INSTRUCTIONS FOR USE

32 SAMPLES

SOPHiA DDM™ Dx Homologous Recombination Deficiency Solution

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

SOPHiA GENETICS™

SUMMARY INFORMATION

Product Name	SOPHiA DDM™ Dx Homologous Recombination Deficiency Solution
Product Type	Bundle Solution
Product Family	Molecular diagnostic application (kit + analytics)
Algorithm ID	ILL1XG1S7_FFPE_CNV_NextSeq_2
Sequencer	Illumina® - NextSeq® 500/550
Gene Panel ID	HRD_v1
Product Version	v1.0
Sample Type	Somatic DNA isolated from formalin-fixed, paraffin embedded (FFPE) tumor tissue specimens
Release Version	SOPHiA DDM™ Core: v6.4.0 / SOPHiA DDM™ Dx mode: v2.4.0
Document ID	SG-00661
Document Version	v11.0
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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 BS0121ILLCSMY08-32

PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	LIBRARY PREPARATION KIT
REF	BS0121ILLCSMY08-32	B1.H1.0021.C-32	B2.0021.C-32	5C0232 (x 2)

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REVISION HISTORY

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
SG-00661 (v11.0)	January 2026	• Change to version numbering system, no additional versions between v5.1 and v11. • Section 2 – Removal of paragraph describing the SOPHiA DDM Desktop App and research use only functionalities. • Section 3 – Removal of description of research use only components, refined the IVD scope of the product. • Figure 1: Change in the name of the analysis platform from SOPHiA DDM Web App to SOPHiA DDM Dx Mode, removal of components outside of IVD scope. • Section 5.1.1 – Addition of freeze/thaw cycle limitations for reagents contained within Box 1 and Library Prep Kit III. • Warnings and Precautions: Addition of CAS identification numbers and concentrations for each identified hazardous component. • Section 8 – Addition of Endogenous and Exogenous Interferences subsection (8.1.5) and carry over and cross contamination subsection (8.1.6). • Section 9.1 – Removal of out of IVD scope warnings from device warnings. • Section 9.2.1 merged into Section 9.2, General Limitations. • Section 9.3.1 – Removal of CDS Support Procedures, SNP/Indels, Proposed BRCA and SOPHiA DDM HRD Status, Copy Number Variation. • Removal of Appendix on CDS SOPHiA DDM HRD. • Removal of Appendix on Genomic Coordinates of the SNP targets of the HRD solution. • Removal of Appendix on Genomic coordinates of the CNV target regions of the HRD solution. • Minor formatting changes and number sequence changes regarding figure captions, table captions, *section* numbers, and appendix numbers. • Minor aesthetic changes.
SG-00661 (v5.1)	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-00661 (v5.0)	October 2024	• Appendix I section *2. CDS SOPHiA DDM™ HRD Components*: Replaced "Gene amplifications" with "CNVs" • Appendix I section *3.2. Review/Edit the BRCA status*: Added warning regarding the inclusion of CNVs in the BRCA variant report • Appendix I section *3.4. Define HRR Status*: Added warning regarding the inclusion of non-BRCA CNVs in the HRR status report • Replaced Appendix I section *7.2.3. Gene Amplification/CNV* with new section *7.2.3. Combined Gene-level and Exon-Level CNV Analysis* • Appendix I section *7.2.6 Proposed BRCA status*: Added warning regarding BRCA status computation • Appendix I sections *8.1. Location Of Results* and *8.2. File Composition*: Updated screenshots and extended list of downloadable files

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
		• Section *9.3.1. For Clinical Decision Support Procedures*: Updated limitations for CNV detection module • Added new section *Appendix V. Genomic Coordinates of the CNV "Target Regions" of the HRD Solution* • Minor formatting changes and number sequence changes regarding figure and table captions across the document
SG-00661 (v4.5)	August 2024	• "SOPHiA DDM™ Web App" changed to "SOPHiA DDM™ Dx mode" • Minor rephrasings related to the change above
SG-00661 (v4.4)	July 2024	• 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*)
SG-00661 (v4.3)	December 2023	• Section 5.2.1 - Changed DQN fragment length threshold from "300" to "375" bp • Section 5.2.1 - Added part number "DNF-464-0500" for HS Large Fragment 50kb kit on Agilent Fragment Analyzer™ • Sections 5.1.1, 5.3.1, and 5.3.2 - Removed third-party provider's intellectual property
SG-00661 (v4.2)	October 2023	• Section 9.2.1 - new limitation added regarding the rounding of QA and GI status for obtaining GI QA metrics and GI index.
SG-00661 (v4.1)	March 2023	• Sections 2, 3, 4, 5.1.1, 5.2.2, 6.1, 6.2 6.3, 9.3, 11 and Appendix I - harmonization of text. • SOPHiA GENETICS™ changed to SOPHiA GENETICS. • Revision history v4.0 updated DNA quality to DNA quantity.
SG-00661 (v4.0)	December 2022	• Section 5.2.1 - corrections made in the Important section regarding DNA quantity. • Section 9.1 - correction made in the final point of the Warnings section. • Appendix I, Section 4.2 - images in Table 4b were updated.
SG-00661 (v3.0)	September 2022	• Inserted TM symbol throughout the document wherever applicable. • Change of Library Prep Kit name to SOPHiA GENETICS™ Dx DNA Library Prep Kit.
SG-00661 (v2.0)	May 2022	• Change of Library Prep Kit name • Correction of typo in Section 5.2.4 • Symbols table updated
SG-00609 (v1.0)	May 2022	• Initial Release

TABLE OF CONTENTS

1. INTENDED PURPOSE

The SOPHiA DDM™ Dx Homologous Recombination Deficiency (HRD) Solution is an in vitro diagnostic medical device that can be used as an aid to determine the Homologous Recombination Deficiency (HRD) status of tumors in patients with ovarian cancer by the semi-quantitative detection of the Genomic Instability biomarker. The device is intended to be used by healthcare professionals and is based on a next generation sequencing (NGS) workflow taking genomic DNA extracted from FFPE treated tumor material as input.

2. GENERAL STATEMENT OF THE TEST PRINCIPLES AND PROCEDURE

The SOPHiA DDM™ Dx HRD Solution is comprised of an NGS kit and a bioinformatics pipeline used in combination with an IVD accessory, the cloud-based platform SOPHiA DDM™ Dx mode. The product is intended for processing and analyzing DNA samples extracted from formalin-fixed, paraffin-embedded (FFPE) biopsy or surgical resection Ovarian Cancer specimens.

The NGS kit and protocol is designed for users to process 50-100 ng of DNA for NGS library construction, hybridization-based capture enrichment of 28 genes and sequencing using the Illumina® NextSeq® 500/550 platform. The resulting NGS data simultaneously features low coverage across the whole-genome with an expected coverage of ~1x (low pass Whole Genome Sequencing data, lpWGS) and high coverage across the targeted sequencing regions (expected coverage: ~3000x mean).

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 HRD Solution report as a PDF. The report describes the sample SOPHiA DDM™ Dx HRD Solution status determined based on Genomic Integrity (GI) analysis.

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).

3. PRODUCT COMPONENTS

SOPHiA DDM™ Dx HRD is a complete solution that bundles the target enrichment kit with the analytical power of AI combined with access to the secure, cloud-based SOPHiA DDM™ Dx mode. The product consists of two components: the library preparation and capture kit (SOPHiA GENETICS™ Dx DNA Library Prep Kit III – 32 reactions) and the analytical bioinformatics pipeline used in combination with the SOPHiA DDM™ Dx mode.

- The kit is composed of reagents and protocols to support the preparation and enrichment of targeted, Illumina®-compatible libraries from DNA libraries from Ovarian Cancer FFPE samples suitable for enrichment and sequencing on an Illumina® NextSeq® 500/550 sequencer. The target enrichment kit allows users to generate within a single sequencing run both low-pass WGS data (~1x coverage) and targeted deep sequencing data (~3,000x coverage).
- The main purpose of the bioinformatics pipeline is to process low-pass WGS data, via a deep-learning algorithm capable of assessing genomic integrity, to establish the SOPHiA DDM™ Dx HRD status.
- SOPHiA DDM™ Dx mode hosts the bioinformatics pipeline and the Web App 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 HRD Report. Refer to the SOPHiA DDM™ Dx mode for Web App operational instructions.

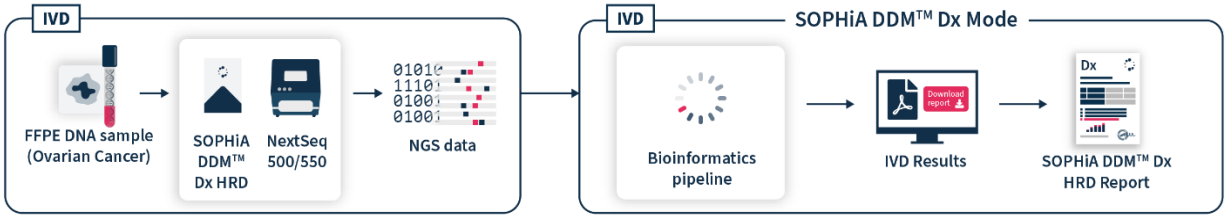


Figure 1. Overview of the different components of SOPHiA DDM™ Dx HRD Solution

4. SOPHIA DDM™ DX MODE

SOPHiA DDM™ Dx mode is a web application that provides a solution to clinicians and researchers to aid them 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™ is intended for use by trained lab professionals, clinical geneticists, and molecular pathologists.

To access the platform for the first time, you will need to sign-up. You 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)
- HRD_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 (122 µl)
- Stubby Universal Adapter (220 µl)

† Stability of frozen reagents in the Box 1 are guaranteed for a maximum of 4 freeze thaw cycles before use.

BOX 2 (STORE AT +2°C TO +8°C)

- Dynabeads® M-270 Streptavidin (440 µl)
- Agencourt® AMPure® XP (8.7 ml)
- IDTE Low TE Buffer (10 ml)
- Nuclease-free water (20 ml)

SOPHiA GENETICS™ Dx DNA Library Prep Kit III* (STORE AT -25°C TO -15°C)‡

- PCR Master Mix 2x (520 µl x 2)
- Fragmentation Buffer (77 µl x 2)
- Fragmentation Enzyme Mix (116 µl x 2)
- Ligation Mix (836 µl x 2)
- Ligation Enhancer (87 µl x 2)

* For 32 samples, two 16-sample kits are provided.

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

‡ Stability of frozen reagents in the SOPHiA GENETICS™ Dx DNA Library Prep Kit III is guaranteed for a maximum of 2 freeze thaw cycles before use.

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

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

Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
Fragmentation Buffer		• H302 - Harmful if swallowed. • H370 - Causes damage to organs. • H412 - Harmful to aquatic life with long lasting effects. • P273 - Avoid release to the environment. • P260 - Do not breathe vapor. • P270 - Do not eat, drink or smoke when using this product. • P264 - Wash thoroughly after handling. • P308 + P311 - IF exposed: Call a POISON CENTER or doctor. • P301 + P312, P330 - IF SWALLOWED: Call a POISON CENTER or doctor if you feel unwell. Rinse mouth. • P405 - Store locked up. • P501 - Dispose of contents and container in accordance with all local, regional, national and international regulations.	Danger	Tetramethyl-ammonium chloride Concentration: 1%-3% CAS: 75-57-0

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

LABORATORY EQUIPMENT

To Qualify Samples: Agilent Fragment Analyzer™ system

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 reagents
    - 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. DNA Preparation

MATERIALS

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

IMPORTANT

The SOPHiA DDM™ Dx HRD Solution is intended for samples with a tumor content of at least 30 percent. Processing DNA from samples with a tumor content below 30 percent is not supported by the solution.

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.

DNA integrity of FFPE DNA samples must be assessed prior experiment using high resolution gel electrophoresis on Fragment Analyzer (Agilent).

Assessing DNA quality: Run 5-10 ng of genomic DNA on the Agilent Fragment Analyzer™ System using the HS large fragment 50 kb kit (DNF-464-0500). The DNA quality of genomic DNA is evaluated with the DNA quality number (DQN). Set the threshold for the DQN to 375 bp and extract the DQN for your DNA samples.

Important: Processing a sample without measuring DQN or a DQN lower than 3 might lead to suboptimal whole genome library preparation yield and insufficient NGS data quality possibly resulting in inconclusive results (see Warnings and Limitations section).

Depending on the DNA quality, adjust the protocol according to *Table 1* on page 19.

Table 1. Recommended amounts of DNA to be used as input based on DNA Quality

INPUT RECOMMENDATIONS BASED ON DNA QUALITY			
Quality of DNA	DQN < 3 Less than 30% of DNA fragments larger than 375 bp	3 ≤ DQN ≤ 5 30% - 50% of DNA fragments larger than 375 bp	5 < DQN ≤ 10 More than 50% of DNA fragments larger than 375 bp
Amount of DNA (ng)	Do not process the sample	100 ng*	50 ng

*If using 100 ng of input material is not possible due to insufficient amounts of starting material, we recommend using 50 ng of input material and increasing the number of PCR cycles to 10 PCR cycles in step 5.2.6 to obtain sufficient library yield.

To avoid mistakes with DNA input, an initial dilution to obtain a concentration in the 10 to 20 ng/µl range is recommended. The DNA concentration should be confirmed by a fluorometric quantification (e.g., Qubit®, Thermo Fisher) and the obtained value used to calculate the final dilution.

PROCEDURE

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

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

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

DNA DILUTION	
DNA	Amount of DNA (cf. *Table 1* on page 19)
IDTE	Complete to 40 µl

3. 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.

5.2.2. Pre-Mixes and Reagents Preparation

COMPONENTS AND REAGENTS

- Fragmentation Enzyme Mix
- Fragmentation Buffer
- Ligation Enhancer
- Ligation Mix
- PCR Master Mix 2x
- Nuclease-free water
- AMPure® XP beads
- Ethanol

PREPARATION

- Remove the SOPHiA GENETICS™ Dx DNA Library Prep Kit III components from -20°C storage and thaw on ice.
- 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 number of reactions).

80% ETHANOL				
Number of Reactions	8	16	24	32
80% Ethanol (in ml)	10	20	30	40

- Ensure that the Fragmentation Buffer in the SOPHiA GENETICS™ Dx DNA Library Prep Kit III is completely thawed.
- Mix well and spin all reagents prior to use and place on ice.

PRE-MIXES

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

FRAGMENTATION PRE-MIX				
Number of Reactions	**8**	**16**	**24**	**32**
Fragmentation Buffer (in µl)	38.4	76.8	115.2	153.6
Fragmentation Enzyme (in µl)	57.6	115.2	172.8	230.4

   - Keep on ice.

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

LIGATION PRE-MIX				
Number of Reactions	**8**	**16**	**24**	**32**
Ligation Mix (in µl)	418	836	1254	1672
Ligation Enhancer (in µl)	14.4	28.8	43.2	57.6

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

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

5.2.3. Enzymatic Fragmentation, End Repair and A-Tailing

MATERIALS

- Diluted double stranded DNA in 40 µl
- Fragmentation pre-mix
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

- Program the thermal cycler for Fragmentation with the following settings:

TEMPERATURE (°C)		TIME (MINUTES)
Lid	99	
Step 1	4	1
Step 2	37	20
Step 3	65	30
Hold	4	∞

Start the Fragmentation 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 Fragmentation 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
Fragmentation pre-mix (in µl)	23	23	35	46

2. Assemble the reaction as follows:
    - Using a multichannel pipette, add 10 µl of Fragmentation pre-mix to each of the 40 µl of DNA samples (total of 50 µl in 4 or 8-tube strips).
    - Using a multichannel pipette set to 35 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 Fragmentation program.

    Proceed immediately to Ligation.

5.2.4. Ligation

MATERIALS

- Fragmentation reaction products in 50 µl each
- Ligation pre-mix
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

- During the Fragmentation, prepare new PCR strips with 5 µl of Stubby Universal Adapter per tube according to the following scheme:

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 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

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 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)	100	100	150	200

2. Using a multichannel pipette, transfer the 50 µl of each Fragmentation 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 45 µl of Ligation pre-mix to each Fragmentation reaction product (55 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).

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.

5.2.5. Post-Ligation Clean Up

MATERIALS

- Ligation reaction products in 100 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 80 µl of AMPure® XP beads to each of the 100 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.
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.

5.2.6. 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	8 cycles
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

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	85	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 (50 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.

5.2.7. 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.
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 30 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 briefly if required.
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 28 µl of the supernatant to a new, labeled library storage tube.

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

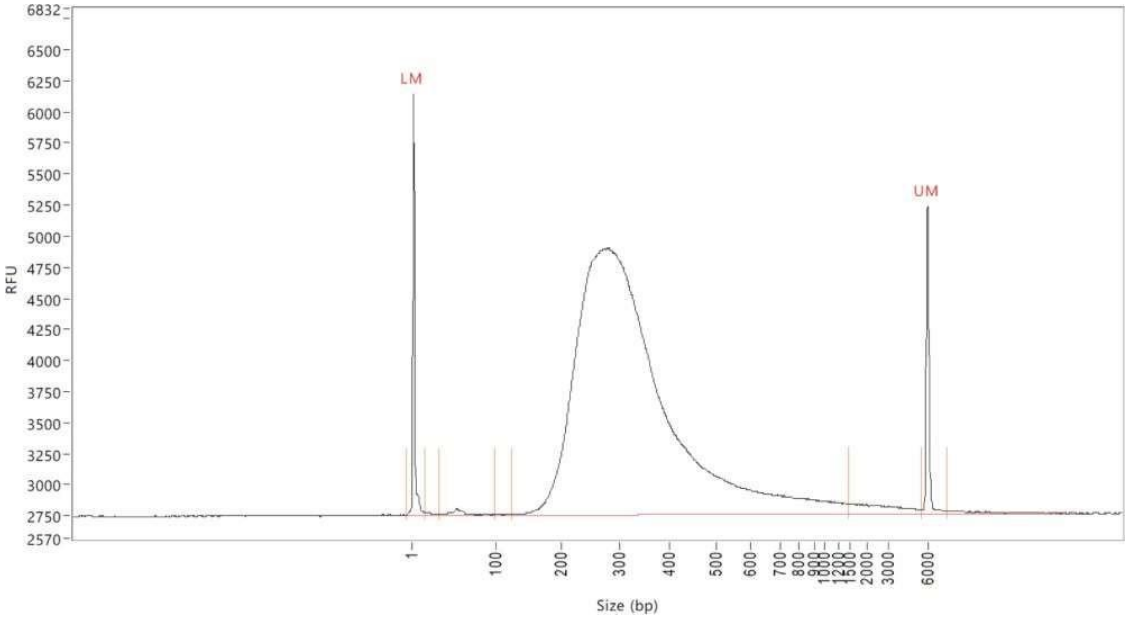
5.2.8. Individual Library Quantification and Quality Control

MATERIALS

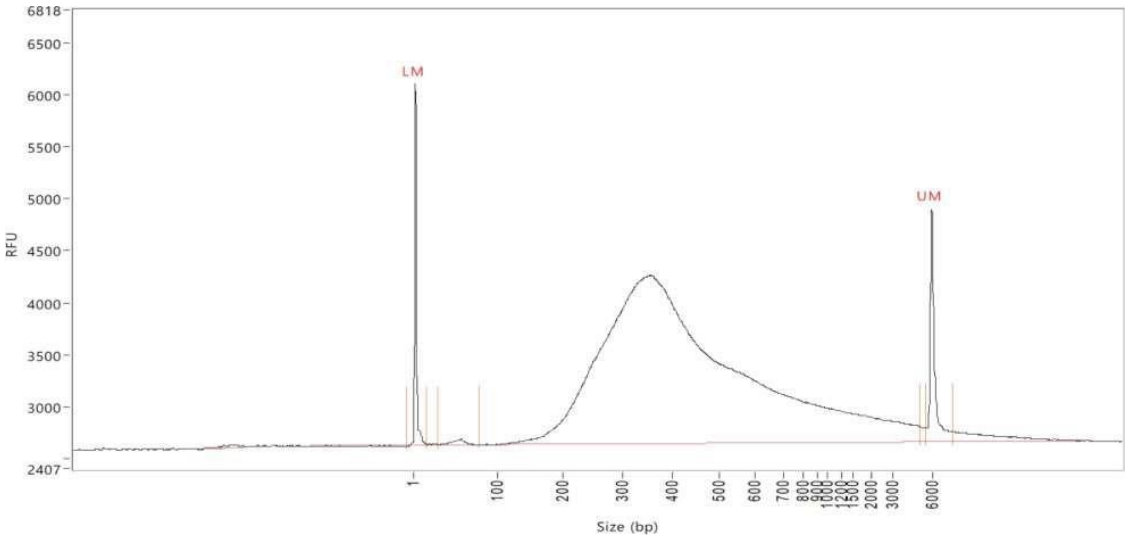
- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

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. LM refers to Lower Marker, UM refers to Upper Marker.



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

5.2.9. Library Pooling for Low-Pass Whole Genome Sequencing (WGS)

MATERIALS

- Individual libraries
- IDTE
- DNA low-binding 1.5 ml tubes

PROCEDURE

1. Determine the molarity of each library with average size of the library (peak size in base pairs) and concentration (ng/µl) obtained during step 5.2.8 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. Transfer 2 µl of each library individually into a new tube and dilute to 10 nM with IDTE.
3. Pool individual libraries at 10 nM by combining 5 µl from each library dilution. Combine the same libraries into one pool that will be combined in the capture pool. When processing multiple captures, create the same number of WGS pools as there are capture pool.

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

5.3. Capture

5.3.1. 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.

5.3.2. Hybridization

MATERIALS

- Lyophilized libraries
- 2X Hybridization Buffer
- Hybridization Buffer Enhancer
- HRD_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 an aliquot of 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 an aliquot of 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 an aliquot of 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 an aliquot of 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

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.

5.3.6. 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	-	
Step 1: Initial Denaturation	98	120	
Step 2: Denaturation	98	20	
Step 3: Annealing	60	30	15 Cycles
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.

5.3.7. 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 if required 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.

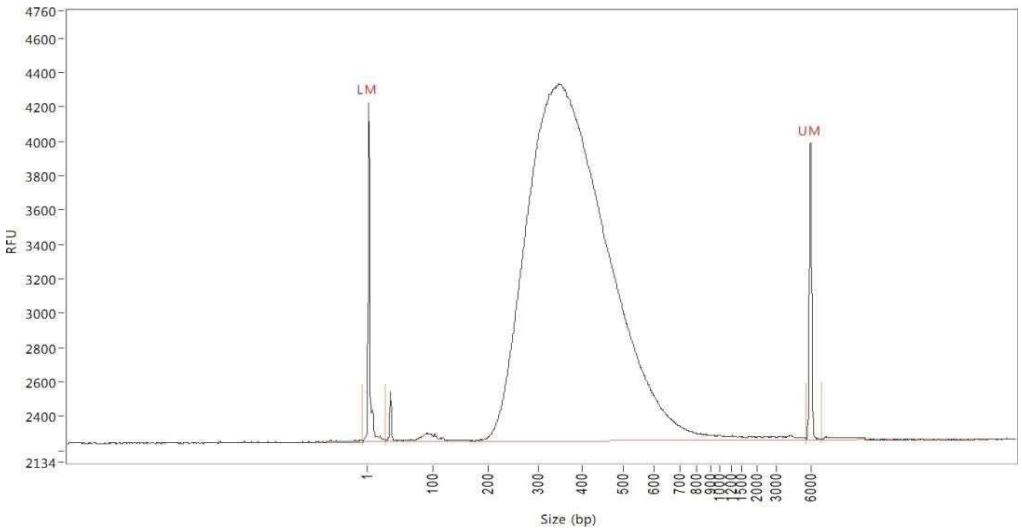
5.3.8. Final Library Quantification and Quality Control

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.



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.

5.4. Sequencing

5.4.1. Library Preparation for Sequencing

MATERIALS

- Illumina® NextSeq® Sequencing Kit
- Final captured libraries
- EBT Buffer or similar

PROCEDURE

1. Determine the molarity of each captured pool with average size of the library (peak size in base pairs) and concentration (ng/µl) obtained during step 5.3.8 as follows:

2.

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

3. Dilute each captured pool to 10 nM.
4. Thaw the WGS pool generated in 5.3.1.
5. To generate the sequencing pool, combine each captured pool with its corresponding WGS pool by mixing 3.3 µl captured pool with 6.7 µl WGS pool.
6. If processing multiple sequencing pools, mix them in equal amounts (e.g., 5 µl of each) following the sequencing recommendation table below:
7.

NEXTSEQ 500/ 550 SYSTEM KIT TYPE	NUMBER OF SAMPLES PER RUN
Mid-Output	8 samples (1 pool)
High-Output	24 samples (3 pools)

8. Mix it well and use this dilution according to Illumina® standard denaturation recommendation.

9. For loading dilution, see the table below:

NEXTSEQ 500/550 SYSTEM KIT TYPE	LOADING DILUTION
Mid-Output	1.3 pM
High-Output	1.4 pM
[Adjust the dilution (1.1 pM to 1.5 pM range) according to the number of clusters obtained in the first run]	

10. 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	32 million	16 million

Note: Increasing the number of reads per sample is expected to provide more confident and sensitive variant calls, especially in regions with otherwise relatively low read depth. Refer to section *9. Warnings, Limitations and Precautions*.

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® 500/550 sequencer (e.g., 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.

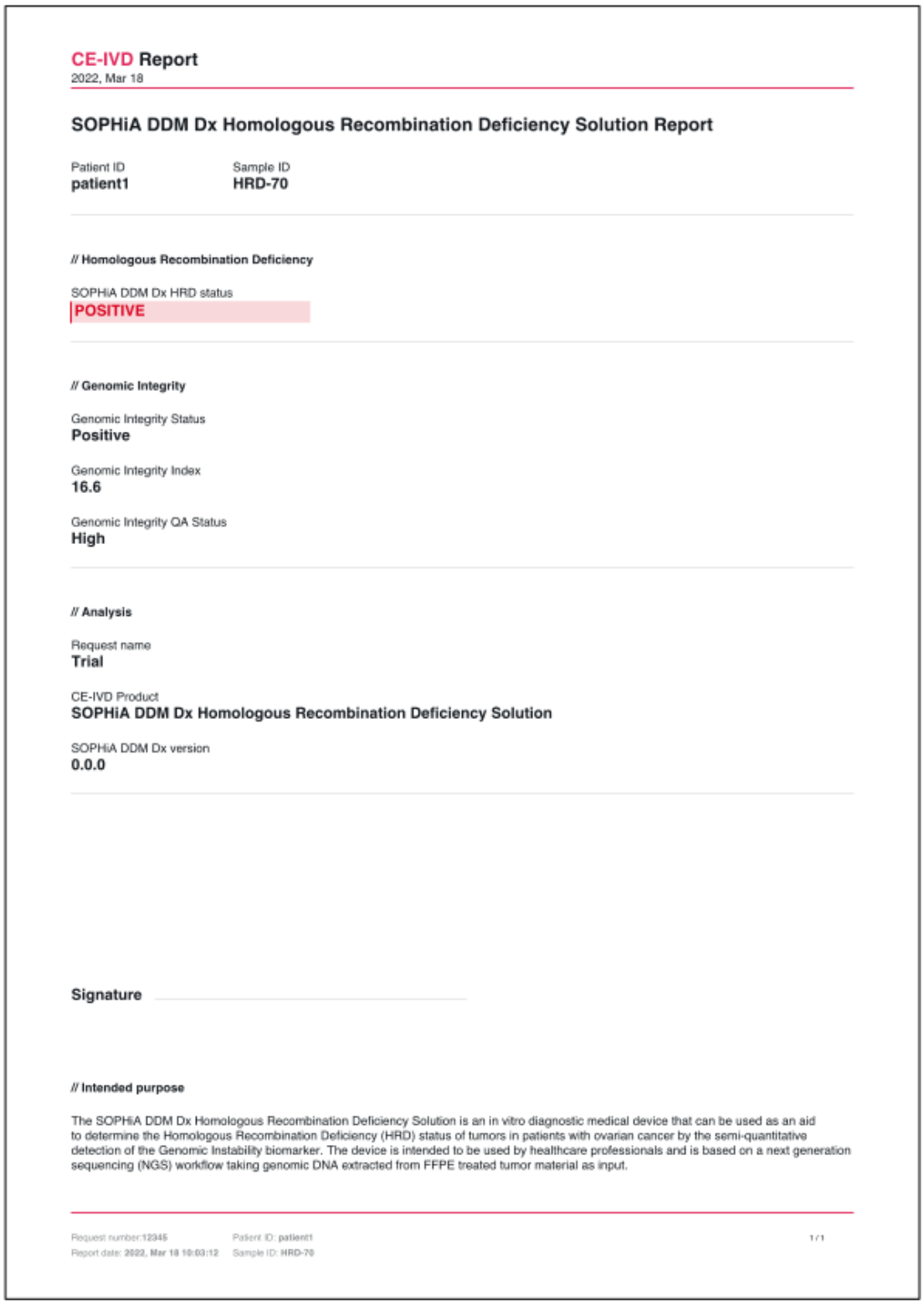
6.2. Data Upload and Analysis

The user logs into SOPHiA DDM™ Dx mode and selects the SOPHiA DDM™ Dx Homologous Recombination Deficiency 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 User Manual for IVD SOPHiA DDM™ Dx mode 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 new 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 “My Genomic Analysis Requests” widget in the dashboard or from the browser. Once completed, the generated SOPHiA DDM™ Dx HRD Solution report for each sample in the request can be accessed and downloaded in PDF format. The SOPHiA DDM™ Dx mode User Manual can be used to obtain detailed instructions regarding the analysis.



CE-IVD Report
2022, Mar 18

SOPHiA DDM Dx Homologous Recombination Deficiency Solution Report

Patient ID
patient1

Sample ID
HRD-70

// Homologous Recombination Deficiency

SOPHiA DDM Dx HRD status
POSITIVE

// Genomic Integrity

Genomic Integrity Status
Positive

Genomic Integrity Index
16.6

Genomic Integrity QA Status
High

// Analysis

Request name
Trial

CE-IVD Product
SOPHiA DDM Dx Homologous Recombination Deficiency Solution

SOPHiA DDM Dx version
0.0.0

Signature

// Intended purpose

The SOPHiA DDM Dx Homologous Recombination Deficiency Solution is an in vitro diagnostic medical device that can be used as an aid to determine the Homologous Recombination Deficiency (HRD) status of tumors in patients with ovarian cancer by the semi-quantitative detection of the Genomic Instability biomarker. The device is intended to be used by healthcare professionals and is based on a next generation sequencing (NGS) workflow taking genomic DNA extracted from FFPE treated tumor material as input.

Request number:12345 Patient ID: patient1 1/1
Report date: 2022, Mar 18 10:03:12 Sample ID: HRD-70

Figure 2. Example of a SOPHiA DDM™ Dx HRD Solution report.

The SOPHiA DDM™ Dx HRD Solution report displays: the SOPHiA DDM™ Dx HRD status, the Genomic Integrity Status, the Genomic Integrity Index, the Genomic Integrity QA status. *Table 2* on page 42 provides an overview of the possible outcomes.

Table 2. SOPHiA DDM™ Dx HRD Solution report possible outcomes

SOPHiA DDM™ Dx HRD Status	Genomic Integrity Status	Genomic Integrity QA Status	Description
Positive	Positive	High	High confidence positive call.
Positive	Positive	Medium	High confidence positive call. The NGS data meets the quality criteria required to deem a sample positive. However, the NGS data is of suboptimal quality and/or the sample tumor content is low.
Negative	Negative	High	High confidence negative call.
Negative	Negative	Medium	High confidence negative call. The NGS data meets the quality criteria required to deem a sample negative. However, the NGS data is of suboptimal quality and/or the sample tumor content is low.
Negative	Negative*	Medium	Medium confidence negative call with increased false negative risk. The NGS data does not meet the quality criteria required to deem a sample negative with high confidence. The signal-to-noise ratio in the NGS data is low, possibly reflecting insufficient sample tumor content. We recommend ensuring that the DNA sample met tumor content and DNA quality requirements.
Undetermined	Inconclusive	Medium	The SOPHiA DDM™ Dx HRD status cannot be determined because of insufficient signal-to-noise ratio in the NGS data used for Genomic Integrity analysis. This result can reflect either the absence of large copy number alterations in the sample or an insufficient sample tumor content. We recommend repeating the experiment with a new DNA sample and make sure its tumor content and quality are sufficient.
Undetermined	Rejected	Low	The SOPHiA DDM™ Dx HRD status cannot be determined because the NGS data used for Genomic Integrity analysis are of insufficient quality. We recommend repeating the experiment by ensuring sample tumor content and DNA quality are sufficient and the protocol instructions are followed.

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

7.2. Raw Data Pre-Processing

Preprocessing

- Collect quality metrics based on the raw fastq files.
- Truncate the fastq files to a maximum size of 2500MB.

Alignment

- Cut adapters and trim low-quality ends from reads (base quality below 20).
- Align reads to the hg19 reference genome in paired end mode.
- Compute alignment statistics and coverage metrics on the raw alignment files.
- Trim overhanging adapters sequences.

7.3. Genomic Integrity Analysis

Genomic Integrity analysis aims at detecting HRD by assessing the degree of genomic instability. Functional homologous recombination repair is necessary for the error-free repair of double strand breaks and for maintaining genome integrity. A consequence of HRD is the loss of genomic integrity via the accumulation of genomic aberrations due to the cell's inability to repair double strand breaks. Our Genomic Integrity analysis aims at assessing these genomic aberrations through the use of Whole Genome Sequencing (WGS). More specifically, our method uses a deep learning algorithm that has been specifically trained to recognize patterns of genomic instability in the WGS coverage profile. The algorithm analyzes low-pass WGS (lpWGS) data and produces a Genomic Integrity (GI) index that reflects the level of genomic integrity. A GI index above 0 indicates low genomic integrity. A GI index below 0 indicates high genomic integrity.

During GI analysis the following algorithmic steps are performed sequentially for each sample of interest: first low pass WGS (lpWGS) data are preprocessed and undergo quality assessment, next the GI index is computed, and lastly a GI status is assigned to the sample. These steps are described in the sections below.

Step 1: Data preprocessing and sample quality assessment:

1. WGS paired-end reads are mapped to the human reference genome and processed to trim adaptors and low-quality base calls. The WGS coverage profile is computed and normalized.

2. The normalized WGS coverage profile undergoes QA based on the metrics defined at the end of this section. One of the following GI QA statuses is assigned to the sample:
    - High quality: the quality of the data is sufficient to confidently compute a GI index and a GI status.
    - Medium quality: the quality of the data is lower (compared to high quality) and, consequently, the deep learning algorithm may not succeed in computing a GI index.
    - Low quality: the quality of the data does not meet the criteria required to compute a GI index.

Step 2: GI Index calculation:

The GI index is obtained by processing the normalized WGS coverage profile using a proprietary deep learning algorithm that has been trained to recognize patterns of genomic instability.

Step 3: GI Status determination:

A GI status that applies to Ovarian Cancer samples is determined by combining the sample QA status and the GI index. Five outcomes are possible:

- GI positive: samples with a GI index larger or equal than 0.
- GI negative: samples with a GI index smaller than 0.
- GI negative*: samples with a GI index smaller than 0 but featuring an increased risk of false negative calls due to low signal to noise ratio.
- GI inconclusive: medium quality samples for which the deep learning algorithm did not succeed in computing a GI index due to insufficient signal to noise ratio.
- GI rejected: low quality samples discarded from GI analysis. Samples are rejected if the number of DNA fragments available for WGS coverage profile calculation is insufficient, if the noise of the WGS coverage profile is excessive, or if the proportion of coverage outliers is excessive.

Definition of QA and GI metrics

- Total nb. of fragments: Total number of DNA fragments (paired-end reads) that are properly mapped.
- Nb. WGS fragments: Total number of DNA fragments available for the raw coverage WGS profile calculation. DNA fragments mapping to the genomic regions enriched for variant calling are excluded. This QA metric is compared against a threshold of 4 million to determine the GI QA status.
- Percentage WGS fragments: Fraction of the total number of WGS fragments over the total number of fragments.
- Proportion of Coverage Outliers: Percentage of WGS regions considered for GI analysis which feature an artefactual and excessive localised coverage which is compensated by the coverage normalisation algorithm. This QA metric is compared against a threshold of 20% to determine the GI QA status.
- Purity/ploidy ratio: Ratio between sample tumor content and sample ploidy, estimated by measuring the strength of the signal induced in the normalized WGS coverage profile by a copy number change. This GI QA metric is compared against a threshold of 0.1 (suggesting low tumor content) to determine the GI QA status. The inability to measure purity-ploidy ratio from the data is also used to determine the GI QA status.
- Residual noise: Residual noise is computed by measuring the standard deviation of the normalized WGS coverage profile with respect to the smoothed WGS coverage profile. This QA metric is compared against a threshold of 0.17 to determine the GI QA status.

- SNR: Strength of the signal induced in the normalized WGS coverage profile by all copy number aberrations present in the sample divided by the residual noise. This GI QA metric is compared against a threshold of 0.55 to determine the GI QA status. SNR is also considered by the algorithm to assign the GI Negative* and GI Inconclusive status.
- QA Status: GI quality assessment status of a sample.
- GI Index: The GI index is a scalar value, ranging between −20 and 20, that reflects the level of genomic integrity. High GI indices reflect low levels of genomic integrity. Low GI indices reflect high levels of genomic integrity.
- GI Status: The genomic integrity status of a sample.

7.4. SOPHiA DDM™ Dx HRD Status Calculation

The bioinformatics pipeline computes the SOPHiA DDM™ Dx HRD status based on the Genomic Integrity status according to the following table.

Genomic Integrity status	SOPHiA DDM™ Dx HRD status
Positive	Positive
Negative	Negative
Negative*	Negative
Inconclusive	Undetermined
Rejected	Undetermined

8. PERFORMANCE EVALUATION

8.1. Analytical Performance Evaluation

8.1.1. Genomic Integrity Status Concordance with Comparator NGS Assay

The analytical performance of the Genomic Integrity Status was evaluated on 238 DNA samples extracted from Ovarian Cancer FFPE specimens with DQN ≥3, sequenced on Illumina® NextSeq® 550, for which a conclusive genomic instability result was obtained with the comparator NGS assay.

Out of the 238 samples included in the analysis, 5 led to an inconclusive Genomic Integrity Status (rejection rate: 2.1%). The following table shows the performance computed on the remaining 233 samples for which a conclusive Genomic Integrity Status was obtained.

Metric	Value
Positive Percent Agreement (PPA)	92.9%
Negative Percent Agreement (NPA)	95.8%
Overall Percent Agreement (OPA)	94.4%

8.1.2. Genomic Integrity Status Sample Tumor Content LOD

The Limit of Detection for the Genomic Integrity Status with respect to sample tumor content was established by measuring the lowest tumor content for which sensitivity is larger or equal to 95%. The measured LOD was 28.5% tumor content.

8.1.3. Genomic Integrity Status Repeatability

The repeatability of the Genomic Integrity Status was established based on results obtained in independent experiments performed using multiple samples.

Characteristic	GI status repeatability	GI index standard deviation (mean / range across samples)
Repeatability	100%	0.26 / [0.23 - 0.32]

8.1.4. Genomic Integrity Status Reproducibility

The reproducibility of the Genomic Integrity Status was established based on results obtained by independent laboratories provided with aliquots of the same reference samples.

Characteristic	GI status reproducibility	GI index standard deviation (mean / range across samples)
Inter-run reproducibility	100%	0.47 / [0.00 - 1.48]
Inter-site reproducibility	100%	1.00 / [0.43 – 2.14]
Inter-lot reproducibility	100%	-

8.1.5. Endogenous and Exogenous Interferences

A risk assessment was performed to evaluate the potential impact of endogenous and exogenous interfering substances on assay performance. Potential interferents were identified from the literature and from similar NGS-based products.

Several substances (e.g., Proteinase K, Formalin, Hemoglobin, Tissue-marking dyes, Ethanol) are known inhibitors of enzymatic reactions used in DNA library preparation. At sufficiently high concentrations, these could reduce enzymatic efficiency and lead to test failure ("no result"), but not to incorrect variant calls or GI status misclassification. Other substances evaluated (e.g., Triglycerides, Paraffin, Xylene) were not found to interfere with the assay workflow.

No mechanisms were identified by which interfering substances could cause false positive or false negative GI status results. In conclusion, the presence of interfering substances, if any, is expected to increase the likelihood of assay failure but does not impact analytical specificity or the determination of GI status.

8.1.6. Carry Over and Cross Contamination

The risks linked to run-to-run and sample-to-sample carryover events, as well as potential cross-reaction, were addressed and mitigated through both experimental and computational strategies.

Carryover risks were evaluated through investigations of the functioning of the NextSeq550 sequencer, implementation of existing quality-check procedures, and analysis of datasets simulating carryover processes. In parallel, cross-reaction risks were mitigated through the design of the algorithm implemented in the SOPHiA DDM Dx HRD Solution for the calculation of the GI index and GII status, which ensures robustness against genomic coverage patterns that could otherwise lead to erroneous assignments. The robustness of this algorithm was further confirmed through analyses of targeted and randomly selected coverage outliers, which did not affect the accuracy of GII status determination. Taken together, the evidence generated in this risk assessment indicates that neither carryover nor cross-reaction events represent a significant risk for the processes of variant calling and GII status calling implemented as part of the SOPHiA DDM Dx HRD Solution.

8.2. Clinical Performance Evaluation

The clinical performance of the SOPHiA DDM™ Dx HRD Solution was established in a retrospective observational clinical study on 110 FFPE-derived DNA extracts from tumor material. All samples were derived from ovarian cancers. The defined tumor content of all the samples ranged from 30% to 90%. The quality of the DNA extracts was evaluated by DQN determination on the Fragment Analyzer (Agilent Technologies, Inc.) using 300bp as threshold. All DNA extracts had a DQN >3, 105 DNA extracts had a DQN > 5, 5 DNA extracts had a DQN ≤ 5.

The reference HRD status of all samples had been established by a marketed IVD genomic assay combining genomic instability and BRCA1/BRCA2 mutation analysis. Comparison of this reference HRD status with the SOPHiA DDM™ Dx HRD status defined in the clinical study allowed the calculation of the diagnostic sensitivity, the diagnostic specificity, the

positive and negative predictive values and the positive and negative likelihood ratios. Out of the 110 samples, 15 samples were excluded from the calculations because of invalid results: 8 samples had an Undetermined SOPHiA DDM™ Dx HRD status, 12 samples had a Failed reference HRD status, and 5 samples had an invalid status for both methods.

Metric	Value (95% C.I.)
Diagnostic sensitivity	80.9% [67.5%-89.6%]
Diagnostic specificity	93.7% [83.2%-97.9%]
Positive Predictive Value	92.7%
Negative Predictive Value	83.3%
Positive Likelihood Ratio	12.9
Negative Likelihood Ratio	0.2

9. WARNINGS, LIMITATIONS AND PRECAUTIONS

9.1. 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 HRD Solution has been validated on DNA extracted from Ovarian Cancer FFPE samples with DQN ≥3 (measured using the Agilent Fragment Analyzer™ system), tumor content ≥30%, and sequenced on Illumina® NextSeq® 500/550.
- Poor quality of raw NGS data can confound the data analysis and cause False Positives, False Negatives and inconclusive results.
- Users must verify the quality of their DNA samples meet the minimum criteria for qualification:
    - Tumor content ≥ 30%
    - DQN ≥3, measured using the Agilent Fragment Analyzer™ system
    - 50ng or 100ng input DNA depending on DQN (see protocol)
- Devices for high resolution gel electrophoresis other than the Agilent Fragment Analyzer™ system (e.g., Agilent TapeStation), were observed to lead to biased estimation of DQN on FFPE DNA samples. Agilent Fragment Analyzer™ system is the only suitable device for qualifying samples for SOPHiA DDM™ Dx HRD Solution.
- Processing of unqualified samples could lead to erroneous results. SOPHiA GENETICS is not liable for the results and consequent decisions taken based on these results.
- A DQN larger or equal to 3 cannot exclude the presence of other quality issues in the DNA sample. Because analysis accuracy is dependent upon DNA quality and quantity, users must ensure that the full workflow used to obtain the input DNA is compatible with the assay, before proceeding with clinical analyses.
- The SOPHiA DDM™ Dx HRD status is exclusively based on Genomic Integrity analysis results and does not take into consideration the mutational status of other genes. A negative SOPHiA DDM™ Dx HRD status does not exclude the presence of mutations in other genes.

9.2. Limitations

- The product is designed to process the volume of data produced by a single Illumina® NextSeq® 500/550 run (MidOutput and HighOutput flowcells). The maximal request size cannot exceed 200 Gb. The maximal file size per sample cannot exceed 50Gb. The pipeline performs random read subsampling to all samples exceeding 5 Gb of compressed fastq files.
- Even if sample multiplexing recommendations are followed, the total number of reads may be insufficient to provide conclusive results for various reasons, including poor sample quality, poor NGS data quality, significant uneven read allocation between WGS and captured libraries, significant uneven read allocation between samples multiplexed in the same run.
- The product does not detect nor report sample cross contamination events.

- Improper execution of DNA extraction, library preparation and/or sequencing could lead to sample rejection, as well as false positive or false negative results.
- Improper execution of the capture step could lead to sample rejection due to excessive off-target read coverage.
- The product does not measure or report sample tumor content.
- The limit of detection for SOPHiA DDM™ Dx HRD status and Genomic Integrity status determination is a tumor content of 30%. Processing samples with tumor content <30% is likely to result in GI inconclusive or GI negative* results but can also result in false negative results.
- Samples with negative SOPHiA DDM™ Dx HRD status and negative* Genomic Integrity status are associated with a lower negative predictive value.
- Low-pass WGS data do not allow to reliably distinguish samples lacking large copy number alterations from samples having insufficient tumor content. Processing samples that do not feature large copy number alterations will lead to GI inconclusive results.
- The GI QA metrics and GI index reported to the end-users are obtained by rounding up the full precision values used for QA status and GI status calculations to the first or second decimal (depending on the metric).

9.3. Precautions

- Good laboratory practice standards and procedures, in addition to strictly following the IFU, are required for adequate performance of the product. Any deviation from instructions regarding sample handling, laboratory protocol, multiplexing and sequencing may negatively affect raw NGS data quality.
- Physically separated pre- and post- PCR rooms should be used to prevent DNA sample contamination.
- Correctly calibrated pipettes and proper lab equipment should be used to perform the experiment.
- Different lot numbers of reagents should not be mixed.
- 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.
- The recommended EDTA concentration in DNA storage buffer is 0.1 mM. An excess of EDTA in sample storage buffers could impair sample processing.
- For detailed instructions on the software, refer to the SOPHiA DDM™ Dx mode User Manual.

10. 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

11. 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.

APPENDIX I. UNIQUE DUAL INDEX PRIMER PLATES

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

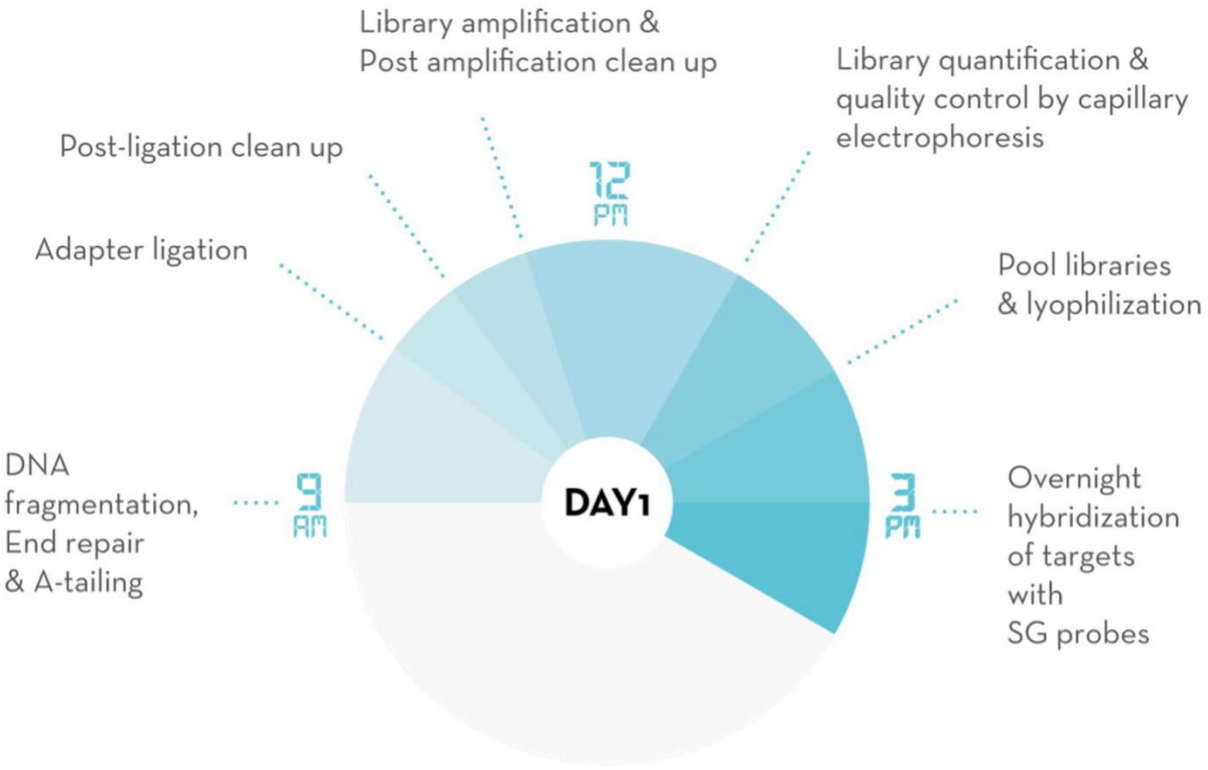
	1	2	3	4	5	6	7	…	12
A	sgUDI-49	sgUDI-57	sgUDI-65	sgUDI-73					
B	sgUDI-50	sgUDI-58	sgUDI-66	sgUDI-74					
C	sgUDI-51	sgUDI-59	sgUDI-67	sgUDI-75					
D	sgUDI-52	sgUDI-60	sgUDI-68	sgUDI-76					
E	sgUDI-53	sgUDI-61	sgUDI-69	sgUDI-77					
F	sgUDI-54	sgUDI-62	sgUDI-70	sgUDI-78					
G	sgUDI-55	sgUDI-63	sgUDI-71	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 NextSeq	i7
sgUDI-49	CAGTGAGC	ACCAAGGA
sgUDI-50	CAATCCTG	CAGACCTG
sgUDI-51	AACTAGAC	CGAGCAAC
sgUDI-52	GCCATTCA	TCTTGACT
sgUDI-53	ATTGTCGT	GACAATGG
sgUDI-54	TGGCGTTC	GTTCTACG
sgUDI-55	CAGGACAG	AACGCTGC
sgUDI-56	GTCCTTAT	GGACATCA
sgUDI-57	GGAATGTA	TTGAGCTC
sgUDI-58	AGCTAACC	ACGTTGAG
sgUDI-59	TCAGCAGG	CTTCAGGA
sgUDI-60	GATTGAGG	TGCCAACT
sgUDI-61	ATTCTTCG	AGGTCATG
sgUDI-62	CTCGACTA	TACTAGCA
sgUDI-63	GTGAGGAT	GTAACTGT
sgUDI-64	ACTGCAGC	TGAGTTGA
sgUDI-65	CGATTGCC	CCTTAGAC
sgUDI-66	TAAGATGC	TATCGCCA

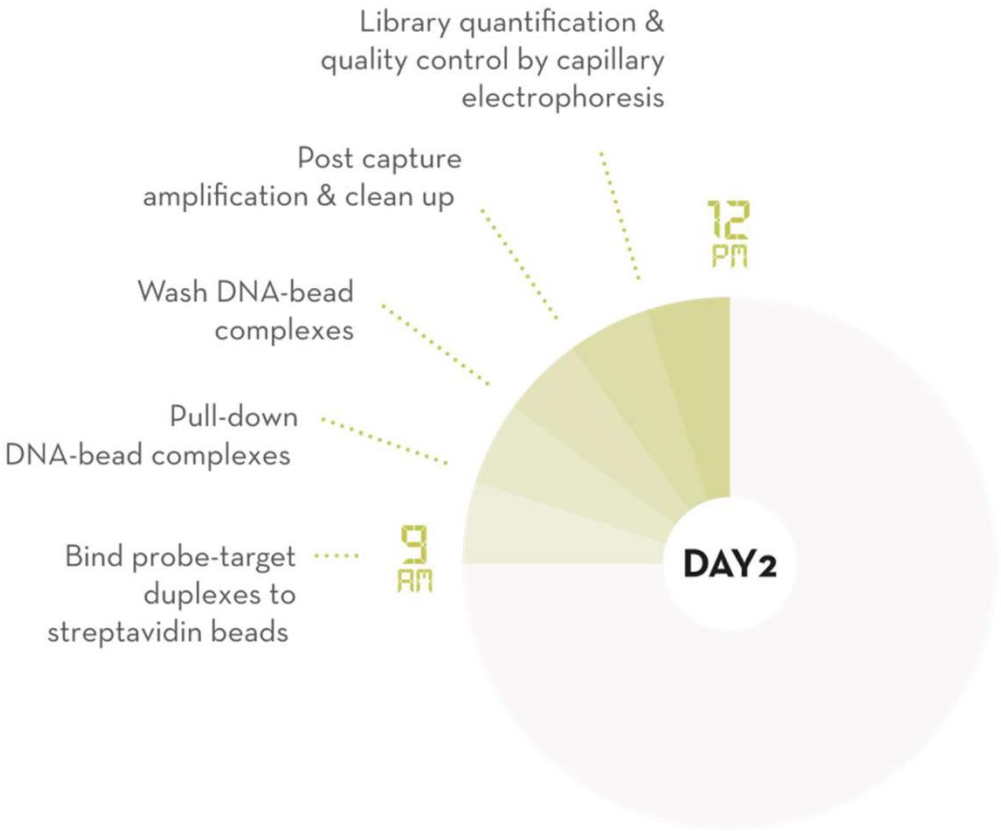
Index	i5 sequences for sample sheet NextSeq	i7
sgUDI-67	TTCAGCAC	GCAGAACA
sgUDI-68	GCGTCAAT	AGGAATGC
sgUDI-69	AATGCGGC	CGTGAGGT
sgUDI-70	TGACTACA	CAATTCAG
sgUDI-71	TCGGAGGA	GGTCCTTC
sgUDI-72	CTCGATTG	TTCCGGCA
sgUDI-73	CGGACTTA	ATGCCTGA
sgUDI-74	AACTGTCC	TCCAGGAC
sgUDI-75	TCACTCAA	GCTGTCAC
sgUDI-76	ATGTTACG	CGACGATT
sgUDI-77	ACTGCGTA	TCGCAACG
sgUDI-78	GGTCAGGT	GAGTTGTA
sgUDI-79	TCAGGTGG	CGCTAAGG
sgUDI-80	CACGGTGA	TTGCGTGC

APPENDIX II. GENERAL WORKFLOW - SOPHIA DDM™ CAPTURE SOLUTIONS



LIBRARY PREPARATION

With SOPHiA GENETICS™ Dx DNA Library Prep Kit III



CAPTURE
EASY WORKFLOW
- ONLY 1-2 TUBES TO HANDLE (MULTIPLEX POOLED LIBRARIES)
- ONLY 3 HOURS HANDS-ON TIME

Document Approvals

Approved Date: 28 Jan 2026

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