INSTRUCTIONS FOR USE

16, 32, 48 AND 96 SAMPLES

SOPHiA DDM™ Dx Solid Tumor Solution

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

SOPHiA GENETICS™

SUMMARY INFORMATION

Product Name	SOPHiA DDM™ Dx Solid Tumor Solution
Product Type	Bundle Solution
Product Family	Molecular diagnostic application (kit + analytics)
Algorithm ID	ILL1XG1S8_FFPE_CNV
Gene Panel ID	STS_v1
Product Version	1.0
Sample Type	Somatic DNA isolated from formalin-fixed, paraffin embedded (FFPE) tumor tissue specimens
Sequencer	Illumina - MiSeq
GMDN Description	Reagent kit IVD / Human genomic analysis interpretive software
Document ID	SG-00660
Document Version	v8.0
Revision Date	January 2026

This Instructions For Use (IFU) is applicable for all SOPHiA DDM™ Dx versions.
Please read the IFU thoroughly before using this product.

SOPHiA GENETICS SA
ZA La Pièce 12
1180 Rolle
Switzerland

SOPHiA GENETICS SAS
Technopole Izarbel
158 allée Fauste d'Elhuyar
F-64210 Bidart, France

EC REP

REF BS0105ILLCSML03-016
BS0105ILLCSML03-032
BS0105ILLCSML03-048

CE IVD

PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	LIBRARY PREPARATION KIT
REF	BS0105ILLCSML03-016	B1.01.0005.C-16	B2.0005.C-16	_
	BS0105ILLCSML03-032	B1.01.0005.C-32	B2.0005.C-32	
	BS0105ILLCSML03-048	B1.01.0005.C-48	B2.0005.C-32	

DISCLAIMER

This document and its contents are the property of SOPHiA GENETICS SA and its affiliates ("SOPHiA GENETICS") and are intended solely for the contractual use by its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced, or referenced to in any way whatsoever without the prior written consent of SOPHiA GENETICS.

SOPHiA GENETICS does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document. The instructions in this document must be strictly and explicitly followed by qualified and adequately trained personnel to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood before using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

SOPHiA GENETICS DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE).

TRADEMARKS

Illumina® and MiSeq® are registered trademarks of Illumina, Inc.

AMPure® and Agencourt® are registered trademarks of Beckman Coulter, Inc.

Qubit® and Dynabeads® are registered trademarks of Thermo Fisher Scientific Inc.

KAPA™ is a trademark of Roche.

QIAseq® is a registered trademark of Qiagen.

SOPHiA GENETICS™and SOPHiA DDM™ Dx are trademarks of SOPHiA GENETICS SA and/or its affiliate(s) in the U.S. and/or other countries. All other names, logos, and other trademarks are the property of their respective owners. UNLESS SPECIFICALLY IDENTIFIED AS SUCH, SOPHiA GENETICS USE OF THIRD-PARTY TRADEMARKS DOES NOT INDICATE ANY RELATIONSHIP, SPONSORSHIP, OR ENDORSEMENT BETWEEN SOPHiA GENETICS AND THE OWNERS OF THESE TRADEMARKS. Any references by SOPHiA GENETICS to third party trademarks are to identify the corresponding third-party goods and/or services and shall be considered nominative fair use under the trademark law.

REVISION HISTORY

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
SG-00660 – 8.0	January 2026	• Change to version numbering system, no additional versions between 5.5 and 8.0. • Section 4: Removal of section detailing SOPHiA DDM Desktop App. • Section 4: Revision of typo and correction with “detecting variants in tumor-related gene.” • Warnings and Precautions: Addition of CAS number and concentration for each hazardous substance identified. • Section 6: Removal of note regarding research use only functionalities. • Section 7: Removal of Warnings and limitations regarding out-of-scope components: large indels, amplification detection, and MSI status.
SG-00660 – 5.5	23.May.25	• Appendix 1: Removed the table “16 Illumina®-compatible Dual Index Adapters in 96-well plate format (7 µl each)” table from Appendix I due to discontinuation of 16 dual index adapters plate format. • Section 5.1.1 Kit Content – BOX 1: Updated to reflect the change above.
SG-00660 – 5.4	19.Mar.25	• 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-00660 – 5.3	14.Aug.24	• "SOPHiA DDM™ Web App" changed to"SOPHiA DDM™ Dx mode" • Minor rephrasings related to the change above
SG-00660 – 5.2	08.Jul.24	• Updated the EC REP address • Reduced content volume of SOPHiA GENETICS hybridization probes from 20 µl to 18 µl (see section *5.1.1 Kit Content – BOX 1*) • Removed third-party provider's intellectual property from sections *5.1.1 Kit Content*, *5.3.1 Library Pooling*, and *5.3.2 Hybridization*
SG-00660 – 5.1	09.Jan.23	• Correction of formatting errors.
SG-00660 – 5.0	14.Sep.22	• SOPHiA DDM™ and SOPHiA GENETICS trademark symbol added. • Document version upgraded to next full integer without any decimal.
SG-00660 – 4.5	19.Apr.22	• SOPHiA DDM™ Web App instructions added
SG-00160 – 4.4	14.Apr.22	• Limitations and Warnings: Modified • SOPHiA GENETICS Office address updated • Wet Lab changes as recommended globally • Minor changes and corrections to typos
ID-60101-20 – 4.3	21.Jul.21	• Page 2, 32- Cosmetic changes • Page 19 - Step order changed. • Page 28 - "48-sample" PCR pre-mix volume corrected.
ID-60101-20 – 4.2	16.Jun.21	• Page 3 - Trademark modified. • Page 47 - Minor addition in file specifications. Page 56, 57 - Heading typo 'Unique' removed.
ID-60101-20 – 4.1	28.May.21	• Page 2 - Summary information table modified. • Page 3 - Disclaimer modified. • Page 11, 15, 16, 32 - Table header modified.

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
		• Page 17 - Step 1 and 2 sequence exchanged. • Page 19 - Ethanol preparation moved under 'Preparation'. • Page 12, 20, 24, 25, 26, 27, 29, 38, 39, 40, 41, 46 - Cosmetic changes and typos. • Page 49 - Bullet point 1,2 and 16 added.
ID-60101-20 – 4.0	30.Mar.21	• Title page, Company logo, Header, Footer, Last page. • Reorganized the topics. • Included SOPHiA DDM™ Web App installation, upload and naming convention instructions. • Combined four kit size "Instructions For Use" together to include different sample numbers. Included tables and made appropriate changes as and when necessary for this purpose. • Following Kit "Instructions For Use" documents were combined: PM_CEIVD_B2.1.1.20_r2en PM_CEIVD_B2.1.1.21_r2en PM_CEIVD_B2.1.1.22_r2en PM_CEIVD_B2.1.1.25_r1en • Minor changes for clarity in the following sections: • Section 5.3.1 Library Pooling • Section 5.3.5 Wash Streptavidin Beads to Remove Unbound DNA

TABLE OF CONTENTS

1. INTENDED USE/PURPOSE

The product is intended to be used to identify variants occurring in 42 genes involved in solid tumors by targeting specific mutation-prone positions within the genomic sequence. The function of the product is to serve as an aid to healthcare professionals to make a clinical decision related to solid tumors, and to provide molecular rationale for appropriate therapy.

The product is intended to be used for in vitro diagnostic and professional use only.

2. SUMMARY AND EXPLANATION OF THE TEST

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

The following table shows genes that are targeted by the SOPHiA DDM™ Dx Solid Tumor Solution (STS) and the tumor types that are associated with those genes.

Table 1. Genes targeted by the SOPHiA DDM™ Dx Solid Tumor Solution (STS)

AKT1	FGFR3	MET
ALK	FOXL2	MYOD1
BRAF	GNA11	NRAS
CDK4	GNAQ	PDGFRA
CDKN2A	GNAS	PIK3CA
CTNNB1	H3F3A	PTPN11
DDR2	H3F3B	RAC1
DICER1	HIST1H3B	RAF1
EGFR	HRAS	RET
ERBB2	IDH1	ROS1
ERBB4	IDH2	SF3B1
FBXW7	KIT	SMAD4
FGFR1	KRAS	TERT
FGFR2	MAP2K1	TP53

3. GENERAL STATEMENT OF THE TEST PRINCIPLE(S)/ PROCEDURE

The validated function of the SOPHiA DDM™ Dx Solid Tumor Solution (STS) analytics is to analyze raw NGS data generated by an Illumina® MiSeq® instrument with MiSeq® Reagent Kit v3, on somatic samples (FFPE) with the KAPA™ Library Amplification Kit and KAPA™ HyperPlus Kit.

The SOPHiA DDM™ Dx STS involves three main steps. The first step is to qualify the DNA sample that can be used for the test. The second is to manually prepare the samples for sequencing, which is called library preparation. Library preparation consists of seven key steps: DNA fragmentation, adapters ligation, PCR amplification of individual libraries, library pooling, probes hybridization, capture, and post- capture PCR amplification. The third procedure is to sequence the prepared sample using SBS (sequencing by synthesis) chemistry on the Illumina® MiSeq® sequencer.

For analysis, the results should be uploaded to the SOPHiA DDM™ Dx mode platform and analyzed using the SOPHiA DDM™ Dx STS application.

4. PRODUCT COMPONENTS

SOPHiA DDM™ Dx STS is composed of two components: the NGS kit and the bioinformatics pipeline used in combination with an IVD accessory, the cloud-based SOPHiA DDM™ Dx mode.

- The purpose of the NGS kit is to prepare and enrich DNA libraries from somatic samples (FFPE) suitable for sequencing on an Illumina® MiSeq® sequencer. The NGS kit allows users to generate targeted sequencing data. The elements are described in the following section 5. Kit Materials and Methods - 5.1. Initial Considerations - 5.1.1 Kit Content.

- The bioinformatics pipeline (“STS pipeline”) processes the raw NGS data via algorithms capable of detecting variants in tumor-related gene.

- SOPHiA DDM™ Dx mode is a front-end web-based application available as a “software-as-a-service” (SaaS) used to generate a downloadable report for genes mentioned in Table 1 for SNVs and INDELs. Limitations apply - please see section 7 - Limitations, Warnings and Precautions.

5. KIT MATERIALS AND METHODS

5.1. Initial Considerations

Please ensure that all tubes are physically intact and stored as per the 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 (16, 32, 48 or 96 samples)

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

Depending on the kit format, the following components are provided:

COMPONENT	NUMBER OF ITEMS DEPENDING ON KIT FORMAT			
	16 samples kit	32 samples kit	48 samples kit	96 samples kit
BOX 1	1	1	1	2 (48 samples each)
Illumina®-compatible Adapters with Dual Index (in a 96-well plate format included in Box 1)	32	32	48	96 (Plate contained in one of the two Box 1s)
BOX 2	1	1	1	2 (48 samples each)

BOX 1 (STORE AT -25°C TO -15°C)

- Universal Blockers - TS Mix (12 µl)
- Human Cot DNA (25 µl)
- Solid Tumor Solution 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)
- Depending on the kit format: 32, 48 or 96 Illumina®-compatible Adapters with Dual Index in a 96-well plate format (7 µl each): see Appendix 1 for adapters display and sequences.

BOX 2 (STORE AT +2°C TO +8°C)

- Dynabeads® M-270 Streptavidin (440 µl)
- Agencourt® AMPure® XP (3 x 1.5 ml for 16 samples, 8.7 ml for 32 samples and 11.6 ml for 48 samples, see Note for 96 samples)
- IDTE Low TE Buffer (10 ml)
- Nuclease-free water (20 ml)

Note: For 96 samples, two times Box 2 of 48 samples is provided (see the table on the previous page).

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. • 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.	Danger	Formamide Concentration: 100% CAS: 75-12-7

Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
		• 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. • P501 Dispose of contents/ container in accordance with national regulations.	Danger	Sodium dodecyl sulfate Concentration: 4.9% CAS: 151-21-3

Please use  and  as personal protective equipment.

5.1.2. Material Required (not provided)

USER-SUPPLIED MATERIALS (TO BE PURCHASED SEPARATELY)

- KAPA™ HyperPlus Kit KK8512 for 24 reactions (Roche Cat. No: 07962401001) or KK8514 for 96 reactions (Roche Cat. No. 07962428001) - recommended due to extra volume of reagent provided in this kit format
- KAPA™ Library Amplification kit KK2620 (Roche Cat. No: 07958978001)
- 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

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

- Table top microcentrifuge (8-tube strips compatible)
- Thermal cycler (programmable heated lid)
- Vortex mixer

5.2. Library Preparation

FFPE 5.2.1. DNA Preparation

MATERIALS

- Formalin-fixed paraffin-embedded tissue extracted DNA (FFPE DNA)
- KAPA™ Frag Conditioning Solution
- IDTE
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

IMPORTANT

The quality of FFPE extracted DNA is variable and might impact sequencing data. Exposure to formalin damages integrity of the molecule by generating DNA fragmentation. It also induces sequencing artefacts due to deamination events.

We highly recommend using the maximum amount of DNA stated in the protocol to generate high- quality sequencing data.

Depending on the amount of DNA used, adjust the number of PCR cycles according to the following table:

TABLE 1. CORRELATION BETWEEN DNA INPUT AND REQUIRED PCR CYCLES (5.2.7 LIBRARY AMPLIFICATION)

Amount of FFPE DNA (ng)	10 ≤ n < 50	50 ≤ n < 100	100 ≤ n < 200
PCR cycles	12	10	8

In order to avoid mistakes with DNA input, an initial dilution to obtain a concentration in the 50 to 100 ng/µl range is recommended. The DNA concentration should be confirmed by a fluorometric quantitation (e.g., Qubit®, Thermo Fisher) and the obtained value used to calculate the final dilution.

PREPARATION

Remove the KAPA™ Frag Conditioning Solution from -20°C storage and thaw at room temperature. After thawing, mix the reagent by gently inverting the tube 5 times and briefly spin in a microcentrifuge.

Depending on the kit format, the number of DNA samples to be pooled per capture reaction will vary according to the following table. This must be taken into consideration before starting.

KIT FORMAT	16 samples kit	32 samples kit	48 samples kit	96 samples kit*
Number of individual libraries per capture	4	8	12	12

* For 96 samples two 48 sample kits are provided, which includes 8 capture reactions.

PROCEDURE

1. Prepare the following PCR strips according to the number of reactions:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip	4-tube	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
Number of strips	1	2	3	2	3	4	6

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

FFPE DNA DILUTION	
FFPE DNA	Amount of FFPE DNA (cf. table 1)
IDTE	Complete to 25 µl

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

Tip: Safe stopping point overnight at 4°C.

3. Prepare the following dilution for the KAPA™ Frag Conditioning Solution:

KAPA™ FRAG CONDITIONING SOLUTION							
Number of Reactions	4	8	12	16	24	32	48
Frag Conditioning Solution (in µl)	3.1	3.1	3.1	6.2	6.2	9.3	12.4
Nuclease-free water (in µl)	196.9	196.9	196.9	393.8	393.8	590.7	787.6

4. FFPE DNA preparation for the fragmentation reaction:
    - Depending on the number of samples, proceed as follows:
        - If processing **4 samples**, add 10 µl diluted KAPA™ Frag Conditioning Solution to each tube of the 4-tube strip containing 25 µl FFPE DNA samples (total of 35 µl in each tube of the 4- tube strip).
        - If processing **8 or more samples**, proceed as follows:
            a. To facilitate pipetting, create a reservoir of diluted KAPA™ Frag Conditioning Solution by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
Diluted KAPA™ Frag Conditioning Solution (in µl)	30	45	30	45	50	75

            b. Using a multichannel pipette, add 10 µl of the diluted KAPA™ Frag Conditioning Solution from the above tubes to the 25 µl of FFPE DNA samples (total of 35 µl in each tube of the 4 or 8-tube strips).
    - Using a multichannel pipette set to 20 µl, mix gently by pipetting up and down 5 times and briefly spin in a microcentrifuge.
5. Keep on ice until enzymatic fragmentation reaction setup.

5.2.2. Pre-mixes and Reagents Preparation

COMPONENTS AND REAGENTS

- KAPA™ Frag Enzyme
- KAPA™ Frag Buffer 10x
- Ligation Buffer
- End Repair & A-tailing Buffer
- End Repair & A-tailing Enzyme Mix
- DNA Ligase
- KAPA™ HiFi HotStart Ready Mix 2x
- Library Amplification Primer Mix 10X
- Nuclease-free water
- AMPure® XP beads
- Ethanol

PREPARATION

- Remove the KAPA™ HyperPlus kit components from -20°C storage and thaw on ice.
- Remove the Dual Index Adapters 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.
- Prepare fresh 80% Ethanol (volume according to the following scheme based on number of reactions):

80% ETHANOL							
Number of Reactions	4	8	12	16	24	32	48
80% Ethanol (in ml)	10	20	30	30	40	50	70

- Once the KAPA™ HyperPlus kit components are thawed, mix the reagents by inverting the tube 5-10 times and briefly spin in a microcentrifuge.

PRE-MIXES

Important: Volumes listed in the following tables are calculated based on reagents supplied in the KAPA™ HyperPlus (24 reactions). Extra reagents volumes provided in the KAPA™ HyperPlus (96 reactions) allow to prepare an additional 5% volume for every mix listed below.

1. Prepare the **Frag pre-mix** as follows:

FRAG PRE-MIX							
Number of Reactions	4	8	12	16	24	32	48
KAPA™ Frag Buffer 10x (in µl)	22	44	65	88	130	176	260
KAPA™ Frag Enzyme (in µl)	44	88	130	176	260	352	520

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

2. Prepare the **End Repair & A-tailing (ER&AT) pre-mix** as follows:

END REPAIR & A-TAILING (ER&AT) PRE-MIX							
Number of Reactions	4	8	12	16	24	32	48
ER&AT Buffer (in µl)	30.8	61.6	92.4	123.2	186.7	246.4	373.4
ER&AT Enzyme (in µl)	13.2	26.4	39.6	52.8	80	105.6	160

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

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

LIGATION PRE-MIX							
Number of Reactions	4	8	12	16	24	32	48
Ligation Buffer (in µl)	132	264	396	528	810	1056	1620
DNA Ligase (in µl)	44	88	132	176	270	352	540
Nuclease-free water (in µl)	22	44	66	88	135	176	270

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

Important: The Ligation Buffer is highly viscous, pipette gently and make sure to obtain a homogeneous Ligation pre-mix.

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

PCR PRE-MIX							
Number of Reactions	4	8	12	16	24	32	48
KAPA™ HiFi HotStart ReadyMix 2x (in µl)	110	220	330	440	675	880	1350
Library Amplification Primer Mix 10x (in µl)	11	22	33	44	67.5	88	135
Nuclease-free water (in µl)	11	22	33	44	67.5	88	135

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

5.2.3. Enzymatic Fragmentation

MATERIALS

- Diluted and conditioned double stranded FFPE DNA in 35 µl
- Frag pre-mix
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

- Preheat the thermal cycler to 37°C (set lid to 45°C).

PROCEDURE

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

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, to facilitate pipetting, create a reservoir of Frag 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	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
Frag pre-mix (in µl)	32	48	32	48	65	96

2. Assemble the reaction as follows:
    - Using a multichannel pipette if processing 8 or more samples, add 15 µl of Frag pre-mix to each of the 35 µl of FFPE DNA samples (total of 50 µl in 4 or 8-tube strips).
    - Using a pipette set to 40 µl (multichannel if processing 8 or more samples), mix thoroughly by pipetting up and down 5 times and briefly spin in a microcentrifuge.
3. Incubate in the thermal cycler at 37°C for 20 minutes.
   Put on ice immediately to stop the fragmentation reaction.

Proceed immediately to End Repair and A-tailing.

5.2.4. End Repair and A-Tailing

MATERIALS

- Fragmented FFPE DNA in 50 µl
- End Repair and A-Tailing (ER&AT) pre-mix

PREPARATION

- Preheat the thermal cycler to 65°C (set lid to 75°C).

PROCEDURE

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, to facilitate pipetting, create a reservoir of ER&AT 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	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
ER&AT pre-mix (in µl)	21.5	32.5	21.5	32.5	43	65

2. Assemble the reaction as follows:
    - Using a multichannel pipette if processing 8 or more samples, add 10 µl of ER&AT pre-mix to each of the 50 µl of FFPE DNA samples (total of 60 µl in 4 or 8-tube strips).
    - Using a pipette set to 40 µl (multichannel if processing 8 or more samples), mix thoroughly by pipetting up and down 5 times and briefly spin in a microcentrifuge.
3. Incubate in the thermal cycler at 65°C for 30 minutes then put on ice immediately.

Proceed immediately to Ligation.

5.2.5. Ligation

MATERIALS

- ER&AT reaction products in 60 µl each
- Ligation pre-mix
- Dual Index Adapters
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

- Remove the Dual Index Adapters plate from 4°C (transferred from -20°C to 4°C earlier) and briefly spin the plate to collect all the liquid. Refer to Appendix 1 for the respective plate format.
- During the ER&AT incubation, prepare new PCR strips with 5 µl of different Dual Index Adapters per tube as per your indexing strategy, according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR Strip	4-tube	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
Number of strips	1	2	3	2	3	4	6

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

PROCEDURE

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, 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	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
Ligation pre-mix (in µl)	100	160	100	160	200	320

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 Dual Index Adapters.
3. Mix thoroughly by pipetting up and down 10 times and spin briefly.
4. Using a multichannel pipette if processing 8 or more samples, add 45 µl of Ligation pre-mix to each ER&AT reaction product (65 µl in each tube of the 4 or 8-tube strip).
5. 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).

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.

Proceed to Post Ligation Clean Up.

5.2.6. Post-Ligation Clean Up

MATERIALS

- Ligation reaction products in 110 µl each
- 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 88 µl of AMPure XP beads to each of the 110 µl ligation reaction products. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly to collect all liquid.
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 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.
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.

5.2.7. Library Amplification

MATERIALS

- Ligated reaction products and beads resuspended in 20 µl of IDTE each
- PCR pre-mix

PREPARATION

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

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	
Step 1: Initial denaturation	98	45	
Step 2: Denaturation	98	15	
Step 3: Annealing	60	30	n cycles*
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

* Follow the table 1 (section 5.2.1 FFPE DNA Preparation) to determine the number of PCR cycles based on the amount of starting material.

PROCEDURE

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, proceed as follows:
        a. To facilitate pipetting, create a reservoir of PCR 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	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
PCR pre-mix (in µl)	65	100	65	100	130	200

2. Assemble the reaction as follows:

- Using a multichannel pipette if processing 8 or more samples, add 30 µl of PCR pre-mix to the ligated reaction products and beads (total volume 50 µl = 30 µl + 20 µl).
- Mix thoroughly by pipetting up and down 10 times and spin briefly.

3. Place the tubes in the thermal cycler and run the LibAmp program.

Tip: Safe stopping point overnight at 4°C.

5.2.8. 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 to collect all liquid.
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 it/them stand for 30 seconds to 1 minute.
6. Carefully discard the ethanol using a multichannel pipette.
7. Repeat steps 5 and 6 once.
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 collect all liquid.

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.

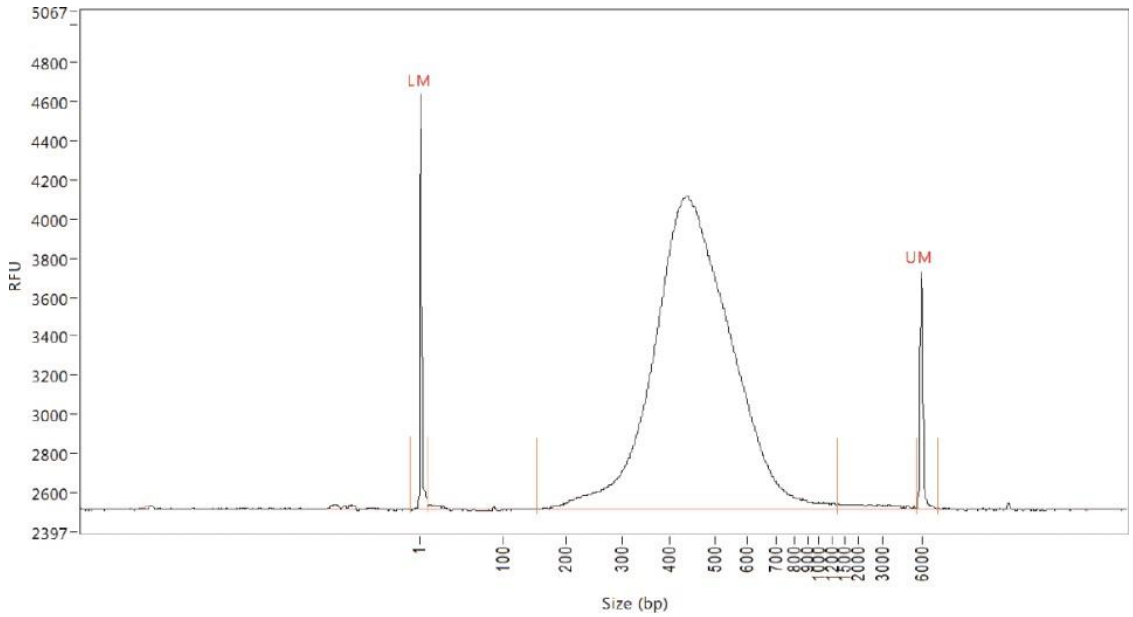
5.2.9. Individual Library Quantification and Quality Control

MATERIALS

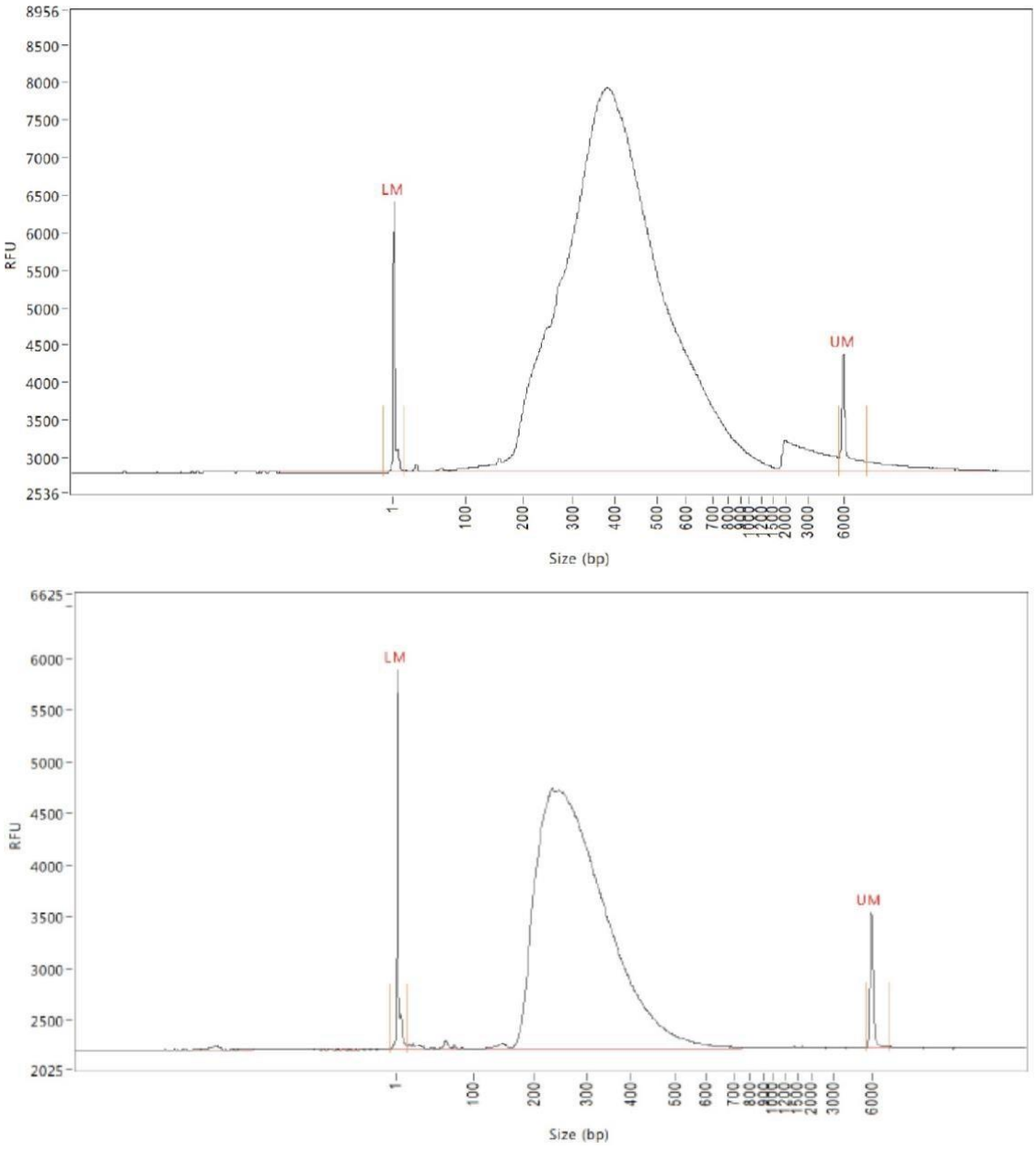
- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

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.



Example of a DNA library distribution obtained with the Agilent Fragment Analyzer capillary electrophoresis system. UM – Upper Marker, LM – Lower Marker



Examples of a DNA library distribution obtained with the Agilent Fragment Analyzer capillary electrophoresis system. UM: Upper Marker , LM : Lower Marker

5.3. Capture

5.3.1. Library Pooling

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 (Refer to the table in point 3)	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. To the individual tubes containing the above pre-mix, add a pool of individual libraries according to the kit format:

KIT FORMAT	16 samples kit	32 samples kit	48 samples kit	96 samples kit
Number of individual libraries per capture	4	8	12	12
Amount of each library per capture	300 ng	200 ng	150 ng	150 ng
Total amount of libraries per capture	1200 ng	1600 ng	1800 ng	1800 ng

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.

Proceed to Hybridization to perform an overnight incubation.

5.3.2. Hybridization

MATERIALS

- Lyophilized libraries
- 2x Hybridization Buffer
- Hybridization Buffer Enhancer
- Solid Tumor Solution 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 IIIŸ
- 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 3 to 5 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 440 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.

5.3.3. 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.

5.3.4. 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 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.

5.3.5. 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

Important: Work to 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 1st incubation of 5 min (step 5), start a timer.
2. Begin processing the second tube.
3. When placing the second tube at 65°C, notice the time separating the tubes and ensure to respect this time gap along steps 2 to 8 in order 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 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.

5.3.6. Post-capture Amplification

MATERIALS

- Streptavidin beads/nuclease-free water suspension (20 µl)
- 2x KAPA™ HiFi HotStart ReadyMix
- 10x Library Amplification Primer Mix
- Nuclease-free water

PREPARATION

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

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	
Step 1: Initial Denaturation	98	45	
Step 2: Denaturation	98	15	15 Cycles
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
2x KAPA™ HiFi HotStart ReadyMix (in µl)	25	55	82.5	110
10x Library Amplification Primer Mix (in µl)	2.5	5.5	8.25	11
Nuclease-free water (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.

5.3.7. 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 collect all the liquid.
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 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 it/them 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 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 collect all liquid.
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.

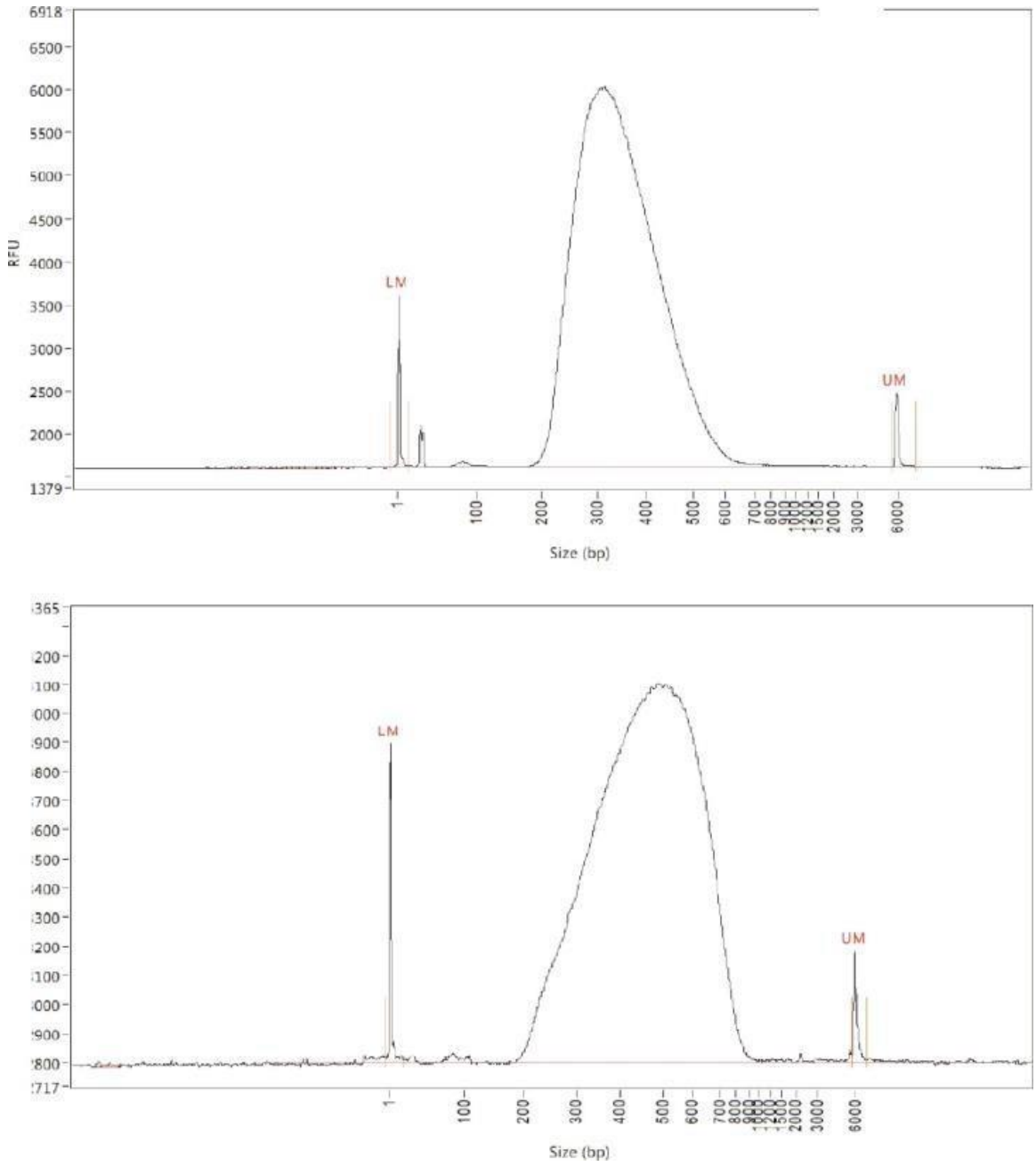
5.3.8. Final Library Quantification and Quality Control

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.



Examples of captured library pool size distribution obtained with the Agilent Fragment Analyzer capillary electrophoresis system. UM-Upper Marker, LM-Lower Marker

5.4. Sequencing

5.4.1. Library Preparation for Sequencing

MATERIALS

- Illumina MiSeq® Reagent Kit v3
- 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 5.3.8 as follows:

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

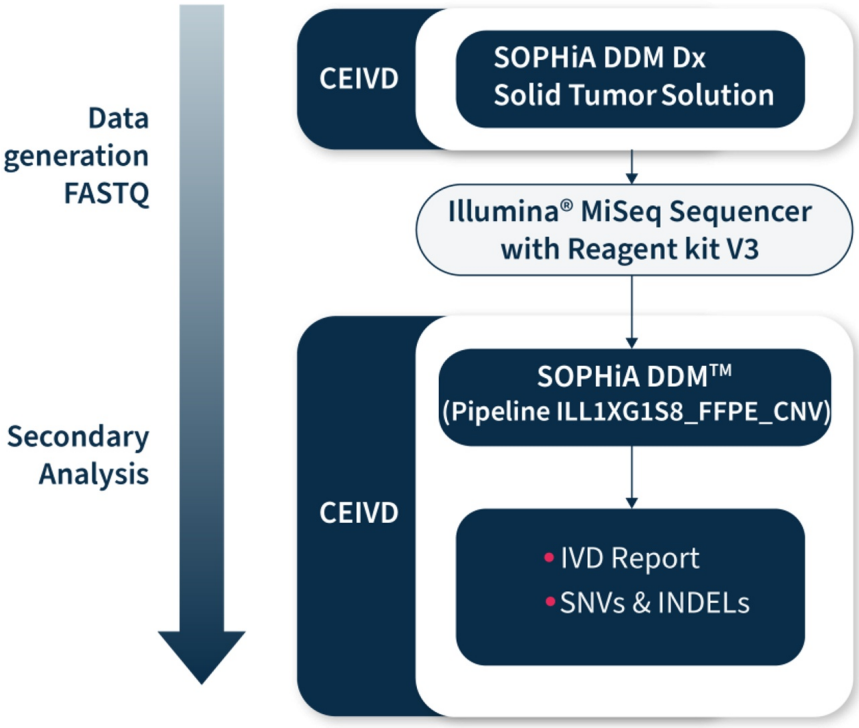
2. Dilute each pool to 4 nM and mix them in equal amount (e.g., 5 µl of each). Mix it well and use this dilution according to Illumina® standard denaturation recommendation.
3. Load a 10 pM dilution of the denatured libraries on MiSeq®.
4. The recommended minimum reads are 2.1 million reads per sample, with a read length 150 bp.

6. ANALYSIS PROCEDURE

6.1. SOPHiA DDM™ Dx Mode Installation Instructions

No installation is necessary for using SOPHiA DDM™ Dx mode. An email will be provided with instructions and a link to SOPHiA DDM™ Dx mode. Please refer to the SOPHiA DDM™ Dx mode User Manual for information on account management, browser compatibilities and other important notices. Support documents are also available through SOPHiA DDM™ Dx mode directly.

6.2. Analysis Workflow Description for IVD Results Generation



Analysis workflow description for the SOPHiA DDM™ Dx Solid Tumor Solution

Please refer to the SOPHiA DDM™ Dx mode User Manual for the full description of the upload workflow.

7. LIMITATIONS, WARNINGS AND PRECAUTIONS

GENERAL WARNINGS

- For detailed instructions on the software, refer to the SOPHiA DDM™ Dx mode user manual.
- If any part of the handling, protocol, sequencer, multiplexing etc. is changed, the analyzes are not covered by the described instructions for use.
- The data provided in the Quality Report (available for download from the SOPHiA DDM™ Platform) is for information only and is not intended to be used for diagnosis.
- The accuracy of the results of the analysis cannot be guaranteed. Sequencing laboratories need to fulfill quality checks of the samples and flag the unqualified samples. Unqualified samples (e.g., insufficient biopsy sample) could lead to compromised results. SOPHiA GENETICS is not liable for the results and consequent decisions taken on the basis of these results.
- Good laboratory practice standards and procedures in addition to strictly following the IFU is required in order to obtain proper performance of the product. For specific safety information, please refer to the corresponding Material Safety Data Sheets (MSDS) provided with each component of the product.
- Physically separated pre- and post- PCR rooms should be defined to prevent DNA sample contamination. Always use fresh reagents, correctly extracted and stored DNA. For details on DNA quality and integrity see IFU Section 5. Kit Materials and Methods - Section 5.2.1 Genomic DNA Preparation.
- Correctly calibrated pipettes and proper lab equipment should be used to perform experiment.
- Different lot numbers of reagents should not be mixed.
- Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.
- 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.

GENERAL LIMITATIONS

- Poor quality of the data due to issues in the sample preparation or sequencing step can confound the data analysis and cause False Positives and/or False Negatives.
- The absence of a variant in the report does not rule out the presence of a variant below the limits of detection of the assay.

SNV / INDELs

- Variant detection in this product has been optimized for SNVs and short INDELs (up to 1/2 of the read length) detection. Please note, that any other type of alteration can be missed by the algorithm.

1. Gene fusions or inversions cannot be detected.
2. Deletions or insertions with a breakpoint outside of the target region might not be detected.

- Variant detection in this product has been optimized on the regions defined as “target regions”. Please note that regions outside this definition might present false negatives and/or false positives.
- For robust performance of the algorithm, we recommend a coverage of at least 1,000x to meet statistical significance compared to noise for most positions. Lower coverage will increase the risk of False Negative variant calls significantly.
- Variants can be missed or wrongly called due to limitations in the kit design. Variants in the regions for which the relevant fragment of the DNA is not pulled out by the probes cannot be detected by the product. Capturing variants can be limited by:
    1. INDELs that affect the hybridization of probes. This can lead to no detection or incorrect variant fraction estimation.
    2. The presence of additional mutation(s) in the target region of the probe on the same allele.
- SNVs or INDELs in homopolymers with a length of ten base pairs or higher cannot be called confidently as their detection is confounded by high background noise.
- Complex Delins may be reported as multiple variants in case it is represented in the alignment as multiple smaller variants that are separated by more than 2 nucleotides.
- Flagged regions in Appendix 4 present characteristics which render variant detection unreliable. They are reported with warnings in the SOPHiA DDM™ interface, and variants detected in these regions are reported with a flag in the “Warnings” tab in SOPHiA DDM™. In addition, the reporting threshold is adapted to systematic errors which have been identified during internal evaluation.
- Regions with high sequence homology can cause uncertainty of mapping and risk of missing or calling wrong variants. One such region excluded from analysis using flagging is: H3F3A exon 2, chr1: 226252120-226252180. It is homologous to several other genomic regions.
- Variants may be represented in different forms in a given region. If one portion of the reads reports the variant in a different form or does not capture the variant, the variant fraction may be underestimated, or the variant may be missed due to low variant fraction.
- In the case of multiple insertions / duplications present within the same region, not all might be reported correctly. Especially, where a smaller insertion is completely contained within a larger insertion, there may be no reads uniquely identifying the smaller insertion and it might be missed and only the larger insertion reported
- In the case of multiple insertions / duplications present within the same region, not all might be quantified correctly. Especially, where a smaller insertion is completely contained within a larger insertion, there may be no reads uniquely identifying the smaller insertion and the variant fraction reported for the smaller insertion might be under- and the variant fraction of the larger insertion over-estimated.
- Sample crosstalk due to index-hoping can be aggravated by the following factors:
    1. Very high coverage in one or few samples due to problems during the sample quantification / normalization or high-level gene amplifications
    2. Overloading of the flow-cell, i.e., high cluster density
    3. Differences in library conversion rate which can be influenced by the quality of FFPE input material

- Sample crosstalk in the presence of low-level index contamination (<1%) can be aggravated by the following factors:
    1. Very high coverage in one or few samples due to problems during the sample quantification / normalization or high-level gene amplifications
    2. Overloading of the flow-cell, i.e., high cluster density
    3. Differences in library conversion rate which can be influenced by the quality of FFPE input material
- Any issue during NGS processing or issues with sample degradation can cause low signal-to- noise ratio and negatively affect accuracy of variant calling. In particular, deaminated samples where more than 10 variants under 5% AF are suspected to be the result of deamination will lead to filtering of all A->G and C->T variants under 5% AF.
- Variant fractions below 6% may not be detected.
- It is recommended that the user exercise caution in interpreting variants reported below 6% and possibly use alternative testing methods to confirm.

8. NON-CLINICAL PERFORMANCE EVALUATION

8.1. METHODS

General

In this study, the performance of the SOPHiA DDM™ Dx STS_v1 kit and the SOPHiA DDM™ Dx pipeline ILL1XG1S8_FFPE_CNV was evaluated with data generated on an Illumina MiSeq® instrument using the SOPHiA DDM™ Dx STS_v1 assay. Variant filtering was set-up according to background noise measured in germline dilution samples and the present claims concern variants above the limit of detection determined by our experiments.

Homopolymers of 10 bp or more were excluded. For each sample, the variants detected by the pipeline were compared to the 'gold-standard' confirmed variants provided by each sequencing centre. Any variants detected outside of the target regions were not considered.

Definitions of Sensitivity, Specificity, Accuracy, Precision, Repeatability and Reproducibility

Each position that was analysed by both the reference method and the method combining the use of the MiSeq® instrument and the SOPHiA GENETICS STS_v1 panel was taken into consideration to calculate analytical parameters such as sensitivity, specificity, accuracy, precision and consistency.

For all positions covered by the SOPHiA DDM™ Dx STS_v1 panel and for which reference information was available, the numbers of the following categories were determined: True Positives (TP) are present in both sets, False Positives (FP) are present only in the variants detected by SOPHiA DDM™ Dx and False Negatives (FN) are only present in the confirmed variants table. All screened positions (TP+FP+TN+FN) were determined by subtracting undetermined positions from the target region of the SOPHiA DDM™ Dx STS_v1 panel, which is CDS ± 25bp of the genes and exons specified in SOPHiA DDM™ Dx STS_v1 documentation:

- Undefined regions: Regions containing low confidence variants, or confirmed variants under the reporting threshold, and for reproducibility and repeatability, regions under 1000x depth to assess coverage reproducibility and repeatability
- Known regions with artefacts (flagged regions)
- Regions where no reference data was available or where it was ambiguous (see also 3.2.3).

Additionally, the INDELs located in homopolymer regions of at least 10 bp were excluded from the calculations.

All the parameters were calculated with the following formulas:

1. Sensitivity was determined as the percentage of confirmed variants detected:

$$Sensitivity = \frac{TP}{TP+FN} \times 100$$

2. Specificity was determined as the percentage of negative positions that were correctly identified as negative:

$$Specificity = \frac{TP}{TN+FP} \times 100$$

(with TN= all screened positions-TP-FP-FN)

3. Accuracy was determined as the percentage of correct calls (positive and negative):

$$Accuracy = \frac{TP+TN}{TP+FP+TN+FN} \times 100$$

4. Precision was determined as the percentage of correct positive calls from all positive calls:

$$Precision = \frac{TP}{TP+FP} \times 100$$

5. Sequencing Repeatability was determined, for each pair of intra-run replicates A and B, as the percentage of well-defined bases (SP) in both samples among bases which were well- defined in at least one sample. Bases are considered well-defined if they are sufficiently covered and do not contain low confidence variant calls.

$$Sequencing\ Repeatability = \frac{\sum_{i\epsilon[SP_A \cap SP_B]} 1}{\sum_{i\epsilon[SP_A \cup SP_B]} 1}$$

6. Variant Repeatability - all positions that were well defined in both replicates are taken into account to calculate the fraction of bases that are identical in both replicates. Given intra-run replicates A and B with well-defined positions SPA and SPB, SPA[i] and SPB[i] are the variant status at position i. dx being the operator returning 1 when x=0 and 0 when x¹0, Variant Repeatability is defined as:

$$Variant\ Repeatability = \frac{\sum_{i\epsilon[SP_A \cap SP_B]} \delta_{SP_A[i]-SP_B[i]}}{\sum_{i\epsilon[SP_A \cap SP_B]} 1}$$

7. Repeatability was defined as the product of the two above measures:

$$Repeatability = Variant\ Repeatability \times Sequencing\ Repeatability \times 100$$

8. Reproducibility was defined equivalently to Repeatability (see Formulas (5)-(7)) for inter-run replicates A and B.

TP, FP, TN and FN were calculated by summing over all samples from each run. Sensitivity, Specificity, Accuracy and Precision were calculated based on those total counts according to the formulas stated above. Specificity, Accuracy and Precision were only calculated using fully characterized samples due to the lack of True Negative positions in the partially characterized samples. Repeatability and Reproducibility were calculated using all positions for all samples and all runs considered.

To determine the confidence intervals in case of 100% sensitivity or other measures, the methods described by Mattocks et al (2010) were used (Mattocks CJ et al, EuroGentest Validation Group, 2010). In cases where the measured criterion was less than 100%, the exact method (Clopper, et al 1934) was used to obtain the confidence interval on the binomial probability for sensitivity, specificity, accuracy, and precision (Clopper C et al, Biometrika, 1934). To reflect the true diversity of variants only unique TP, FP, FN and TN were used in confidence interval calculations.

8.2. DATA

Available samples are grouped in two categories depending on the high confidence characterization available for benchmark comparisons. First, partially characterized samples where known high confidence variants have been identified were processed either by SOPHiA GENETICS (Site A) or by external partners (Sites B to F) using recommended experimental conditions. These results contain a large array of representative clinical cases and enable estimates of sensitivity for vari ant calling. In addition, replicates were included to estimate repeatability and reproducibility.

The second category of samples consists in reference samples with vendor-supplied regions of high confidence. This well-characterized sample enables us to determine specificity, precision, and accuracy as well as sensitivity, across the regions which have been independently characterized using two different NGS assays, each over 3 replicates.

The input data comes from several multi-patient runs (one sequence file per patient) executed by six distinct laboratories: Site A-F. All files of a run were analysed together as part of the same batch process.

8.3. GENERAL CONCLUSIONS

The following table compiles the complete summaries of detected variants compared to the list of confirmed variants (TP/FN) for each run containing partially characterized samples.

Table 2. Variant summaries for each run of partially characterized samples

Run	# Samples	TP	FN
SiteA_03	6	6	0
SiteA_04	3	6	0
SiteA_05	14	44	0
SiteB_01	9	37	0
SiteB_02	11	25	0
SiteB_03	13	14	0
SiteC_01	18	50	1
SiteC_02	15	33	0
SiteC_03	15	33	0
SiteD_01	14	41	0
SiteD_02	16	29	0
SiteE_01	1627	44	0
SiteE_02	5	6	0
SiteF_01	6	10	0
SiteF_02	5	6	0
Total	**166**	**384**	**1**

The table below displays the unique TN base positions and variants detected in this performance evaluation study. The total number of base positions in the analysed regions was 3,610 bases. Eighty-one (81) unique confirmed variants were detected in the study. Unique true negatives where high confidence reference data was available and covered a total of 3'590 bp, which cover 16.8% of the target (21,343 bp).

Table 3. Variant Summaries across all runs

TP	80
FP	0
FN	1
TN	3577

8.4. RESULTS

The combination of the Illumina MiSeq® instrument, SOPHiA DDM™ Dx STS assay and SOPHiA DDM™ Dx leads to an observed performance of 98.77% sensitivity, 100% specificity, 99.97% accuracy, 100% precision, 96.54% repeatability and 89.13% reproducibility.

Table 4. Performance summary

N°	Performance Measurement	Mean	5th Percentile
A	On-target Rate	77.0%	[51.7%]
B	Uniformity	98.7%	[92.5%]

N°	Performance Measurement	Observed*	[Reporting Limit]
C	Limit of detection	6%	[1%]

N°	Performance Measurement	Observed	[lower 95% CI]*
1	Sensitivity	98.77%	[93.31%]
2	Specificity	100%	[99.92%]
3	Accuracy	99.97%	[99.85%]
4	Precision	100%	[96.25%]
5	Repeatability	96.45%	[96.41%]
6	Reproducibility	89.13%	[89.05%]

* For SNVs and INDELs, based on reference samples.

** The 95% CI was calculated on the unique variants in the performance evaluation study in order to reflect the real diversity of the variants.

** The 95% CI was calculated on all positions for all samples, using clinical inter-site reproducibility.

9. 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
	In vitro diagnostic medical device
	Contains sufficient for <n> tests
	Importer
	Date of manufacture
	Refer to **Warnings and Precautions** in "Section 5. Kit Materials and Methods"
	Refer to **Warnings and Precautions** in "Section 5. Kit Materials and Methods"

10. SUPPORT

In case of difficulty using SOPHiA DDM™ Dx mode, please consult the troubleshooting section of the SOPHiA DDM™ Dx mode User Manual available on SOPHiA DDM™ Dx mode 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™ Dx mode.

APPENDIX 1. DUAL INDEX ADAPTER PLATES

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

	1	2	3	4	5	6	7	...	12
A	701-501	701-502	701-503	701-504					
B	702-501	702-502	702-503	702-504					
C	703-501	703-502	703-503	703-504					
D	704-501	704-502	704-503	704-504					
E	705-501	705-502	705-503	705-504					
F	706-501	706-502	706-503	706-504					
G	707-501	707-502	707-503	707-504					
H	708-501	708-502	708-503	708-504					

48 Illumina®-compatible Dual Index Adapters in 96-well plate format (7 µl each)

	1	2	3	4	5	6	7	...	12
A	701-501	703-502	705-503	707-501	709-502	711-503			
B	702-501	704-502	706-503	708-501	710-502	712-503			
C	703-501	705-502	701-504	709-501	711-502	707-504			
D	704-501	706-502	702-504	710-501	712-502	708-504			
E	705-501	701-503	703-504	711-501	707-503	709-504			
F	706-501	702-503	704-504	712-501	708-503	710-504			
G	701-502	703-503	705-504	707-502	709-503	711-504			
H	702-502	704-503	706-504	708-502	710-503	712-504			

96 Illumina®-compatible Dual Index Adapters in 96-well plate format (7 µl each)

	1	2	3	4	5	6	7	8	9	10	11	12
A	701-501	702-501	703-501	704-501	705-501	706-501	707-501	708-501	709-501	710-501	711-501	712-501
B	701-502	702-502	703-502	704-502	705-502	706-502	707-502	708-502	709-502	710-502	711-502	712-502
C	701-503	702-503	703-503	704-503	705-503	706-503	707-503	708-503	709-503	710-503	711-503	712-503
D	701-504	702-504	703-504	704-504	705-504	706-504	707-504	708-504	709-504	710-504	711-504	712-504
E	701-505	702-505	703-505	704-505	705-505	706-505	707-505	708-505	709-505	710-505	711-505	712-505
F	701-506	702-506	703-506	704-506	705-506	706-506	707-506	708-506	709-506	710-506	711-506	712-506
G	701-507	702-507	703-507	704-507	705-507	706-507	707-507	708-507	709-507	710-507	711-507	712-507
H	701-508	702-508	703-508	704-508	705-508	706-508	707-508	708-508	709-508	710-508	711-508	712-508

i5	i5 sequences for sample sheet
D501	TATAGCCT
D502	ATAGAGGC
D503	CCTATCCT
D504	GGCTCTGA
D505	AGGCGAAG
D506	TAATCTTA
D507	CAGGACGT
D508	GTACTGAC

i7	i7 sequences for sample sheet
D701	ATTACTCG
D702	TCCGGAGA
D703	CGCTCATT
D704	GAGATTCC
D705	ATTCAGAA
D706	GAATTCGT
D707	CTGAAGCT
D708	TAATGCGC
D709	CGGCTATG
D710	TCCGCGAA
D711	TCTCGCGC
D712	AGCGATAG

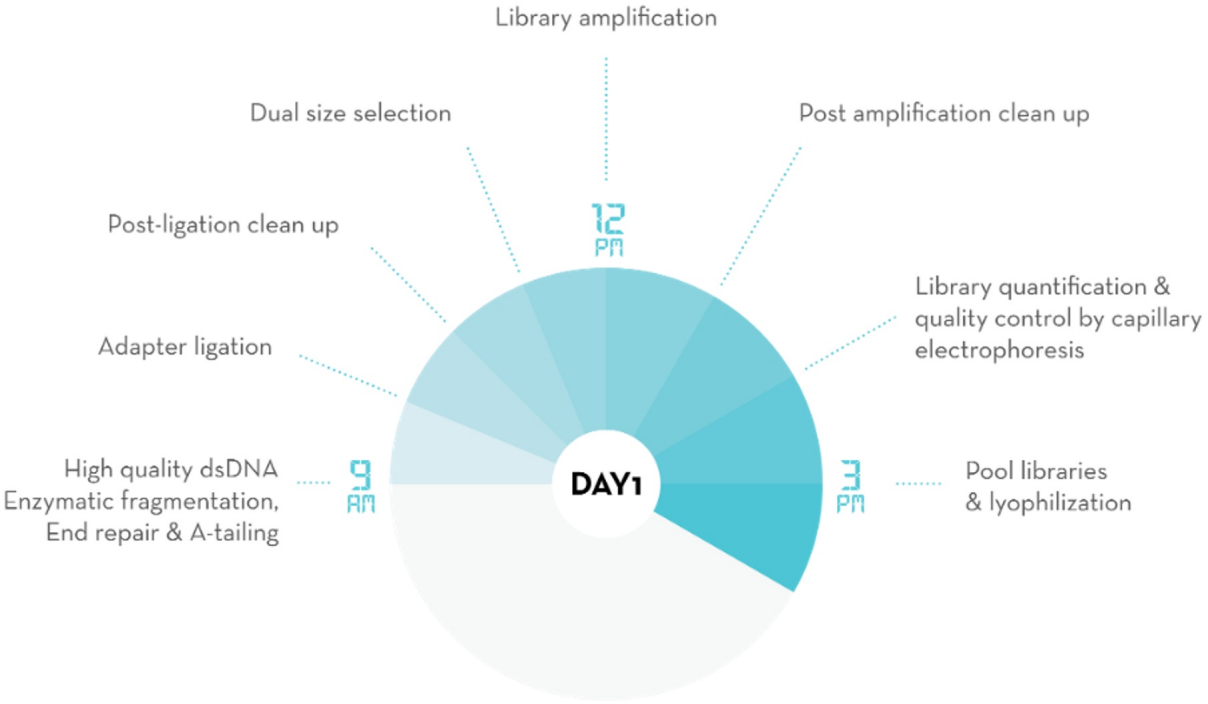
APPENDIX 2. LABORATORY EQUIPMENT USED IN SOPHIA GENETICS LABORATORY

USER-SUPPLIED MATERIALS	SUPPLIER	PRODUCT NAME	CATALOG N°
RNase/DNase-free 8-tube strips (0.2 ml)	Thermo Fisher Scientific	EasyStrip Snap Tubes	AB-2000
DNA low binding tubes (1.5 ml)	Axygen	MaxyClear Microcentrifuges Tubes	MCT-175-C
Tubes (1.5 ml)	Eppendorf	Eppendorf Tubes	3810X
Conical tubes (15 ml and 50 ml)	Falcon	15 ml & 50 ml Conical Centrifuge Tubes	352096 & 352070
Filter tips	Starlab	TipOne RPT	S1180-3710, S1183- 1740, S1180-8710, S1180-9710, S1182- 1730
Ethanol (molecular biology grade)	Merck	Ethanol Absolute	1.00983.1000

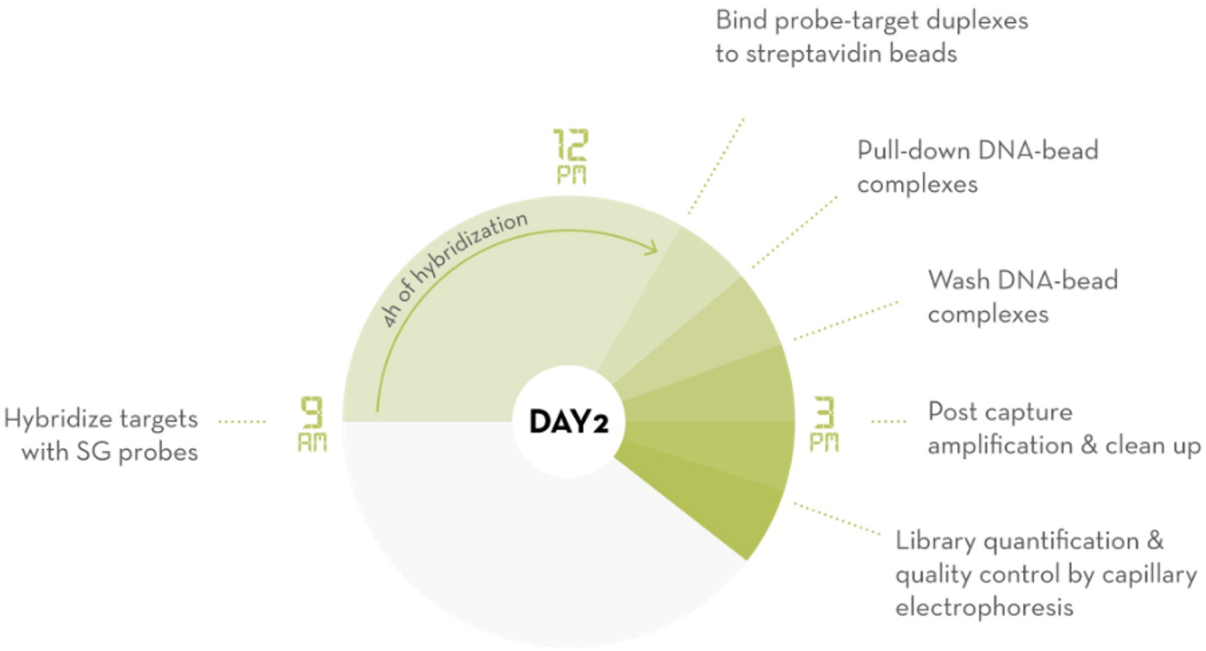
PRE-PCR ZONE	SUPPLIER	PRODUCT NAME	CATALOG N°
Vortex mixer	Scientific Industries	Vortex Genie 2	SI-0236
Table top microcentrifuge (8-tube strips compatible)	Starlab	Mini Centrifuge	N2631-0007
Magnetic separation rack 96-well type	Alpaqua	96S Super Magnet Plate	A001322
Magnetic separation rack 96-well type	Thermo Fisher Scientific	DynaMag-96 Side Magnet	12331D
Multichannel pipettes (P10; P100; P300)	StarLab	ErgoOne	S7108-0510, S7108- 1100, S7108-3300
Thermal cycler with pro-grammable heated lid	Biometra	TAdvanced 96	
Fluorometric quantitation equipment and reagent	Thermo Fisher Scientific	Qubit 3.0 Fluorometer & Qubit dsDNA HS Assay kit	Q33216 & Q32854
Single channel pipettes (P10;P100; P200; P1000)	StarLab	ErgoOne	S7100-0510, S7100- 1100, S7100-2200, S7100-1000

POST-PCR ZONE	SUPPLIER	PRODUCT NAME	CATALOG N°
Thermal cycler with programmable heated lid	Biometra	TAdvanced 96	
Capillary electrophoresis system	Advanced Analytical	Agilent Fragment Analyzer	
Vacuum concentrator (SpeedVacTM or similar)	Thermo Fisher Scientific	Savant DNA120-230	
Dry block heater or water bath(1.5 ml tube compatible)	Techne	Dri-Block DB-1	
Magnetic separation rack (1.5 ml tube compatible)	Thermo Fisher Scientific	MagJET Separation Rack, 12 x 1.5 mL tube	MR02
Magnetic separation rack (96-well type)	Alpaqua	96S Super Magnet Plate	A001322
Magnetic separation rack 96-well type	Thermo Fisher Scientific	DynaMag-96 Side Magnet	12331D
Vortex mixer	Grant instrument	Multi-tube Vortex Mixer, V32	
Vortex mixer	Scientific Industries	Vortex Genie 2	SI-0236
Table top microcentrifuge (8- tube strips compatible)	StarLab	Mini Centrifuge	N2631-0007
Multichannel pipettes (P10; P100; P300)	StarLab	ErgoOne	S7108-0510, S7108- 1100, S7108-3300
Fluorometric quantitation equipment and reagent	Thermo Fisher Scientific	Qubit 3.0 Fluorometer & Qubit dsDNA HS Assay kit	Q33216 & Q32854
Single channel pipettes (P10; P100; P200; P1000)	StarLab	ErgoOne	S7100-0510, S7100- 1100, S7100-2200, S7100-1000

APPENDIX 3. GENERAL WORKFLOW – SOPHIA DDM™ CAPTURE SOLUTIONS



Library Preparation
With KAPA™ HYPERPLUS Kit



CAPTURE
EASY WORKFLOW
- ONLY 1-4 TUBES TO HANDLE (MULTIPLEX POOLED LIBRARIES)
- ONLY 3 HOURS HANDS-ON TIME

APPENDIX 4. LIST OF THE TARGET REGIONS AND APPLICABLE FLAGGED REGIONS

TARGET REGIONS*

Target Regions

CHROMOSOME	START	END
1	115252179	115252359
1	115256410	115256609
1	115258660	115258791
1	162748359	162748529
1	226252119	226252180
2	29432641	29432754
2	29436839	29436957
2	29443561	29443711
2	29445199	29445284
2	29445372	29445483
2	198266113	198266259
2	198266455	198266622
2	198266698	198266864
2	209113082	209113394
2	212566681	212566901
2	212570032	212570126
3	12626619	12626635
3	12627269	12627290
3	12629089	12629110
3	12632286	12632483
3	12641199	12641250
3	12645624	12645798
3	41265962	41266274
3	138664423	138664550
3	138664919	138665574

CHROMOSOME	START	END
3	178916859	178916965
3	178917467	178917697
3	178922319	178922330
3	178927963	178928136
3	178935987	178936132
3	178951871	178952162
4	1803551	1803762
4	1806047	1806257
4	1807846	1807910
4	1808262	1808420
4	55140997	55141150
4	55144052	55144183
4	55151967	55152140
4	55589739	55589874
4	55592012	55592226
4	55593373	55593500
4	55593561	55593728
4	55594166	55594297
4	55599225	55599368
4	55602653	55602785
4	153245325	153245556
4	153247147	153247393
4	153249349	153249551
4	153250813	153250947
4	153251873	153252030
5	1258776	1258787
5	1268743	1268754
5	1271227	1271238
5	1271317	1271328
5	1295080	1295297
6	26031867	26032298
6	117630054	117630070

CHROMOSOME	START	END
6	117638334	117638350
7	6431544	6431682
7	55241603	55241746
7	55242404	55242523
7	55248975	55249181
7	55259401	55259577
7	116339128	116340348
7	116411837	116412069
7	116414924	116415175
7	116417432	116417533
7	116418819	116419021
7	116422031	116422161
7	116423347	116423533
7	116435698	116435855
7	140453064	140453203
7	140481365	140481503
8	38272286	38272429
8	38274813	38274944
9	21968217	21968251
9	21970890	21971217
9	21974666	21974836
9	80409368	80409518
9	80412425	80412574
10	43609917	43610194
10	43613810	43613938
10	43615518	43615661
10	43617383	43617474
10	123247494	123247637
10	123257998	123258129
10	123279482	123279693
11	533442	533622
11	533755	533954

CHROMOSOME	START	END
11	534201	534332
11	17741319	17741969
12	25378537	25378717
12	25380157	25380356
12	25398197	25398328
12	58145272	58145510
12	112888111	112888326
14	95557529	95557712
14	95560214	95560503
14	105246414	105246563
15	66727354	66727585
15	66729073	66729240
15	90631808	90631989
17	7572916	7573018
17	7573894	7574043
17	7576839	7577170
17	7577449	7577660
17	7578114	7578570
17	7579264	7579600
17	7579664	7579731
17	7579824	7579922
17	37868170	37868310
17	37879561	37879720
17	37880968	37881174
17	73775139	73775155
18	48586225	48586296
18	48591782	48591986
18	48593378	48593567
18	48602997	48603156
18	48604615	48604847
19	3114931	3115080
19	3118911	3119061

CHROMOSOME	START	END
20	57484394	57484488

*A target region is the position where the pipeline will report high confidence variants when they are present. Coordinates are 1-based and the end coordinate is included in the region.

APPLICABLE FLAGGED REGIONS**

CHROMOSOME	START	END	DESCRIPTION
1	226252120	226252180	This region is highly homologous to the following regions: chr2:175584612-175584763 (intergenic), chrX:122648386-122648447 (intergenic) and chr4:140619580-140619731 (intronic region of MGST2), and variants can be confounded because of low mappability

**A flagged region is a certain region overlapping an exon and could cause some uncertainty in variant calling, e.g. low complexity, noisy, pseudogene, etc. It could be sequencing technology dependent. A variant detected in this region will not be classified as low confidence in SOPHiA DDM™ Dx but will be associated with a warning triangle and a detailed warning message can be found in the warnings tab. The corresponding variant's filter column in the final full variant table will not be marked due to this region. Coordinates are 1-based and the end coordinate is included in the region.

Document Approvals

Approved Date: 29 Jan 2026

Approval Verdict: Approve	Coleman Spence, (cspence@sophiagenetics.com) Regulatory Approval 27-Jan-2026 16:51:28 GMT+0000

Approval Verdict: Approve	Slawomir Kubik, (skubik@sophiagenetics.com) Technical Approval 28-Jan-2026 16:09:05 GMT+0000

QA Approval Verdict: Approve	Nora Kormos, Quality Specialist (nkormos@sophiagenetics.com) Quality Assurance Approval 29-Jan-2026 14:38:41 GMT+0000