetting up and down 5 times followed by a brief spin in a microcentrifuge to collect all liquid. Keep the samples on ice.

  Tip: Safe stopping point overnight or longer at -80°C.

5.2.2. Pre-mixes and Reagents Preparation

COMPONENTS AND REAGENTS

- RT Reaction Buffer
- RT Enzyme Mix
- Second Strand Reaction Buffer
- Second Strand Enzyme Mix
- End Prep Reaction Buffer
- End Prep Enzyme Mix
- Ligation Enhancer
- Ligation Master Mix
- PCR Master Mix 2x
- Nuclease-free water
- AMPure® XP beads equilibrated at room temperature

PREPARATION

- Remove the SOPHiA GENETICS™ Dx RNA Library Prep Kit IV components from -20°C storage and thaw on ice.
- Remove the Unique Dual Index Primer Plate from -20°C storage and put it into 4°C refrigerator for later use.
- Remove the AMPure® XP beads from 2-8°C storage and let them equilibrate at room temperature for at least 30 minutes. Vortex the AMPure® XP beads thoroughly, to ensure proper resuspension of the beads, prior to use in all subsequent steps.
- Prepare fresh 80% Ethanol (volume according to the following scheme based on the number of reactions):
-

80% ETHANOL				
Number of Reactions	8	16	24	32
80% Ethanol (in ml)	20	30	40	50

- Ensure the End Prep Reaction Buffer in the SOPHiA GENETICS™ Dx RNA Library Prep Kit IV is completely thawed. If a precipitate is seen in the buffer, pipette up and down several times to break it up and vortex to mix until full resuspension. Place on ice until use.
- Mix well and spin all reagents prior to use and place on ice.

PRE-MIXES

1. Prepare the RT pre-mix as follows:
2.

RT PRE-MIX				
Number of Reactions	8	16	24	32
RT Reaction buffer (in µl)	38.4	76.8	115.2	153.6
RT Enzyme Mix (in µl)	19.2	38.4	57.6	76.8

- Mix thoroughly by pipetting up and down 10 times and briefly spin down. Keep on ice.

2. Prepare the Second Strand Synthesis pre-mix as follows:

SECOND STRAND SYNTHESIS PRE-MIX				
Number of Reactions	8	16	24	32
Second Strand Reaction Buffer (in µl)	76.8	153.6	230.4	307.2
Second Strand Enzyme Mix (in µl)	38.4	76.8	115.2	153.6
Nuclease-free water (in µl)	460.8	921.6	1382.4	1843.2

- Mix thoroughly by pipetting up and down 10 times and briefly spin down. Keep on ice.

3. Prepare the **End Prep pre-mix** as follows:

END PREP PRE-MIX				
Number of Reactions	**8**	**16**	**24**	**32**
End Prep Buffer (in µl)	67.2	134.4	201.6	268.8
End Prep Enzyme Mix (in µl)	28.8	57.6	86.4	115.2

- Mix thoroughly by pipetting up and down 10 times and briefly spin down. Keep on ice.

4. Prepare the **Ligation pre-mix** as follows:

LIGATION PRE-MIX				
Number of Reactions	**8**	**16**	**24**	**32**
Ligation Master Mix (in µl)	290	580	870	1160
Ligation Enhancer (in µl)	9.7	19.3	28.95	38.6

- Mix thoroughly by pipetting up and down 10 times and briefly spin down. Keep on ice.

Important: The Ligation Mix and the Second Strand Enzyme Pre-mix are highly viscous, pipette gently and make sure to get a homogeneous ligation pre-mix.

5.2.3. Reverse Transcription (RT) - FFPE RNA

MATERIALS

- Diluted FFPE RNA samples (12 µl)
- RT primers
- RT pre-mix
- RNAse/DNAse-free 0.2 ml 8-tube strips

PREPARATION

1. Prepare the thermal cycler for RT with the following settings:

	TEMPERATURE (°C)	TIME (MINUTES)
Lid	99	
Step 1: Primer Annealing	65	5
Step 2: Hold	4	∞
Step 3: Primer Extension	25	10
Step 4: Reverse Transcription	42	15
Step 5: Enzyme Deactivation	70	15
Step 6: Hold	4	∞

Note: If your PCR machine does not allow you to put your program on hold and then continue it for the next step, you can use two separate programs for Steps 1-2 and Steps 3-6.

2. Start the RT program. When the block reaches Step 1, pause the program.

PROCEDURE

Important: Always keep the samples and pre-mix on ice before and after the incubation to prevent RNA degradation and to block the enzymatic reactions.

1. To facilitate pipetting create a reservoir of RT primers into a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	4-tube	8-tube	8-tube	8-tube
RT Primers (in µl)	4.5	4.5	7.0	9.0

2. To facilitate pipetting create a reservoir of RT pre-mix into a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	4-tube	8-tube	8-tube	8-tube
RT pre-mix (in µl)	13	13	19.5	26.5

3. Assemble the reaction as follows:
    - Using a multichannel pipette, add 2 µl of RT primers to the 12 µl of FFPE RNA samples.
    - Mix by pipetting up and down 5 times and briefly spin in a microcentrifuge.
4. Place the tubes in the thermal cycler preheated at 65°C with the RT program. Leave the tubes in the thermal cycler during steps 1 and 2 (incubation at 65°C and cooling to 4°C). Once the samples have reached 4°C remove the tubes from the thermocycler, spin them briefly, and continue to assemble the reaction (on ice) as follows:
    - Using a multichannel pipette, add 6 µl of RT pre-mix to your 14 µl of RNA samples and RT primers (20 µl in 4 or 8-tube strips).
    - Mix by pipetting up and down 5 times and briefly spin in a microcentrifuge.

    Important: The tubes should be kept on ice before and after the incubation at 65°C to avoid degradation of RNA molecules.
5. Place the reaction in the thermal cycler and continue the RT program (steps 3-6).

Proceed immediately to Second Strand cDNA synthesis.

5.2.4. Second Strand cDNA synthesis

MATERIALS

- RT reaction product in 20 µl
- Second Strand pre-mix

PREPARATION

- Set up the thermal cycler at 16°C (open lid).

PROCEDURE

Important: Always keep the samples and pre-mix on ice before and after the incubation to prevent RNA degradation and to block the enzymatic reactions.

1. To facilitate pipetting, create a reservoir of Second Strand pre-mix by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	4-tube	8-tube	8-tube	8-tube
Second Strand pre-mix (in µl)	135	135	203	270

2. Assemble the reaction as follows:
    - Using a multichannel pipette, add 60 µl of Second Strand pre-mix to the 20 µl of RT product.
    - Mix by pipetting up and down 5 times and briefly spin in a microcentrifuge.
3. Incubate in the thermal cycler at 16°C for 1h (open lid).

Proceed immediately to cDNA Clean Up.

5.2.5. cDNA Clean Up

MATERIALS

- Second Strand cDNA synthesis product in 80 µl
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- IDTE
- RNase/DNase-free 0.2 ml 8-tube strips

PROCEDURE

1. Using a multichannel pipette, add 120 µl of AMPure® XP beads to the 80 µl Second Strand cDNA synthesis product. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly to remove the liquid from the tube walls.
3. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 3 minutes or until the liquid becomes clear.
4. Carefully discard the supernatant using a multichannel pipette.

   Keep tubes on the magnetic rack for the following steps.
5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Incubate for 30 seconds to 1 minute.
6. Carefully discard the ethanol using a multichannel pipette.
7. Repeat steps 5 and 6 once more.
8. Remove the residual ethanol using a P10 or P20 multichannel pipette.

9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered nucleic acid.

   Remove tubes from the magnetic rack.

10. Using a multichannel pipette, add 53 µl of IDTE to the beads to elute the cDNA.
11. Mix thoroughly by pipetting up and down 10 times and spin briefly.
12. Incubate at room temperature for 5 minutes and spin briefly if required to remove the liquid from the tube walls.
13. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 3 minutes or until the liquid becomes clear.
14. Carefully transfer 50 µl of the eluted cDNA to new, labeled 0.2 ml 8-tube strips tube(s).

   Tip: Safe stopping point overnight at -20°C or for longer storage.

5.2.6. End Repair and A-tailing

MATERIALS

- Purified cDNA in 50 µl each
- End Prep pre-mix
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

- Program the thermal cycler for End Prep with the following settings:

	TEMPERATURE (°C)	TIME (MINUTES)
Lid	75	
Step 1	4	1
Step 2	20	30
Step 3	65	30
Hold	4	∞

- Start the End Prep program. When the block reaches Step 1 - 4°C, pause the program.

PROCEDURE

Important: Always keep the samples and pre-mix on ice before and after the incubation to block the enzymatic reaction.

1. To facilitate pipetting, create a reservoir of End Prep pre-mix by adding the following volumes in a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	4-tube	8-tube	8-tube	8-tube
End Prep pre-mix (in µl)	22	22	33	44

2. Assemble the reaction as follows:
    - Using a multichannel pipette, add 10 µl of End Prep pre-mix to the 50 µl of cDNA samples (60 µl in 4 or 8-tube strips).
    - Using a multichannel pipette set to 40 µl, mix thoroughly by pipetting up and down 5 times and briefly spin in a microcentrifuge.
3. Place in the thermal cycler and continue the End Prep program.

Proceed immediately to Ligation.

5.2.7. Ligation

MATERIALS

- End Repair and A-Tailing (ER&AT) reaction products in 60 µl each
- Ligation pre-mix
- Stubby Universal Adapter
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

- During the ER&AT incubation, prepare new PCR strips with 5 µl of Stubby Universal Adapter per tube as per your indexing strategy, according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip	4-tube	8-tube	8-tube	8-tube
Number of strips	2	2	3	4

- Set up the thermal cycler at 20°C (open lid).

PROCEDURE

1. To facilitate pipetting, create a reservoir of Ligation pre-mix in a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	4-tube	8-tube	8-tube	8-tube
Ligation pre-mix (in µl)	70	70	105	140

2. Using a multichannel pipette, transfer the 60 µl of each ER&AT reaction product to the 4 or 8-tube strips containing 5 µl of Stubby Universal Adapter.
3. Mix thoroughly by pipetting up and down 10 times and spin briefly.
4. Using a multichannel pipette, add 31 µl of Ligation pre-mix to each ER&AT reaction product (96 µl in each tube of the 4 or 8-tube strip).
5. Using a multichannel pipette set to 60 µl, mix thoroughly by pipetting up and down 10 times and spin briefly.
6. Incubate in the thermal cycler at 20°C for 15 minutes (open lid).

Proceed to Post Ligation Clean Up.

Important: Do not place the strip(s) on ice at the end of the ligation as it might decrease the binding of the DNA to the beads.

5.2.8. Post-Ligation Clean Up

MATERIALS

- Ligation reaction products in 96 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- Nuclease-free water
- IDTE
- RNase/DNase-free 0.2 ml 8-tube strips

PROCEDURE

1. Using a multichannel pipette, add 80 µl of AMPure® XP beads to each of the 96 µl ligation reaction products. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 170 µl of supernatant using a multichannel pipette.

   Keep the tubes on the magnetic rack for the following steps.
5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Incubate for 30 seconds to 1 minute.

6. Carefully discard the ethanol using a multichannel pipette.
7. Repeat steps 5 and 6 once more.
8. Remove the residual ethanol using a P10 or P20 multichannel pipette.
9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

   Remove the tubes from the magnetic rack.
10. Using a multichannel pipette, add 20 µl of IDTE to the beads.

    Mix thoroughly by pipetting up and down 10 times and spin briefly.

Proceed to Library Amplification.

5.2.9. Library Amplification

MATERIALS

- Ligated reaction products and beads resuspended in 20 µl of IDTE each
- PCR Master Mix 2x
- 32 Unique Dual Index Primer Plate for Illumina®

PREPARATION

- Program the thermal cycler for Library Amplification with the following settings:

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99		
Step 1: Initial Denaturation	98	120	
Step 2: Denaturation	98	20	
Step 3: Annealing	60	30	n cycles*
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

* Follow Table 2 (Section 5.2.1 Total RNA Preparation) to determine the number of PCR cycles based on the RNA quality and the amount of starting material.

PROCEDURE

1. To facilitate pipetting, create a reservoir of PCR Master Mix 2x by adding the following volumes to a new set of 4 or 8-tube strips, according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	4-tube	8-tube	8-tube	8-tube
PCR Master Mix 2x (in µl)	60	60	90	120

2. Assemble the reaction as follows:
    - Using a multichannel pipette, add 5 µl of different Unique Dual Index Primer per tube to the ligation products and beads, according to your indexing strategy.
    - Mix thoroughly by pipetting up and down 10 times and spin briefly.
    - Using a multichannel pipette add 25 µl of PCR Master Mix 2x to the ligation products and beads (25 µl in 4 or 8-tube strips). Mix thoroughly by pipetting up and down 10 times and spin briefly.
3. Place the tubes in the thermal cycler and run the Library Amplification program.

Tip: Safe stopping point overnight at 4°C.

5.2.10. Post-Amplification Clean Up

MATERIALS

- PCR reaction products in 50 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- Nuclease-free water
- DNA low-binding tubes for the storage of libraries

PROCEDURE

1. Using a multichannel pipette, add 50 µl of AMPure® XP beads to each 50 µl of the PCR product. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 90 µl supernatant using a multichannel pipette.

    Keep the tubes on the magnetic rack for the following steps.

5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Let the tubes stand for 30 seconds to 1 minute.
6. Carefully discard the ethanol using a multichannel pipette.
7. Repeat steps 5 and 6 once more.
8. Remove the residual ethanol using a P10 or P20 multichannel pipette.
9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

   Remove the tubes from the magnetic rack.
10. Using a multichannel pipette, add 20 µl of nuclease-free water to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin to remove the liquid from the tube walls.
11. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until liquid becomes clear.
12. Carefully transfer 18 µl of the supernatant (transferring two times 9 µl is recommended at this step) to a new, labeled library storage tube.

Tip: Safe stopping point overnight at 4°C or -20°C for longer storage.

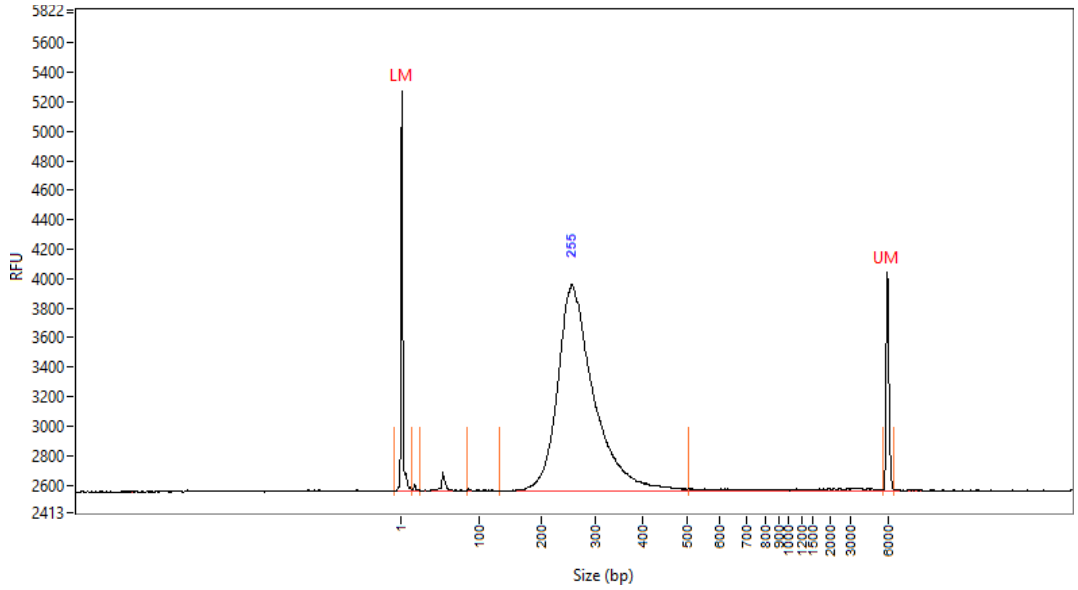
5.3. Individual Library Quantification and Quality Control

MATERIALS

- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system
- Nuclease-free water
- RNase/Dnase-free 0.2 ml 8-tube strips

PROCEDURE

1. Prepare a 4-time dilution of each library with nuclease-free water (e.g., 2 µl of library in 6 µl nuclease-free water).
2. Quantify the libraries with a fluorometric method (e.g., Qubit HS quantification using 2 µl of the 4x library dilution mentioned above).
3. Quality control the libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 200bp and 800bp with a clear peak in the range of 200-400 bp.



Example of a pre-capture library distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system; UM – Upper Marker, LM – Lower Marker.

5.4. Capture

5.4.1. Library Pooling for Hybridization and Capture

MATERIALS

- Individual libraries
- Human Cot DNA
- Universal Blockers - TS Mix
- DNA low-binding 1.5 ml tubes

PROCEDURE

1. Prepare a pre-mix of the following in a DNA low-binding tube:

NUMBER OF CAPTURES	1	2	3	4
Human Cot DNA (in µl)	5	11	16.5	22
Universal Blockers – TS Mix (in µl)	2	4.4	6.6	8.8

2. If performing two or more captures, pipette 7 µl of the above pre-mix into individual DNA low- binding tubes.
3. Add a pool of 8 individual libraries using 200 ng of each one (total of 1600 ng) per capture to the individual tubes containing the above pre-mix.

4. Mix thoroughly by pipetting up and down 10 times and spin briefly.
5. Dry each mix using a vacuum DNA concentrator until mix is completely lyophilized. Use mild heating (45-50°C) to speed up the lyophilization.

Tip: Safe stopping point overnight at -20°C.

5.4.2. Hybridization

MATERIALS

- Lyophilized libraries
- 2X Hybridization Buffer
- Hybridization Buffer Enhancer
- ROS_v1 Probes
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips
- 1.5 ml Tubes
- 10X Wash Buffer I
- 10x Wash Buffer II
- 10x Wash Buffer III
- 10X Stringent Wash Buffer
- 2X Beads Wash Buffer

PREPARATION

1. Pre-warm the thermal cycler to 95°C (set lid to 99°C).
2. After the 10-minute denaturation, switch directly to 65°C (set lid to 75°C).

Important: We recommend the use of different thermal cyclers for 95°C and 65°C incubations, if available.

PROCEDURE

1. Prepare a Hybridization pre-mix according to the number of capture reactions:

NUMBER OF CAPTURES	1	2	3	4
2x Hybridization Buffer (in µl)	8.5	18.7	28.05	37.4
Hybridization Buffer Enhancer (in µl)	3.4	7.48	11.22	14.96
Nuclease-free Water (in µl)	1.1	2.42	3.63	4.84

2. Resuspend the lyophilized pellet in 13 µl of the hybridization pre-mix.
3. Transfer the resuspended pellet to a PCR tube (one tube per capture reaction).
4. Incubate in the thermal cycler at 95°C for 10 minutes.

Important: Do not let the tube temperature drop below 65°C from step 4 to 6 as this can lead to incorrect probe annealing.

5. Move the PCR tube from the 95°C to 65°C thermal cycler, then add 4 µl of probes to the mix. Using a pipette set to 13 µl, mix thoroughly by pipetting up and down 5 times.
6. Incubate in the thermal cycler at 65°C for 4 to 16 hours.
7. Prepare the 1x working solutions of different wash buffers in advance as described in the following pages to allow them to reach equilibrium during the hybridization reaction.

WASH BUFFER PREPARATION FOR 1 REACTION

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	33	297	**330**
10x Wash Buffer II	22	198	**220**
10x Wash Buffer III	22	198	**220**
10x Stringent Wash Buffer	44	396	**440**
2x Bead Wash Buffer	275	275	**550**

Important: Pre-warm 1x Stringent Buffer and aliquot 110 µl of 1x Wash Buffer I at 65°C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

WASH BUFFER PREPARATION FOR 2 REACTIONS

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	66	594	**660**
10x Wash Buffer II	44	396	**440**
10x Wash Buffer III	44	396	**440**
10x Stringent Wash Buffer	88	792	**880**
2x Bead Wash Buffer	550	550	**1100**

Important: Pre-warm 1x Stringent Buffer and aliquot 220 µl of 1x Wash Buffer I at 65°C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

WASH BUFFER PREPARATION FOR 3 REACTIONS

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	99	891	**990**
10x Wash Buffer II	66	594	**660**
10x Wash Buffer III	66	594	**660**
10x Stringent Wash Buffer	132	1188	**1320**
2x Bead Wash Buffer	825	825	**1650**

Important: Pre-warm 1x Stringent Buffer and aliquot 330 µl of 1x Wash Buffer I at 65°C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

WASH BUFFER PREPARATION FOR 4 REACTIONS

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	132	1188	**1320**
10x Wash Buffer II	88	792	**880**
10x Wash Buffer III	88	792	**880**
10x Stringent Wash Buffer	176	1584	**1760**
2x Bead Wash Buffer	1100	1100	**2200**

Important: Pre-warm 1x Stringent Buffer and aliquot 330 µl of 1x Wash Buffer I at 65°C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

5.4.3. Streptavidin Beads Preparation

MATERIALS

- Streptavidin beads equilibrated at room temperature
- 1x Bead Wash Buffer
- 1.5 ml tubes
- RNase/DNase-free 0.2 ml 8-tube strips

PROCEDURE

Perform these steps just before the end of the hybridization incubation.

1. Mix the beads by vortexing them for 15 seconds.
2. Transfer 100 µl of beads per capture (200 µl for 2 reactions, 300 µl for 3 reactions, 400 µl for 4 reactions) to a single 1.5 ml tube.
3. Place the tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
4. Add 200 µl of 1x Bead Wash Buffer per capture (400 µl for 2 reactions, 600 µl for 3 reactions, 800 µl for 4 reactions) to the tube. Vortex for 10 seconds.
5. Place the tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
6. Repeat steps 4 and 5 once.
7. Add 100 µl of 1x Bead Wash Buffer per capture (200 µl for 2 reactions, 300 µl for 3 reactions, 400 µl for 4 reactions) to the tube. Vortex for 10 seconds.
8. Transfer 100 µl of cleaned beads to a new PCR tube (one tube per capture reaction).
9. Place tube(s) on a 96-well plate format magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.

Important: Do not allow the beads to dry.

Proceed immediately to Binding of Hybridized Targets to the Beads.

5.4.4. Binding of Hybridized Targets to the Beads

MATERIALS

- Cleaned Streptavidin beads in PCR tube(s)
- Hybridization reaction(s)

PROCEDURE

Important: Work quickly to ensure that the temperature of the sample remains close to 65°C.

1. Remove the hybridization reaction(s) from the thermal cycler and briefly spin down the tube(s) and place them back on the thermocycler.
2. Place the washed Streptavidin bead tubes in the thermocycler (no more than two tubes at a time to avoid drying of beads).

3. For each hybridization reaction, transfer 17 µl of the hybridization reaction solution to one PCR tube containing cleaned beads. Resuspend the beads by pipetting up and down until the solution is homogeneous.
4. Bind the DNA to the beads by placing the tube(s) into a thermal cycler set at 65°C (lid at 75°C). Incubate for 45 minutes.
5. During the incubation, gently pipette up and down the tube(s) every 15 minutes to ensure that the beads remain in suspension.

Proceed directly to Wash Streptavidin Beads to Remove Unbound DNA.

5.4.5. Wash Streptavidin Beads to Remove Unbound DNA

MATERIALS

- Hybridized targets on beads
- RNase/DNase-free 0.2 ml 8-tube strips
- DNA low-binding 1.5 ml tubes
- 1x Wash Buffer I (1/3 at 65°C and 2/3 at room temperature)
- 1x Wash Buffer II
- 1x Wash Buffer III
- 1x Stringent Wash Buffer (at 65°C)
- Nuclease-free water
- IDTE

PROCEDURE

Ensure that the temperature remains close to 65°C for steps 1 to 7

Note:If working with 2 or more capture tubes, work in a staggered manner from steps 2 to step 8, including the following:

1. When placing the first tube in thermoblock at 65°C for the first incubation of 5 min (step 5), start a timer.
2. Begin processing the second tube.
3. When placing the second tube at 65°C, note the time separating the tubes and ensure to respect this time gap along step 2 to step 8 to ensure each tube incubates exactly 5 min at 65°C with the stringent wash.

1. Add 100 µl of 1x Wash Buffer I (at 65°C) to each of the hybridized target/streptavidin beads tubes.
2. Working with one tube at a time, resuspend and transfer the mix one by one to a new DNA low- binding 1.5 ml tube. If working with two or more capture tubes, work in a staggered manner as indicated above.
3. Place tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
4. Add 200 µl of 1x Stringent Wash Buffer (at 65°C) to the tube. Gently resuspend the beads by pipetting up and down.

   Strong mixing of beads with the stringent wash buffer could decrease the quality of the capture.

5. Incubate at 65°C for 5 minutes.
6. Place the tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
7. Repeat steps 4 to 6 once.

   Work at room temperature.

8. Add 200 µl of 1x Wash Buffer I (at room temperature) to your tube. Gently resuspend the beads by pipetting up and down.

   Note: If working with 2 or more capture tubes; from this step on, process all the tubes at the same time.

9. Vortex for 2 minutes.
10. Place tube(s) on a magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.
11. Add 200 µl of 1x Wash Buffer II to each tube(s). Vortex for 1 minute.
12. Place tube(s) on a magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.
13. Add 200 µl of 1x Wash Buffer III to each tube(s). Vortex for 30 seconds. Spin briefly to collect all the liquid.
14. Place tube(s) on a magnetic rack and let them stand until the solution becomes clear. Carefully remove and discard the supernatant.
15. Add 200 µl of 1x IDTE to each tube(s). Resuspend the beads. Spin briefly to collect all the liquid.
16. Place tube(s) on a magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.
17. Remove all the remaining liquid by using a P10 or P20 pipette.
18. Add 20 µl of nuclease-free water to each tube(s), resuspend and transfer the beads/water mix to a new PCR tube.

5.5. Post-Capture Amplification

MATERIALS

- Streptavidin beads/nuclease-free water suspension (20 µl)
- Post Capture PCR Master Mix 2X
- Post Capture Illumina® Primers Mix
- PCR Enhancer
- Nuclease-free water

PREPARATION

- Program the thermal cycler for Post Capture Amplification using the following settings:

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	15 Cycles
Step 1: Initial Denaturation	98	120	
Step 2: Denaturation	98	20	
Step 3: Annealing	60	30	
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

PROCEDURE

1. Prepare the PCR pre-mix as follows:

PCR PRE-MIX				
Number of Reaction(s)	1	2	3	4
Post Capture PCR Master Mix 2X (in µl)	25	55	82.5	110
Post Capture Illumina® Primers Mix (in µl)	2.5	5.5	8.25	11
PCR Enhancer (in µl)	2.5	5.5	8.25	11

2. Add 30 µl of PCR pre-mix to each bead suspension. Mix thoroughly by pipetting up and down 10 times and spin briefly.
3. Place the tube(s) in the thermal cycler and run the Post Capture Amplification program.

Tip: Safe stopping point overnight at 4°C or -20°C for longer storage.

5.6. Post-Capture Amplification Clean Up

MATERIALS

- PCR reaction products in 50 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- IDTE
- DNA low-binding tubes for library storage

PROCEDURE

1. Add 50 µl of AMPure® XP beads to each of the 50 µl PCR reaction products. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly to remove the liquid from the tube walls.
3. Place tube(s) on a magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 90 µl supernatant using a multichannel pipette.

   Keep the tube(s) on the magnetic rack for the following steps.
5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Let the tube(s) stand for 30 seconds to 1 minute.
6. Carefully discard the ethanol.
7. Repeat steps 5 and 6 once.
8. Remove the residual ethanol using a P10 or P20 pipette.
9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

   Remove the tube(s) from the magnetic rack.
10. Add 20 µl of IDTE to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin briefly to remove the liquid from the tube walls.
11. Place tube(s) on a magnetic rack for 5 minutes or until liquid becomes clear.
12. Carefully transfer 18 µl of the supernatant (transferring two times 9 µl is recommended at this step) to a new, labeled library storage tube.

Tip: Safe stopping point overnight at 4°C or -20°C for longer storage.

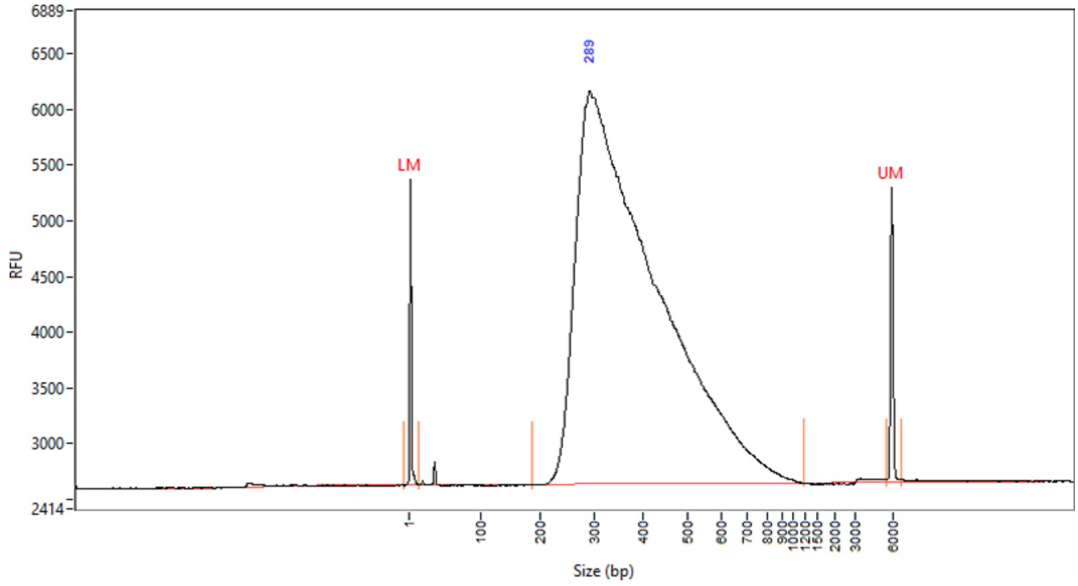
5.7. Final Library Quantification and Quality Control

MATERIALS

- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system

PROCEDURE

1. Quantify each captured library pool with a fluorometric method (e.g., Qubit HS quantification using 2 µl of the library).
2. Control the quality of the captured pool of libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 200bp and 800bp with a clear peak in the range of 200-400 bp.



Example of post-capture library pool size distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM refers to Lower Marker, UM refers to Upper Marker.

5.8. Library Preparation for Sequencing

MATERIALS

- Illumina® NextSeq® 550 Mid-Output Kit v2.5 (300 cycles)
- Final captured libraries
- EBT Buffer or similar

PROCEDURE

1. Determine the molarity of each pool with average size of the library (peak size in base pairs) and concentration (ng/µl) obtained during step 5.7 as follows:

$$\text{Library molarity (nM)} = \frac{\text{Library concentration (ng/µl)}}{\text{Average size in base pairs} \times 649.5} \times 10^6$$

2. Dilute each captured pool to 4 nM.
3. If processing multiple sequencing pools, mix them in equal amounts (e.g., 5 µl of each) following the sequencing recommendation table below:

NEXTSEQ 550 SYSTEM KIT TYPE	MAXIMUM SAMPLES PER RUN
Mid-Output	Up to 86

4. Mix it well and use this dilution according to Illumina® standard denaturation recommendation.
5. For loading dilution, see the table below:

NEXTSEQ 550 SYSTEM KIT TYPE	LOADING DILUTION
Mid-Output	1.3 pM
[Adjust the dilution (1.1 pM to 1.5 pM range) according to the number of clusters obtained in the first run]	

6. For recommended reads per sample, see the table below:

READ LENGTH (IN BP)	RECOMMENDED TOTAL READS PER SAMPLE	RRECOMMENDED READ-PAIRS (FRAGMENTS) PER SAMPLE
2 X 150	3 million	1.5 million

6. ANALYSIS PROCEDURE

6.1. NGS Data Demultiplexing

The user should perform NGS data demultiplexing following the instructions provided by the user guide of the Illumina® NextSeq® 550 sequencer (NextSeq 550 Systems Guide, Document # 15069765 v07). As indicated by Illumina®, standalone demultiplexing for third party analyzes can be performed with bcl2fastq v2.0 or higher (bcl2fastq2 Conversion Software v2.20 Software Guide, Document #15051736 v03).

Note: Users should note that usage of the no-lane-splitting option in bcl2fastq or usage of other automated demultiplexing workflows can result in file names incompatible with SOPHiA DDM™ Dx mode. Please refer to the User Manual of SOPHiA DDM™ Dx mode for clear instructions.

6.2. Data Upload and Analysis

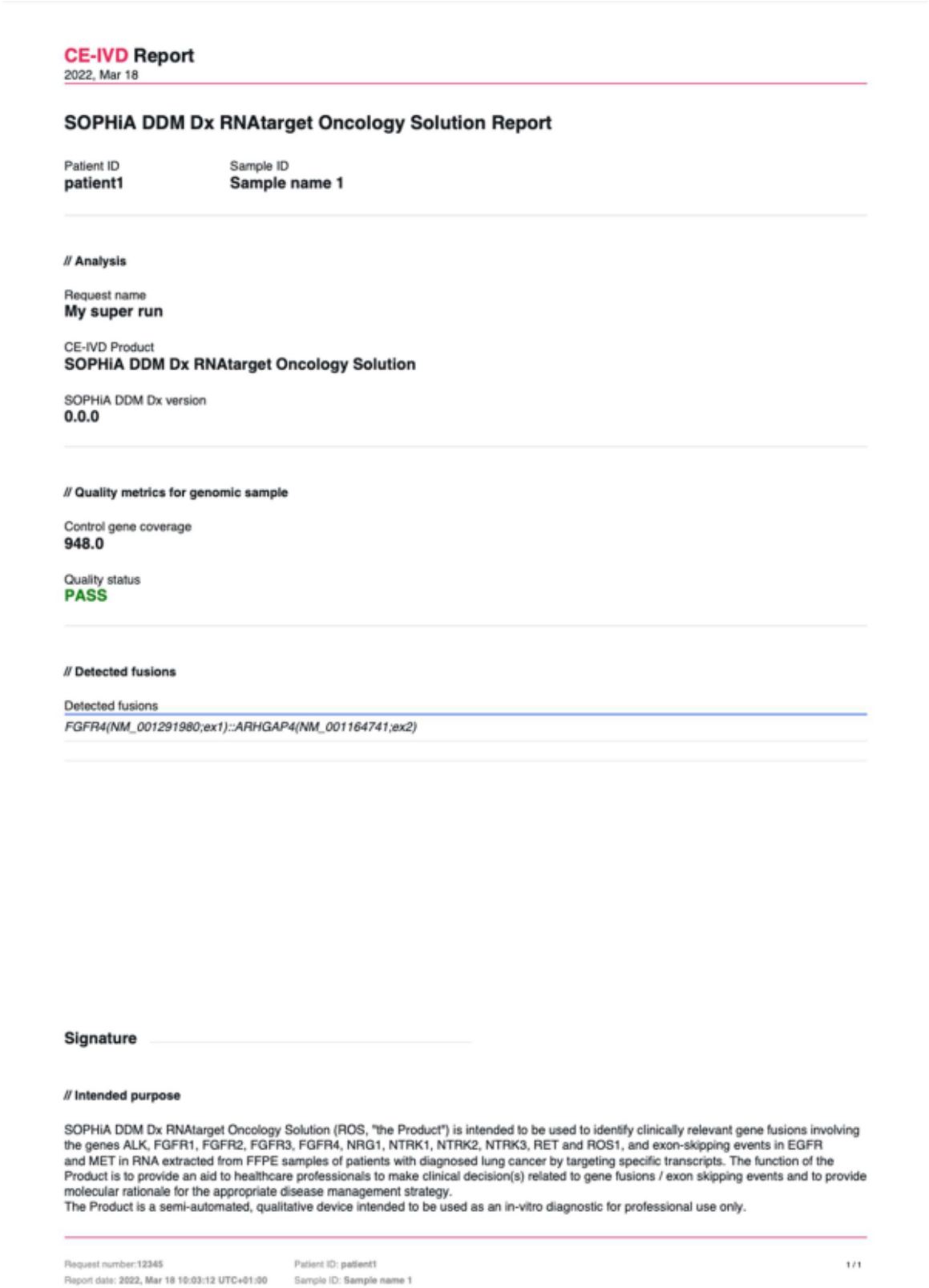
The user logs into SOPHiA DDM™ Dx mode and selects the SOPHiA DDM™ Dx RNAtarget Oncology Solution within the “My product(s)” window, to initiate NGS data upload. To create a new request for a genomic analysis, please refer to the “Create an analysis request" section in the SOPHiA DDM™ Dx mode User Manual for further instructions. After completion of the analysis, users -will receive a notification by email. If a notification is not received within 24 h from the initiation of the data upload process, the users should contact support.

Note: All samples in the analysis request must have been processed within the same sequencing run and prepared with the same assay reagents.

6.3. Report Generation

After sequencing (FASTQ) files are successfully uploaded, the progress of the analysis can be monitored in the widget “My Genomic Analysis Requests” widget in the dashboard or from the browser. Once completed, the generated SOPHiA DDM™ Dx ROS report for each sample in the request can be accessed and downloaded in the PDF format. The SOPHiA DDM™ Dx mode User Manual can be used to obtain detailed instructions regarding the analysis.

Figure 2. Example of a SOPHiA DDM™ Dx ROS Report with the first possible test outcome, see Table 3.



CE-IVD Report
2022, Mar 18

SOPHiA DDM Dx RNAtarget Oncology Solution Report

Patient ID
patient1

Sample ID
Sample name 1

// Analysis

Request name
My super run

CE-IVD Product
SOPHiA DDM Dx RNAtarget Oncology Solution

SOPHiA DDM Dx version
0.0.0

// Quality metrics for genomic sample

Control gene coverage
948.0

Quality status
PASS

// Detected fusions

Detected fusions
FGFR4(NM_001291980;ex1)::ARHGAP4(NM_001164741;ex2)

Signature

// Intended purpose

SOPHiA DDM Dx RNAtarget Oncology Solution (ROS, "the Product") is intended to be used to identify clinically relevant gene fusions involving the genes ALK, FGFR1, FGFR2, FGFR3, FGFR4, NRG1, NTRK1, NTRK2, NTRK3, RET and ROS1, and exon-skipping events in EGFR and MET in RNA extracted from FFPE samples of patients with diagnosed lung cancer by targeting specific transcripts. The function of the Product is to provide an aid to healthcare professionals to make clinical decision(s) related to gene fusions / exon skipping events and to provide molecular rationale for the appropriate disease management strategy.
The Product is a semi-automated, qualitative device intended to be used as an in-vitro diagnostic for professional use only.

Request number:12345
Report date: 2022, Mar 18 10:03:12 UTC+01:00
Patient ID: patient1
Sample ID: Sample name 1
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The SOPHiA DDM™ Dx ROS Report displays: SOPHiA DDM™ Dx ROS detected fusion and/or exon skipping events, the Sample Quality Score and Sample Quality Status. The following Table 3 provides an overview of the possible outcomes.

Table 3. Overview of possible test outcomes of the SOPHiA DDM™ Dx ROS

SOPHiA DDM™ Dx ROS detected fusions / exon skipping events	Quality Score	Quality Status	Description
Positive	≥50	Pass	High confidence positive call.
Positive	<50	Warning	High confidence positive call with a warning. Increased risk that additional fusions in the sample are not detected. WARNING sample quality status as the quality score (control gene coverage) threshold is not met.
Negative	≥50	Pass	High confidence negative call.
Negative (Inconclusive)	<50	Warning	Negative call with a warning. Increased false negative risk and inconclusive result. WARNING sample quality status as the quality score (control gene coverage) threshold is not met.

7. ANALYSIS DESCRIPTION AND PARAMETERS

7.1. Resource Files

Alignment is performed against the GRCh37 reference genome (also referred to as hg19). Publicly available sources for all these requirements can be found at:

GRCh37 – hg19 reference genome:

https://storage.googleapis.com/genomics-public-data/references/b37/Homo_sapiens_assembly19.fasta.gz

The translation of genomic coordinates into gene labels and genomic ranges (for genes, transcripts, and exons) is carried out using an internal database of transcripts generated on the 23 August 2016 using the RefSeq version available between RefSeq release 77 and release 78.

7.2. Target Genes

Targets comprise a set of transcripts with high clinical relevance in advanced lung tumors. Specifically, the SOPHiA DDM™ Dx ROS product targets exons that are known to be involved in pathogenic rearrangements: exon skipping events (MET ex14, EGFRvIII) or fusions (ALK, FGFR1-4, NRG1, NTRK1-3, RET, ROS1). The full list of exons targeted for the detection of gene fusions is provided in the following Table 4 (black numbers). Exons that are not specifically targeted, but for which fusions would not be excluded from the report if detected, are also included in the table (red numbers).

Table 4. List of exons involved in gene fusion events detected by SOPHiA DDM™ Dx ROS

Gene symbol	Exons permitted when the gene is the 5' partner	Exons permitted when the gene is the 3' partner	Refseq Transcript ID
ALK	NA	2,3,4,5,6-9,10,11-15,16-20,21-29	NM_004304
FGFR1	1,2,3-8,9-11,12,13,14,15-18	2-6,7,8-10,11,12,13,14-16,17,18	NM_023110
FGFR2	1,2,3-5,6,7,8,9,10,11,12,13,14,15,16,17,18	2,3,4,5,6-9,10,11,12,13,14-17,18	NM_000141
FGFR3	2,3,4,5,6,7-12,13,14,15,16,17,18	4,5,6-9,10,11,12,13-17,18	NM_000142
FGFR4	2,3-6,7,8-10,11,12-15,16,17,18	2,3-6,7,8-17,18	NM_213647
NRG1	NA	2,3,4,5,6,7,8,9-12	NM_013964
NTRK1	NA	7-13,14-17	NM_002529
NTRK2	NA	9,10,11-13,14,15,16,17,18-21	NM_006180
NTRK3	NA	7,8-11,12,13,14,15,16,17,18-20	NM_001012338
RET	NA	2,3-10,11,12,13-16,17,18-20	NM_020975
ROS1	NA	32-36,37-42,43	NM_002944

black = target exons for which fusions can be detected and will be reported;

red = exons that are not targeted, but for which fusions will still be reported if detected.

7.3. Raw Data Pre-Processing

7.3.1. Preprocessing

In the initial steps of data processing, the fastq.gz files are truncated to a maximum size of 200MB. The original file size and the size after truncation are reported in the QA Report. Next, the data quality metrics are collected based on the fastq files after truncation.

7.3.2. Alignment

Reads are aligned with STAR-2.7.0f_0328 using single-end settings. The following files are generated:

a. Main bam file: Contains all the reads spanning a single location in the genome (i.e., without breakpoints) or spanning several locations in a linearly spliced way, i.e., consistent with a splicing event. This file is used for the detection of exon skipping.

b. Chimeric junction file: Contains the reads spanning two or more locations in the genome but that do not align in a linearly spliced way. Since these are the type of reads typically involved in a re-arrangement, this file is used for the detection of fusions. For a more detailed description of a chimeric alignment the user can refer to (Dobin et al., 2013)[4].

7.4. List Of Included Modules

The results returned by the analytical pipeline in the IVD report are fusion calls and /or exon skipping calls and a quality indicator for each sample.

7.4.1. Gene Fusion/Exon Skipping Event Calling Module:

Fusion and exon skipping calling

The fusion and exon skipping detection module includes algorithms that apply statistical tests to identify relevant events from chimerically aligned reads, quantification functions, and filtering functions. The module executes the following steps:

a. Identify candidate fusions from the chimeric junction file output by STAR. Candidate fusions involving non-coding RNAs or two regions from the same gene will not be considered for downstream analysis.

b. Identify candidate exon skipping events from the main bam file by examining evidence of exon skipping in the alignments.

c. Assess the likelihood of a candidate being a true fusion/exon skipping event. The statistical model used considers the following:

- The type of breakpoint (only relevant for fusions). For example, in a typical fusion where the breakpoint is intronic for both partners, the breakpoint type will be intron-intron. Other types could be exon-intron or exon-exon. Fusions where the breakpoint is in the middle of an exon are penalized and require additional molecule support to be called as high confidence because these types of events are rare.

- The mapping position of the reads (only relevant for fusions). For example, in a typical fusion where the breakpoint is intronic for both partners, reads will map to the exon-intron boundaries of both partners. In this case the mapping position will be exon-exon. Other types of mapping positions could be exon-intron or intron-intron.
- The number of molecules supporting the event.
- The predicted coding consequence.

In some cases, a pair of fusion genes may have multiple fusions called with different breakpoints. In this case, multiple fusions will be reported.

Fusion and exon skipping filtering and reporting

The list of fusions returned during the calling step may contain low confidence calls generated by artifacts in the data, events with insufficient molecular coverage support or high confidence calls that are not supported by the scope of the product. Any fusions that meet the following criteria are regarded as low confidence and will not appear in the IVD report:

a. Fusion calls where both partners are annotated as belonging to the same gene family based on the HGNC annotation.

b. Fusion calls where both partners have a high degree of homology but are not annotated as in the same gene family. Homology is defined as a BLAST alignment between the 5' gene and the 3' gene yielding the E-value above 50.

c. Fusion calls in which at least one partner is a pseudogene.

d. Fusion calls in which the pair of genes is annotated in HGNC as locus-type “readthrough”.

e. Fusion or exon skipping calls with less than 10 supporting molecules.

f. Fusion calls in which the 5' and 3' genes are overlapping.

g. Fusion calls where intronic sequence comprises part of the mature fusion transcript.

Furthermore, for a fusion to be reported, at least one partner must be listed in Table 4 (with matching transcript ID, orientation within the fusion and exon bordering the breakpoint).

Please note:

a. Partner exons displayed in red in Table 4 are not targeted by the assay, but could still be detected (for example, if an adjacent exon is targeted).

b. Genomic coordinates, instead of gene symbols, will be displayed for genes not present in Table 1 (which lists clinically relevant genes).

Only the following exon-skipping events will be reported: EGFR 1-8 (NM_005228), EGFR 1-9 (NM_005228) and MET 13-15 (NM_001127500).

7.4.2. Quality Indicator

For each analyzed sample the following indicator is computed:

- Control gene coverage: this value represents the computed molecular coverage of an internal control gene. Since the selected control gene is expected to be well expressed across various tissue types, this metric is used as a proxy of the RNA conversion rate, i.e., the efficiency with which RNA molecules available in the sample are converted into fragments that can be sequenced and used for the analysis.
- Samples displaying molecular coverage below 50x are considered of having suboptimal data quality and are labeled with “WARNING” Quality Status in the SOPHiA DDM™ Dx ROS Report. This status indicates that only a small number of unique molecules were sequenced, possibly due to low input material quality or technical problems with the workflow execution. Insufficient data quality may affect the power of detection of fusion or exon skipping events. Coverage of 50x or above is associated with “PASS” Quality Status. See Section 6.3 for further details.

8. PERFORMANCE EVALUATION

8.1. Analytical Performance Evaluation

8.1.1. Fusion/ Exon Skipping Status Concordance with Comparator NGS Assay

The analytical performance of the fusion/exon skipping detection was tested using 50 ng of RNA derived from 54 clinical FFPE samples, including 40 lung tumor samples and 14 derived from other solid tumors. The status of these samples was pre-determined with alternative NGS method(s) and compared with the outcome of SOPHiA DDM™ Dx ROS.

Samples	Performance measurement	Observed	lower 95% confidence interval
Overall (n=54)	Positive Percent Agreement	100%	88.65%
	Negative Percent Agreement	100%	99.99%
	Overall Percent Agreement	100%	NA
Lung only (n=40)	Positive Percent Agreement	100%	79.61%
	Negative Percent Agreement	100%	99.99%
	Overall Percent Agreement	100%	NA

8.1.2. Fusion/ Exon Skipping Limit of Detection

Limit of detection (LoD) was estimated using dilutions of a reference sample, bearing 14 targeted rearrangements, and a clinical sample, both with known number of fusion/exon skipping molecules. 50 ng total RNA was used as input. LoD was measured using as the lowest number of fusion molecules present in the input material at which sensitivity reached ≥95%, with a 95% confidence interval.

Event	LoD [molecules/50 ng of RNA]
FGFR3-BAIAP2L1	280
EML4-ALK	303
EGFRvIII	258
CCDC6-RET	335
METex14	240
ETV6-NTRK3	296
CD74-ROS1	677
KIF5B-RET	78

Event	LoD [molecules/50 ng of RNA]
LMNA-NTRK1	371
FGFR3-TACC3	69
SLC34A2-ROS1	187
NCOA4-RET	241
TFG-NTRK1	369
TPM3-NTRK1	213
CD74-NRG1	357*

*fusion evaluated in a clinical sample

8.1.3. Fusion/ Exon Skipping Status Repeatability

Repeatability was addressed by measuring Positive Percent Agreement (PPA) / Negative Percent Agreement (NPA) in an experiment consisting of 32 replicates of a diluted reference sample, bearing 14 targeted rearrangements, so that different events were present at ~0.9-6-fold of their LoD. 100% of repeatability was measured for all 13 events present above their limit of detection.

8.2. Clinical Performance Evaluation

The clinical performance of the SOPHiA DDM™ Dx RNAtarget Oncology Solution was established in a clinical study on 22 RNA samples isolated from FFPE lung tumor samples. The clinical status (i.e. fusion/exon skipping-positive or -negative) for each sample was pre-determined using the externally validated NGS method. 50 ng of RNA was processed with SOPHiA DDM™ Dx ROS by an external site and the determined status was compared to the pre-determined status.

Metric	Value (95% C.I.)
Diagnostic sensitivity	100% [70.09%-100%]
Diagnostic specificity	100% [77.19%-100%]

9. WARNINGS, PRECAUTIONS AND LIMITATIONS

9.1. General Warnings

- For in-vitro diagnostics only. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
- SOPHiA DDM™ Dx ROS has been validated for RNA FFPE samples from Lung Cancer (input of 50ng) with DV200 ≥ 20% (measured using Agilent Fragment Analyzer™ system) and tumor content ≥20% (measured using standard pathology methods), and sequenced on Illumina® NextSeq® 550 (Mid-Output flow cell).
- Poor quality of raw NGS data can confound the data analysis and cause False Positive, False Negative and inconclusive results..
- If any part of the handling, protocol, sequencer, multiplexing etc. is changed, the analyzes are not covered by the described Instructions for Use.
- Physically separated pre- and post-PCR rooms should be defined to prevent sample contamination. Always use fresh reagents, correctly extracted and stored RNA. For details on RNA quality and integrity see IFU Section 5.3.1 Total RNA Preparation.
- Correctly calibrated pipettes and proper lab equipment should be used to perform experiment.
- Different lot numbers of reagents should not be mixed.
- The maximal run size that can be uploaded for this product is 75Gb. The analytical pipeline may process individual samples with up to 1Gb of data. Higher volumes per sample might cause the pipeline to fail.
- For detailed instructions on the software, refer to the SOPHiA DDM™ Dx mode User Manual.

9.2. General Precautions

- For use only with the SOPHiA GENETICS™ Dx RNA Library Prep Kit IV.
- For IVD use only with SOPHiA DDM™ Dx mode.
- Do not use kits, reagents, or disposable items beyond their expiration dates.
- All biological materials and chemicals are potentially hazardous. While FFPE specimen material and nucleic acids prepared from it are unlikely to pose an infectious hazard, the user should always adhere to local Health and Safety procedures.
- Specimens shall be handled as infectious using safe laboratory procedures such as those outlined in the CLSI Document M29-A4[5].
- Store and handle reagents according to instructions on the kit boxes, and do not use if expired.
- Some reagents may require safety precautions. For specific safety information, please refer to the corresponding Material Safety Data Sheets (MSDS) for each component of the product.
- Dispose of unused reagents, kits, its accessories, and waste in accordance with local regulations.

- The accuracy of the results of the analysis cannot be guaranteed. Sequencing laboratories need to fulfil quality checks of the samples and flag the unqualified samples. Unqualified samples (e.g., input samples outside the range of recommended criteria: RNA quantity, tumor content, and DV200) could lead to compromised results regarding the analytical performance of the assay. SOPHiA GENETICS™ is not liable for the results and consequent decisions taken on the basis of these results.
- It is recommended to store purified RNA in buffered solution composed of 10 mM Tris pH 7.5 or 8.0, and 0.1 mM EDTA.

9.3. General Limitations

- Multiplexing to average read numbers per sample in a run lower than 3M reads (1.5M fragments) may negatively impact product performance.
- The absence of a fusion/exon skipping event in the report does not rule out the presence of such an event present below the limits of detection of the assay. For more details on the limit of detection, please refer to Section 9.3.1.
- The maximum amount of data per fastq.gz file (or four set of files in the case of split-lane inputs) is limited to 200mb which corresponds to 4.5-5M reads per sample. Files larger than that will be downsampled (i.e., reads are output until the maximum size, or the end of the file, is reached).
- The product does not detect nor report sample cross contamination events.
- The product does not measure or report sample tumor content.
- Even when sample multiplexing recommendations are followed and the recommended average number of 1.5M read pairs per sample is achieved for a given sequencing run, molecule depth may be insufficient to provide sensitive and accurate results for various reasons, including: poor sample quality and poor NGS data.
- Quality, uneven number of targeted transcripts between pooled samples, significant uneven read allocation between individual or pooled and captured libraries, significantly uneven read allocation between samples multiplexed in the same run, skewed read depth allocation across the gene panel (e.g., consistently lower read depth in AT-rich regions). It is also possible that libraries derived from samples of poor quality will consume significantly less reads than mean read number in a run.
- The assay is capable of detecting gene fusions, defined as abnormal transcripts containing exons of two otherwise separate genes, and particularly fusion gene exon-exon junctions on the level of mRNA, provided that the exon of the primary target participating in the fusion is included in the target list within Table 4.
- The SOPHiA DDM™ Dx ROS IVD Report provides gene symbol, transcript ID and relevant exons only for fusions involving genes listed in Table 1.
- All fusions will be reported provided they fulfill the restrictions listed in section 7.4.1 Gene Fusion + Exon Skipping Event Calling Module – Fusion and exon skipping filtering and reporting. Any fusions outside of the scope of this product will not be reported.
- Any exon skipping events other than MET exon 14 and EGFRvIII will not be reported.
- Fusion/exon skipping event detection performance is assured as long as data quality is sufficient as indicated by the quality indicator (see Section 7.4.2). Absence of reported events for samples with a WARNING Quality Status does not exclude presence of events. High confidence fusions are still reported for such samples.

- Input material fragmentation might not reflect the extent of the chemical damage of the genomic RNA. Chemical damage is known to decrease conversion of RNA to cDNA and results in low molecule diversity. Therefore, in some cases, poor data quality may be obtained for samples with relatively high DV200 values (refer to Section 5.2.1).
- Impaired performance of the assay, particularly false negative or inconclusive results, may be caused by: issues in the sample preparation or sequencing steps, presence of events below their limit of detection, insufficient starting material (i.e. <50 ng), low input material quality, low tumor content, insufficient sequencing depth, contamination with DNA (particularly at or above 4:1 DNA:RNA ratio), events with high sequence homology to non-targeted regions, events with low sequence complexity, or limitations in the Kit design.
- Low molecular diversity observed in the obtained data is typically reflected by low library yield and may be caused by various factors, including insufficient input material, high input material fragmentation, excessive chemical damage of RNA, contamination with RNases, presence of chemical interferents, issues during library preparation or capture, sequencing problems, insufficient sequencing depth. Indication of poor data quality can be observed as:
    a. Low library yield – samples with library concentration of at least 25 ng/ml generally yield data of sufficient quality as measured by control gene molecule count, lower values might indicate sample quality issues or technical problems.
    b. Low captured library yield – capture yields with concentration of at least 10 ng/ml generally indicate correct execution of the workflow.
    c. Low read number – read numbers significantly lower than 3M reads per sample may negatively impact performance.
    d. Sequencing problems – sequencing run parameters below values recommended by Illumina® will not support good product performance.
- Fusion and exon skipping events require a sufficient amount of starting molecules for the fusion in the sample. Events may not be detected when the fusion is lowly or not expressed. Product is only capable of detecting fusions / exon skipping events if they are expressed at a certain level in any given sample, resulting in a sufficient number of molecules available for conversion. Please see the limits of detection for the tested events in the Table 5 below.

Table 5. Detection limit of the gene fusions / exon skipping events (molecule counts)

Main target	5' gene	3' gene	5' exon	3' exon	Limit of detection [molecules]*
ALK	EML4	ALK	13	20	303
EGFR	EGFR	EGFR	1	8	258
FGFR3	FGFR3	TACC3	17	11	69
FGFR3	FGFR3	BAIAP2L1	17	2	280
MET	MET	MET	13	15	240
NRG1	CD74	NRG1	6	6	357
NTRK1	LMNA	NTRK1	2	11	371
NTRK1	TFG	NTRK1	5	10	369
NTRK1	TPM3	NTRK1	8	10	213

Main target	5' gene	3' gene	5' exon	3' exon	Limit of detection [molecules]*
NTRK3	ETV6	NTRK3	5	15	296
RET	CCDC6	RET	1	12	335
RET	KIF5B	RET	24	11	78
RET	NCOA4	RET	7	12	241
ROS1	CD74	ROS1	6	34	677
ROS1	SLC34A2	ROS1	4	34	187

* Limit of detection was estimated using the fusion molecule counts in the input material determined either by digital droplet PCR or numbers declared by manufacturer of the reference material used. The value is the upper limit of the estimated 95% confidence interval, for LoD ensuring 95% sensitivity, calculated using 5 serial dilutions with at least 10 replicates at each dilution[6].

- Fusion detection was verified with clinical samples bearing a diverse but limited set of fusions. Not all of the tested samples were derived from lung tumors. For a detailed list, please see Table 6 below. Due to the potentially large numbers of possible fusions, it cannot be guaranteed that the product will be able to efficiently detect fusions not included on this list.

Table 6. List of samples used for fusion detection

Main target	5' gene	3' gene	5' exon	3' exon	Samples tested	Sample type
ALK	EML4	ALK	13	20	3	Lung cancer, Lung adenocarcinoma, Reference sample
ALK	EML4	ALK	2	20	1	Lung adenocarcinoma
ALK	EML4	ALK	6	20	1	Lung adenocarcinoma
ALK	PPFIBP1	ALK	12	20	1	Lung cancer
EGFR	EGFR	EGFR	1	8	2	Glioma, Reference sample
FGFR1	FGFR1	TACC1	17	5	1	Glioma
FGFR2	FGFR2	RBFOX3	17	5	1	Glioma
FGFR3	FGFR3	TACC3	17	11	5	Urinary tract tumor, Glioblastoma, Glioma, Reference sample
FGFR3	FGFR3	BAIAP2L1	17	2	1	Reference sample
FGFR3	FGFR3	TACC3	17	8	2	Glioma
FGFR3	FGFR3	AGO3	7	12	1	Glioblastoma
FGFR3	FGFR3	AGO3	8	12	1	Glioblastoma
FGFR3	FGFR3	MYH14	17	24	1	Glioma
FGFR3	FGFR3	MYH14	18	24	1	Glioma

Main target	5' gene	3' gene	5' exon	3' exon	Samples tested	Sample type
MET	MET	MET	13	15	4	Lung cancer, Lung adenocarcinoma, Reference sample
NRG1	CD74	NRG1	6	6	2	Lung cancer
NRG1	SLC3A2	NRG1	4	6	1	Lung cancer
NRG1	SLC3A2	NRG1	5	6	1	Lung cancer
NTRK1	LMNA	NTRK1	2	11	1	Reference sample
NTRK1	TFG	NTRK1	5	10	1	Reference sample
NTRK1	TPM3	NTRK1	8	10	1	Reference sample
NTRK1	NOS1AP	NTRK1	10	10	1	Glioma
NTRK2	UBQLN1	NTRK2	1	14	1	Glioma
NTRK2	UBQLN1	NTRK2	1	16	1	Glioma
NTRK3	ETV6	NTRK3	5	15	1	Reference sample
RET	CCDC6	RET	1	12	1	Reference sample
RET	KIF5B	RET	24	11	1	Reference sample
RET	NCOA4	RET	7	12	1	Reference sample
RET	KIF5B	RET	15	12	1	Lung cancer
ROS1	CD74	ROS1	6	34	2	Lung adenocarcinoma, Reference sample
ROS1	SDC4	ROS1	5	34	3	Lung cancer
ROS1	GOPC	ROS1	8	35	2	Glioblastoma
ROS1	GOPC	ROS1	8	36	2	Glioblastoma
ROS1	SDC4	ROS1	5	33	2	Lung cancer
ROS1	SLC34A2	ROS1	4	34	1	Reference sample
ROS1	CD74	ROS1	6	33	1	Lung adenocarcinoma
ROS1	CD74	ROS1	6	35	1	Lung adenocarcinoma
ROS1	CD74	ROS1	6	36	1	Lung adenocarcinoma
ROS1	CD74	ROS1	6	43	1	Lung adenocarcinoma
ROS1	SDC4	ROS1	4	34	1	Lung cancer
ROS1	TPM3	ROS1	8	35	1	Lung cancer

- The fusion calling algorithm was tested with in silico generated sequencing reads corresponding to gene fusions, for a limited list of fusions (Table 7). Due to the potentially large number of possible fusions, it cannot be guaranteed that the product will be able to correctly report fusions not included on this list. The likelihood to be detected was assigned to each fusion:

a. High: more than 90% of the simulated reads are assigned to the fusion by the fusion caller.

b. Medium: between 60 and 90% of the simulated reads are assigned to the fusion by the fusion caller.

c. Low: less than 60% of the simulated reads are assigned to the fusion by the fusion caller.

Failure to assign 100% of the reads to the expected fusion is observed in situations in which not all the reads could be successfully mapped to the exon-intron boundary. Identified reasons for unsuccessful mapping include low sequence complexity, presence of long homopolymeric regions, presence of repetitive regions and sequences with high homology to non-targeted regions.

Table 7. Likelihood of detection of gene fusion

Main target	5' gene	3' gene	5' exon	3' exon	5' transcript	3' transcript	Likelihood of Detectability
ALK	ATIC	ALK	7	20	NM_004044	NM_004304	High
RET	CCDC6	RET	8	11	NM_005436	NM_020630	High
ALK	SEC31A	ALK	22	20	NM_001077207	NM_004304	High
ALK	TFG	ALK	5	20	NM_001007565	NM_004304	High
ALK	TPM3	ALK	10	20	NM_152263	NM_004304	High
RET	CCDC6	RET	8	12	NM_005436	NM_020630	High
ALK	TPR	ALK	15	20	NM_003292	NM_004304	High
FGFR1	TRIM24	FGFR1	11	9	NM_003852	NM_00117406	High
NTRK2	TRIM24	NTRK2	12	12	NM_003852	NM_00101806	High
RET	TRIM24	RET	9	12	NM_015905	NM_020630	High
NRG1	CD74	NRG1	6	4	NM_001025159	NM_00115999	High
RET	TRIM33	RET	11	12	NM_015906	NM_020630	High
RET	TRIM33	RET	16	12	NM_015906	NM_020630	High
RET	TRIM33	RET	17	11	NM_015906	NM_020630	High
ALK	VCL	ALK	13	20	NM_003373	NM_004304	High
ALK	VCL	ALK	16	20	NM_003373	NM_004304	High
ALK	VCL	ALK	6	20	NM_003373	NM_004304	High
FGFR1	ZMYM2	FGFR1	17	10	NM_001190964	NM_00117406	High
ROS1	CLIP1	ROS1	20	36	NM_001247997	NM_002944	High
ALK	CLTC	ALK	30	20	NM_001288653	NM_004304	High

Main target	5' gene	3' gene	5' exon	3' exon	5' transcript	3' transcript	Likelihood of Detectability
ALK	CLTC	ALK	31	20	NM_001288653	NM_004304	High
ROS1	CLTC	ROS1	31	35	NM_001288653	NM_002944	High
FGFR1	CNTRL	FGFR1	37	8	NM_007018	NM_00117406	High
FGFR1	BCR	FGFR1	4	10	NM_004327	NM_00117406	High
ALK	DCTN1	ALK	13	20	NM_001135040	NM_004304	High
ALK	EML4	ALK	1	20	NM_001145076	NM_004304	High
ALK	EML4	ALK	12	20	NM_001145076	NM_004304	High
ALK	EML4	ALK	13	20	NM_001145076	NM_004304	High
ALK	EML4	ALK	14	20	NM_001145076	NM_004304	High
ALK	EML4	ALK	16	20	NM_001145076	NM_004304	High
ALK	EML4	ALK	17	20	NM_001145076	NM_004304	High
ALK	EML4	ALK	2	20	NM_001145076	NM_004304	High
FGFR1	BCR	FGFR1	4	8	NM_004327	NM_00117406	High
ALK	EML4	ALK	19	18	NM_001145076	NM_004304	High
ALK	EML4	ALK	19	20	NM_001145076	NM_004304	High
ALK	EML4	ALK	3	20	NM_001145076	NM_004304	High
NTRK3	EML4	NTRK3	1	14	NM_001145076	NM_00100715	High
RET	ERC1	RET	10	12	NM_001301248	NM_020630	High
RET	ERC1	RET	12	12	NM_001301248	NM_020630	High
RET	ERC1	RET	17	12	NM_001301248	NM_020630	High

Main target	5' gene	3' gene	5' exon	3' exon	5' transcript	3' transcript	Likelihood of Detectability
RET	ERC1	RET	7	12	NM_001301248	NM_020630	High
ROS1	ERC1	ROS1	10	36	NM_001301248	NM_002944	High
ROS1	ERC1	ROS1	11	36	NM_001301248	NM_002944	High
FGFR3	ETV6	FGFR3	5	10	NM_001987	NM_000142	High
NTRK3	ETV6	NTRK3	3	14	NM_001987	NM_00100715	High
NTRK3	ETV6	NTRK3	4	14	NM_001987	NM_00100715	High
NTRK3	ETV6	NTRK3	4	15	NM_001987	NM_00101233	High
NTRK3	ETV6	NTRK3	5	15	NM_001987	NM_00101233	High
NTRK3	ETV6	NTRK3	5	20	NM_001987	NM_00101233	High
RET	ETV6	RET	5	12	NM_001987	NM_020630	High
FGFR1	FGFR1	PLAG1	1	2	NM_001174063	NM_002655	High
FGFR1	FGFR1	PLAG1	1	4	NM_001174063	NM_00111463	High
ALK	CARS	ALK	17	20	NM_001014437	NM_004304	High
NTRK2	FRMD3	NTRK2	1	12	NM_001244959	NM_00101806	High
RET	GOLGA5	RET	7	12	NM_005113	NM_020630	High
ROS1	GOPC	ROS1	3	36	NM_001017408	NM_002944	High
ROS1	GOPC	ROS1	4	36	NM_001017408	NM_002944	High
ROS1	GOPC	ROS1	7	35	NM_001017408	NM_002944	High
ALK	HIP1	ALK	21	20	NM_001243198	NM_004304	High
ALK	HIP1	ALK	26	20	NM_001243198	NM_004304	High
ALK	HIP1	ALK	28	20	NM_001243198	NM_004304	High
RET	HOOK3	RET	11	12	NM_032410	NM_020630	High
ALK	CARS	ALK	18	20	NM_001014437	NM_004304	High

Main target	5' gene	3' gene	5' exon	3' exon	5' transcript	3' transcript	Likelihood of Detectability
ALK	KIF5B	ALK	15	20	NM_004521	NM_004304	High
ALK	KIF5B	ALK	17	20	NM_004521	NM_004304	High
RET	KIF5B	RET	1	11	NM_004521	NM_020630	High
RET	KIF5B	RET	14	12	NM_004521	NM_020630	High
RET	KIF5B	RET	15	11	NM_004521	NM_020630	High
RET	KIF5B	RET	15	12	NM_004521	NM_020630	High
RET	KIF5B	RET	16	12	NM_004521	NM_020630	High
RET	KIF5B	RET	22	12	NM_004521	NM_020630	High
RET	CCDC6	RET	1	11	NM_005436	NM_020630	High
RET	KIF5B	RET	23	12	NM_004521	NM_020630	High
RET	KIF5B	RET	24	11	NM_004521	NM_020630	High
FGFR2	KLK2	FGFR2	1	5	NM_001002231	NM_000141	High
FGFR2	KLK2	FGFR2	3	5	NM_001002231	NM_000141	High
RET	KTN1	RET	29	12	NM_001079521	NM_020630	High
ROS1	LRIG3	ROS1	16	35	NM_001136051	NM_002944	High
ALK	MSN	ALK	11	20	NM_002444	NM_004304	High
RET	NCOA4	RET	6	12	NM_001145260	NM_020630	High
RET	NCOA4	RET	7	12	NM_001145260	NM_020630	High
RET	CCDC6	RET	1	12	NM_005436	NM_020630	High
FGFR4	NSD1	FGFR4	2	7	NM_022455	NM_00129198	High
RET	PCM1	RET	29	12	NM_001315507	NM_020630	High
RET	PCM1	RET	31	17	NM_001315507	NM_020630	High
ALK	PPFIBP1	ALK	12	20	NM_003622	NM_004304	High
ALK	PPFIBP1	ALK	6	20	NM_001198915	NM_004304	High
ROS1	PPFIBP1	ROS1	6	35	NM_001198915	NM_002944	High

Main target	5' gene	3' gene	5' exon	3' exon	5' transcript	3' transcript	Likelihood of Detectability
RET	CCDC6	RET	2	12	NM_005436	NM_020630	High
ROS1	PWWP2A	ROS1	1	36	NM_001130864	NM_002944	High
NTRK2	QKI	NTRK2	6	12	NM_001301085	NM_00101806	High
ALK	RNF213	ALK	20	20	NM_001256071	NM_004304	High
ROS1	SDC4	ROS1	2	35	NM_002999	NM_002944	High
ALK	SEC31A	ALK	20	20	NM_001077206	NM_004304	High
ALK	TFG	ALK	4	20	NM_001007565	NM_004304	High
RET	NCOA4	RET	8	11	NM_001145260	NM_020630	High
RET	NCOA4	RET	8	12	NM_001145260	NM_020630	High
ROS1	TFG	ROS1	4	35	NM_001007565	NM_002944	High
ALK	MSN	ALK	10	20	NM_002444	NM_004304	Medium
ALK	TPM3	ALK	6	20	NM_001043351	NM_004304	Medium
ROS1	PPFIBP1	ROS1	8	35	NM_001198915	NM_002944	Medium
ALK	STRN	ALK	3	20	NM_003162	NM_004304	Medium
NTRK1	TPM3	NTRK1	7	12	NM_001043351	NM_00100779	Medium
ROS1	TPM3	ROS1	7	35	NM_001043351	NM_002944	Medium
NRG1	CD74	NRG1	5	4	NM_001025158	NM_00115999	Low
ALK	CLIP1	ALK	12	20	NM_001247997	NM_004304	Low
ALK	EML4	ALK	20	20	NM_001145076	NM_004304	Low
RET	BCR	RET	3	12	NM_004327	NM_020630	Low
NRG1	SDC4	NRG1	4	4	NM_002999	NM_00115999	Low

Main target	5' gene	3' gene	5' exon	3' exon	5' transcript	3' transcript	Likelihood of Detectability
ROS1	TFG	ROS1	3	35	NM_001007565	NM_002944	Low
FGFR1	CNTRL	FGFR1	38	10	NM_007018	NM_00117406	Low
ALK	KIF5B	ALK	24	20	NM_004521	NM_004304	Low
RET	PRKAR1A	RET	8	12	NM_001276289	NM_020630	Low
RET	KIF5B	RET	1	12	NM_004521	NM_020630	Low
ALK	TPM3	ALK	4	20	NM_001043351	NM_004304	Low
ALK	DCTN1	ALK	23	20	NM_001135040	NM_004304	Low
ALK	STRN	ALK	2	20	NM_003162	NM_004304	Low
NTRK1	TPM4	NTRK1	8	12	NM_001145160	NM_00100779	Low
ROS1	TPM3	ROS1	3	36	NM_001043351	NM_002944	Low
ALK	TPM4	ALK	8	20	NM_001145160	NM_004304	Low
ALK	RANBP2	ALK	18	20	NM_006267	NM_004304	Low

- Detection of the following fusions is not supported by the product due to issues with read mapping of the fusion junctions.

5' gene	3' gene	5' exon	3' exon
TRIM27	RET	3	12
NPM1	ALK	5	20
RANBP2	FGFR1	19	8

- Fusions where the breakpoint is in the body of an exon can be detected but require additional molecule support comparing to fusions with intronic breakpoints (>10 supporting molecules).
- Detection of FGFR4 fusions was not validated in clinical FFPE RNA samples due to rarity of such events. Feasibility of detecting FGFR4 fusions is based on the following:
    a. It was observed that targeted FGFR4 exons display relatively high molecular coverage across a range of clinical samples, providing support for the capability of the product to capture eventual FGFR4 fusion transcripts involving these exons.

b. Detection of a clinically relevant FGFR4 fusion was verified in silico (see above) to confirm that the limiting amount of data supporting the rearrangement in this target is correctly recognized by the SOPHiA DDM™ Dx ROS analytical pipeline.

c. Capability of detection of multiple fusion events of homologous genes FGFR1, FGFR2 and FGFR3 was confirmed both in clinical samples and in silico.

- Events in the regions for which the relevant fragment of the RNA is not pulled down by the probes cannot be detected by the product. Capturing transcripts can be limited by:

  a. INDELs that affect the hybridization of probes. This can lead to no detection or incorrect molecule count estimation.

  b. The presence of additional mutation(s) in the target region of the probe.

- Individual RNA molecules are discriminated by the algorithm based on the start-end coordinates of the aligned sequencing reads; it may occur that multiple, distinct molecules will yield reads with identical start-end coordinates and will be considered as a single molecule. In such cases, the number of detected molecules will be underestimated.

- IGH is a clinically relevant fusion partner for the IVD genes as defined by COSMIC and fusions that involve this gene are reported with genomic coordinates. This gene is extremely polymorphic and considered a "multi-gene locus", without a RefSeq transcript assigned to it. As there is no unambiguous transcript assigned to this gene, fusion events including it cannot be properly annotated.

- Readthrough fusions that occur when two adjacent genes are spliced together are not reported if the distance of the spliced regions is less than 100 kb (see Fusion and Exon Skipping Filtering and Reporting).

- In rare cases, sample quality can be wrongly estimated for samples with atypically high or low expression of the control gene.

- Presence of extremely high amount of a particular transcript targeted by the assay may decrease molecular coverage at remaining targets and, consequently, decrease assay performance. Please refer to Annex I: coverage plot in the QA Report to assess this scenario.

10. REFERENCES

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11. SYMBOLS

Symbol	Title
	Consult instructions for use
	Catalog number
	Batch code (Lot Number)
	Caution
	Manufacturer
	Temperature Limit
	Use-by date
	European Conformity
	Authorized Representative in the European Community
	Contains sufficient for <n> tests
	Importer
	Refer to **Section 5 - Warnings and Precautions.**
	Refer to **Section 5 - Warnings and Precautions.**
	Refer to **Section 5 - Warnings and Precautions.**
	Refer to **Section 5 - Warnings and Precautions.**
	Refer to **Section 5 - Warnings and Precautions.**
	Box 1
	Box 2

12. SUPPORT

In case of difficulty using SOPHiA DDM™ please consult the “Troubleshooting” section of the SOPHiA DDM™ Dx mode User Manual or contact our support line by telephone at +41 21 694 10 60 or e-mail support@sophiagenetics.com. Please visit www.sophiagenetics.com for further details. Support may also be reached via web request from the Dashboard screen in the Support section of SOPHiA DDM™.

Any serious incident occurring in relation to the device should be promptly reported to SOPHiA GENETICS and the competent authorities of the member state, where the user and/ or the patient is established.

Do not use components that are damaged. Contact support@sophiagenetics.com if there are any concerns with the kits.

13 TROUBLESHOOTING

Table 8. Recommendations in case of a "WARNING" Quality Status in the SOPHiA DDM™ Dx ROS IVD Report for a sample due to insufficient (<50 fold) control gene coverage.

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATIONS FOR MITIGATION
Insufficient quantity of input RNA	• Total library yield of <25 ng/ml • Captured library yield of <10 ng/ml	• Using a fluorometric quantification method, validate that a minimum input quantity of 50 ng of total RNA is used per sample.
Low library conversion rate (rate of conversion of input RNA into molecules compatible for sequencing)	• Total library yield of <25 ng/ml • Captured library yield of <10 ng/ml	• Ensure that the input RNA is free of substances that inhibit enzymatic activity by following the storage buffer composition recommendations defined in Section 5.2.1. • Ensure that RNA is not excessively fragmented (e.g., due to contamination with RNases) before or during protocol execution by adhering to the minimum RNA integrity thresholds and RNA handling best practices defined in Section 5.2.1. • Minimize the risk of chemical modification of input RNA by employing standard histological deparaffinization protocols as recommended in Section 5.2.1. If necessary, reduce Formalin fixation durations.
Poor balancing of samples multiplexed on the same sequencing run resulting in <3 M reads per sample	• Within the QA Report, compare the number of reads between samples sequenced on the same run and check if the read distribution within the run is balanced or if outlier samples exist that display significantly higher or lower read counts than average.	• If a significant imbalance in the number of reads between the samples is observed, repeat the quantification and pooling of the individual sample libraries to ensure they have the same molarity before repeating the sequencing run.
Poor data yield of the sequencing run resulting in <3 M reads per sample	• Within the QA Report, check the total number of reads produced by the sequencing run. Compare this with the expected number of reads as defined by the manufacturer (Illumina®): ~260 M paired-end reads for Mid-Output kits ~800 M paired-end reads for High-Output kits (CDS only)	• Check that the relevant sequencing run metrics (e.g., Raw Cluster Density, Clusters Passing Filter, % Aligned and Q30 distribution) are within normal ranges to identify potential technical problems with the sequencer or the used sequencing consumables and contact the manufacturer's (Illumina®) technical support if necessary. • Repeat the sequencing run with adjusted final library loading concentration to avoid under- or over-clustering.

14. APPENDIX II-UNIQUE DUAL INDEX PRIMER PLATES

32 Illumina®-compatible Unique Dual Index Primers V2 in 96-well plate format (7 µl each)

	1	2	3	4	5	6	7	…	12
A	sgUDI-49	sgUDI-57	sgUDI-65	sgUDI-73					
B	sgUDI-50	sgUDI-58	sgUDI-66	sgUDI-74					
C	sgUDI-51	sgUDI-59	sgUDI-67	sgUDI-75					
D	sgUDI-52	sgUDI-60	sgUDI-68	sgUDI-76					
E	sgUDI-53	sgUDI-61	sgUDI-69	sgUDI-77					
F	sgUDI-54	sgUDI-62	sgUDI-70	sgUDI-78					
G	sgUDI-55	sgUDI-63	sgUDI-71	sgUDI-79					
H	sgUDI-56	sgUDI-64	sgUDI-72	sgUDI-80					

Index sequences for the Illumina®-compatible Unique Dual Index Primers

Index	i5 sequences for sample sheet MiSeq	i5 sequences for sample sheet NextSeq	i7
sgUDI-49	GCTCACTG	CAGTGAGC	ACCAAGGA
sgUDI-50	CAGGATTG	CAATCCTG	CAGACCTG
sgUDI-51	GTCTAGTT	AACTAGAC	CGAGCAAC
sgUDI-52	TGAATGGC	GCCATTCA	TCTTGACT
sgUDI-53	ACGACAAT	ATTGTCGT	GACAATGG
sgUDI-54	GAACGCCA	TGGCGTTC	GTTCTACG
sgUDI-55	CTGTCCTG	CAGGACAG	AACGCTGC
sgUDI-56	ATAAGGAC	GTCCTTAT	GGACATCA
sgUDI-57	TACATTCC	GGAATGTA	TTGAGCTC
sgUDI-58	GGTTAGCT	AGCTAACC	ACGTTGAG
sgUDI-59	CCTGCTGA	TCAGCAGG	CTTCAGGA
sgUDI-60	CCTCAATC	GATTGAGG	TGCCAACT
sgUDI-61	CGAAGAAT	ATTCTTCG	AGGTCATG
sgUDI-62	TAGTCGAG	CTCGACTA	TACTAGCA
sgUDI-63	ATCCTCAC	GTGAGGAT	GTAACTGT
sgUDI-64	GCTGCAGT	ACTGCAGC	TGAGTTGA

Index	i5 sequences for sample sheet MiSeq	i5 sequences for sample sheet NextSeq	i7
sgUDI-65	GGCAATCG	CGATTGCC	CCTTAGAC
sgUDI-66	GCATCTTA	TAAGATGC	TATCGCCA
sgUDI-67	GTGCTGAA	TTCAGCAC	GCAGAACA
sgUDI-68	ATTGACGC	GCGTCAAT	AGGAATGC
sgUDI-69	GCCGCATT	AATGCGGC	CGTGAGGT
sgUDI-70	TGTAGTCA	TGACTACA	CAATTCAG
sgUDI-71	TCCTCCGA	TCGGAGGA	GGTCCTTC
sgUDI-72	CAATCGAG	CTCGATTG	TTCCGGCA
sgUDI-73	TAAGTCCG	CGGACTTA	ATGCCTGA
sgUDI-74	GGACAGTT	AACTGTCC	TCCAGGAC
sgUDI-75	TTGAGTGA	TCACTCAA	GCTGTCAC
sgUDI-76	CGTAACAT	ATGTTACG	CGACGATT
sgUDI-77	TACGCAGT	ACTGCGTA	TCGCAACG
sgUDI-78	ACCTGACC	GGTCAGGT	GAGTTGTA
sgUDI-79	CCACCTGA	TCAGGTGG	CGCTAAGG
sgUDI-80	TCACCGTG	CACGGTGA	TTGCGTGC

Document Approvals

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Approval Verdict: Approve	Coleman Spence, (cspence@sophiagenetics.com) Regulatory Approval 28-Jan-2026 01:10:56 GMT+0000

Approval Verdict: Approve	Slawomir Kubik, (skubik@sophiagenetics.com) Technical Approval 28-Jan-2026 17:18:45 GMT+0000

QA Approval Verdict: Approve	Nora Kormos, Quality Specialist (nkormos@sophiagenetics.com) Quality Assurance Approval 29-Jan-2026 14:39:56 GMT+0000