INSTRUCTIONS FOR USE
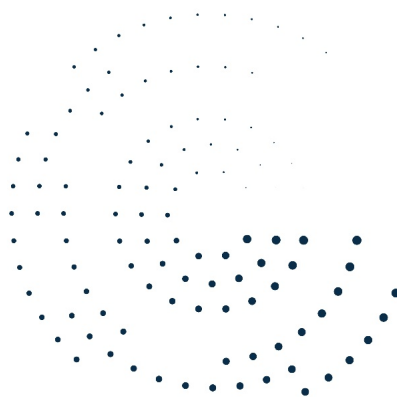
32 Samples

SOPHiA DDM™

Extended Homologous Recombination Solution

Using The SOPHiA GENETICS™ DNA Library Prep Kit III

SOPHiA GENETICS™

SUMMARY INFORMATION

Product Name	SOPHiA DDM™ extended Homologous Recombination Solution
Product Type	Bundle Solution
Product Family	Kit + analytics
Algorithm ID	ILL1XG1S6_FFPE_CNV_NextSeq_3
Gene Panel ID	ExtHRS_v1
Product Version	v1.0
Release Version	v5.10.31 - p5.5.73
Sample Type	Somatic DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens
Sequencer	Illumina® NextSeq 500/550
Document ID	SG-03102
Document Version	v1.0
Revision Date	Feb 2023

This Instructions For Use is applicable to all SOPHiA DDM™ versions.

Please read the Instructions For Use thoroughly before using this product.

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EC | REP

REF   BS0124ILLRSMY08-32

RUO

PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	LIBRARY PREPARATION KIT
REF	BS0124ILLRSMY08-32	B1.H1.0024.R-32	B2.0000.R-32	500232 (x 2)

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REVISION HISTORY

DOCUMENT ID / VERSION	DATE	DESCRIPTION OF CHANGE
SG-03102 – 1.0	Feb 2023	• Initial release

TABLE OF CONTENTS

1 PRODUCT INTRODUCTION

BRCA1 and *BRCA2* play a key role in Homologous Recombination Repair (HRR). Deleterious mutations in *BRCA1* and/or *BRCA2* are known to cause Homologous Recombination Deficiency (HRD). HRD can also result from genetic and epigenetic mutations impairing other HRR genes; however, the clinical significance of non-BRCA HRR gene mutations is still under debate. It is well known that mutations in HRR genes, beyond *BRCA1* and *BRCA2*, can lead to HRD in several cancer types, including ovarian, breast, prostate, and pancreatic cancers.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

2 GENERAL STATEMENT OF THE TEST PRINCIPLES AND PROCEDURE

The SOPHiA DDM™ extended Homologous Recombination Solution (ExtHRS) product focuses mainly on the identification of genomic aberrations which are known or suspected to affect the HRR pathway. The product will include an NGS kit and protocol to allow research professionals to perform targeted sequencing on DNA extracted from FFPE tissue.

Further, the product will include a bioinformatics pipeline for:

i) The detection of somatic and germline mutations and gene amplifications in *BRCA1*, *BRCA2*, as well as in other genes involved in HRR (based on targeted sequencing data), and

ii) Tertiary annotation to identify deleterious mutations in *BRCA1* and *BRCA2*, as well as in other HRR genes.

The ExtHRS product will be integrated into the SOPHiA DDM™ platform. Its user interface will allow research professionals to perform an interpretation of the analytical results produced by the pipeline.

3 PRODUCT COMPONENTS

The SOPHiA DDM™ extended Homologous Recombination Solution (ExtHRS) consists of three major components: the NGS kit, the bioinformatics pipeline, and the SOPHiA DDM™ desktop app.

1. The purpose of the NGS kit is to prepare and enrich DNA libraries from tumor FFPE samples suitable for deep sequencing (~3000x coverage) on an Illumina® NextSeq® 500/550 sequencer.

2. The main purpose of the bioinformatics pipeline is to

    - i) detect somatic and germline mutations and gene amplifications in *BRCA1* and *BRCA2*, as well as in other genes involved in HRR (based on targeted sequencing data), and

    - ii) provide tertiary annotation for identifying deleterious mutations in *BRCA1* and *BRCA2*, as well as in other HRR genes.

3. The SOPHiA DDM™ desktop app hosts the bioinformatics pipeline and serves as the interface for NGS data upload, and SOPHiA DDM™ extended Homologous Recombination Solution report generation and download.
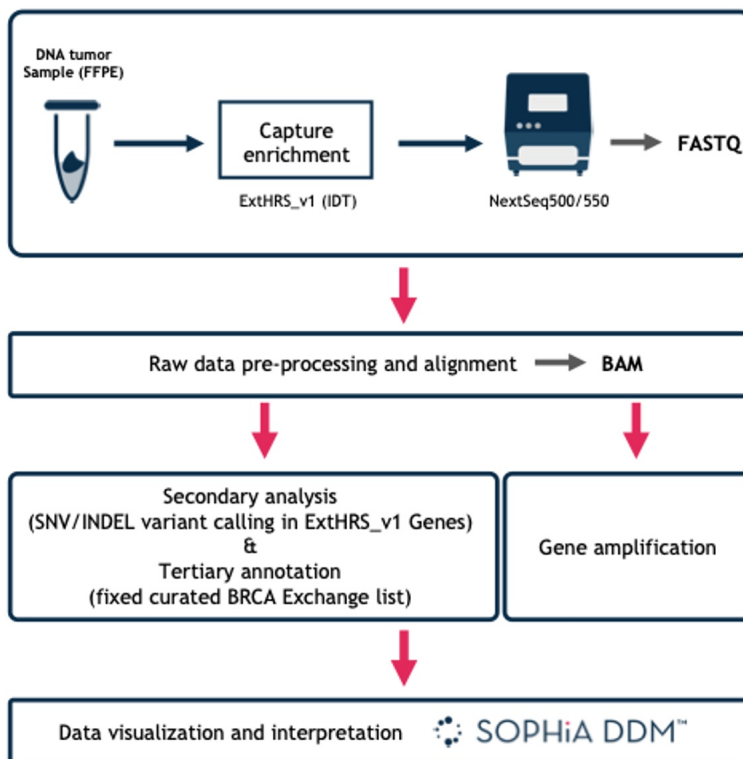
Overview of the different components of SOPHiA DDM™ extended Homologous Recombination Solution

4 PROTOCOL

4.1 Materials

4.1.1 Initial Considerations

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

4.1.2 Kit Content (32 Samples)

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

Box 1 (Store At -25°C To -15°C)

- xGen® Universal Blockers - TS Mix (12 µl)

- Human Cot DNA (25 µl)

- HRD_v1 xGen® Lockdown® Probes by SOPHiA GENETICS (20 µl)
    - *Note:* The product contains ExtHRS_v1 probes, although being labeled as HRD_v1.

- xGen® 2x Hybridization Buffer (50 µl)

- xGen® Hybridization Buffer Enhancer (20 µl)

- xGen® 2x Bead Wash Buffer (1250 µl)

- xGen® 10x Stringent Wash Buffer (200 µl)

- xGen® 10x Wash Buffer I (160 µl)

- xGen® 10x Wash Buffer II (110 µl)

- xGen® 10x Wash Buffer III (110 µl)

- 32 Illumina®-compatible Unique Dual Index Primers v2 in 96-well plate format (7 µl of primer per well). Please refer to Appendix I for primers display and sequences.

- Post Capture Illumina® Primers Mix (20 µl)

- PCR Enhancer (20 µl)

- Post Capture PCR Master Mix 2x (122 µl)

- Stubby Universal Adapter (220 µl)

Box 2 (Store At +2°C To +8°C)

- Dynabeads® M-270 Streptavidin (440 µl)

- Agencourt® AMPure® XP (8.7 ml)

- IDTE Buffer pH 8.0 (10 ml)

- Nuclease-free water (20 ml)

4.1.3 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 preparation kit.

⚠ **Refer to Warnings and Precautions below for additional details.**

4.1.4 Warnings And Precautions

PRODUCT NAME	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
Hybridization Buffer Enhancer		• H351 Suspected of causing cancer. • H360 May damage fertility or the unborn child. • H373 May cause damage to	Danger	Formamide

PRODUCT NAME	GHS PICTOGRAM	H&P STATEMENTS	SIGNAL WORD	HAZARDOUS COMPONENT
		organs through prolonged or repeated exposure. • P201 Obtain special instructions before use. • P202 Do not handle until all safety precautions have been read and understood. • P260 Do not breathe vapour/ spray. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P308+P313 IF exposed or concerned: Get medical advice/ attention. • P314 Get medical advice/ attention if you feel unwell. • P405 Store locked up. • P501 Dispose of contents/ container in accordance with national regulations.		
10x Stringent Wash Buffer		• H302 Harmful if swallowed. • H315 Causes skin irritation. • H319 Causes serious eye irritation	Danger	Ethylenediaminetetraacetic acid disodium salt
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	Danger	Sodium dodecyl sulfate

PRODUCT NAME	GHS PICTOGRAM	H&P STATEMENTS	SIGNAL WORD	HAZARDOUS COMPONENT
		center/ doctor. • P501 Dispose of contents/ container in accordance with national regulations.		
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

Please use and as personal protective equipment.

4.1.5 Materials Required (Not Provided)

User-Supplied Materials (To Be Purchased Separately)

Lab-related plasticware

- DNA low binding 1.5 ml tubes

- 50 ml conical tubes

- Filter tips

- 1.5 ml tubes

- RNase/DNase-free 0.2 ml 8-tube strips

Reagents

- Ethanol (molecular biology grade)

Other

- Ice to ensure reagents and pre-mixes are kept at 4 °C

- Illumina® sequencing reagents

Laboratory Equipment

To qualify samples use the: Agilent Fragment Analyzer™ system

To avoid sample contamination, separate the workspace into:

- *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

4.2 Library Preparation

4.2.1 DNA Preparation

Materials

- DNA extracted from formalin-fixed paraffin-embedded tissue (FFPE DNA)

- IDTE

- Nuclease-free water

- RNase/DNase-free 0.2 ml 8-tube strips

Input Recommendations

The quality of FFPE-extracted DNA is variable and might impact sequencing data. Exposure to formalin damages the integrity of DNA molecules and can lead to DNA fragmentation. It also induces sequencing artifacts due to deamination events.

DNA integrity of FFPE DNA samples should be assessed prior experiment using high-resolution gel electrophoresis on the Agilent Fragment Analyzer™ system.

Assessing DNA quality: Run 5–10 ng of genomic DNA on the Agilent Fragment Analyzer™ system using the HS large fragment 50 kb kit. The DNA quality of genomic DNA is evaluated with the DNA quality number (DQN). Set the threshold for the DQN to 300 bp and extract the DQN for your DNA samples.

⚠️ **Processing a sample without measuring DQN or a DQN lower than 3 might lead to suboptimal 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 the following table:

DNA INPUT RECOMMENDATIONS BASED ON DNA QUALITY			
DNA Quality (DQN)	DQN < 3 <30% of DNA fragments larger than 300 bp	3 ≤ DQN ≤ 5 30%–50% of DNA fragments larger than 300 bp	5 < DQN ≤ 10 >50% of DNA fragments larger than 300 bp
DNA Amount (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 cycles in step *4.2.6 Library Amplification* to obtain sufficient library yield.

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

Safe stopping point overnight at 4 °C.

4.2.2 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

1. Remove the SOPHiA GENETICS™ DNA Library Prep Kit III components from -20 °C storage and thaw on ice.

2. 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.

3. Prepare fresh 80% Ethanol (volume according to the following scheme based on number of reactions).

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

4. Ensure that the Fragmentation Buffer in the SOPHiA GENETICS™ DNA Library Prep Kit III is completely thawed.

5. 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 (µl)	38.4	76.8	115.2	153.6
Fragmentation Enzyme (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 (µl)	418	836	1254	1672
Ligation Enhancer (µl)	14.4	28.8	43.2	57.6

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

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

4.2.3 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

⚠️ **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:

2.

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	**4-tube**	**8-tube**	**8-tube**	**8-tube**
Fragmentation pre-mix (µl)	23	23	35	46

3. 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.

4. Place in the thermal cycler and continue the Fragmentation program.

5. Proceed immediately to Ligation.

4.2.4 Ligation

Materials

- Fragmentation reaction product in 50 µl each

- Ligation pre-mix

- Stubby Universal Adapters

- 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

⚠️ **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:

2.

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	4-tube	8-tube	8-tube	8-tube
Ligation pre-mix (µl)	100	100	150	200

3. 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.

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

5. 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).

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

7. Incubate in the thermal cycler at 20 °C for 15 minutes (open lid).

Proceed to Post-Ligation Clean Up.

⚠️ **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.**

4.2.5 Post-Ligation Clean Up

Materials

- Ligation reaction product 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.

4.2.6 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®

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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:

2.

NUMBER OF REACTIONS	8	16	24	32
PCR strip (1 strip)	**4-tube**	**8-tube**	**8-tube**	**8-tube**
PCR Master Mix 2x (µl)	60	60	85	120

3. 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.

4. Place the tubes in the thermal cycler and run the Library Amplification program.

   ✓ Safe stopping point overnight at 4 °C.

4.2.7 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.

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

4.2.8 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

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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 200 bp and 800 bp.
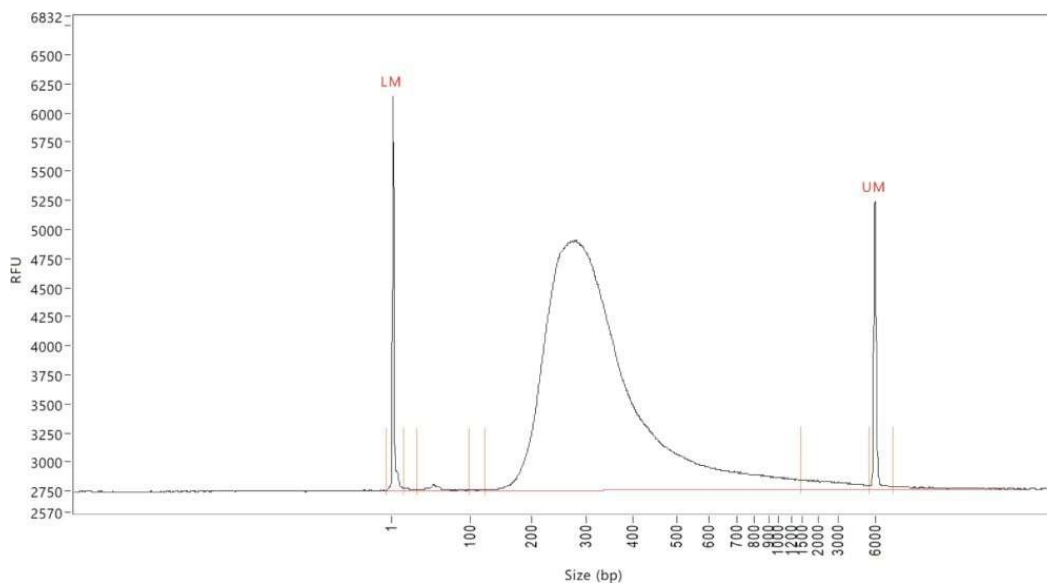
Example of a DNA library distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM refers to Lower Marker, UM refers to Upper Marker.
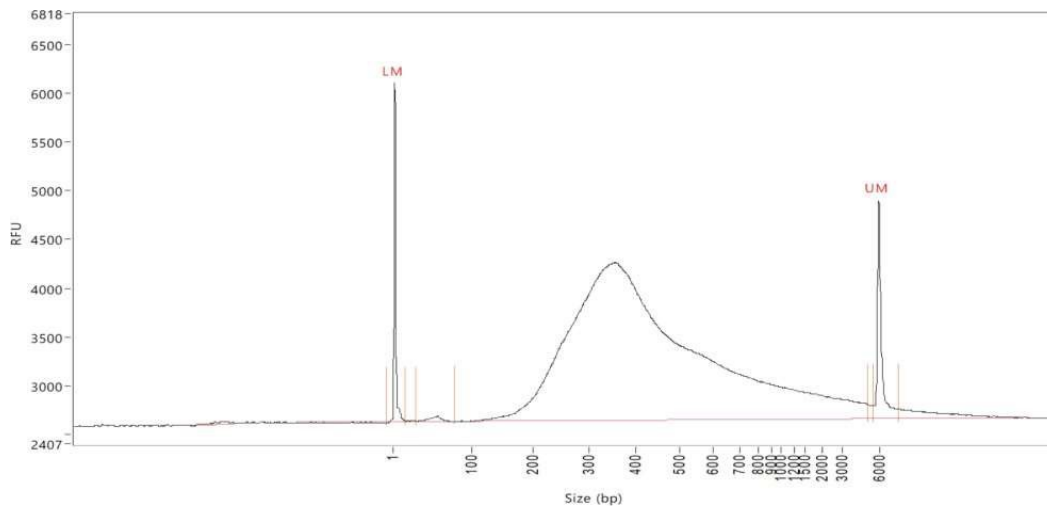
Example of a DNA library distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM refers to Lower Marker, UM refers to Upper Marker.

4.3 Library Pooling

4.3.1 Library Pooling For Hybridization And Capture

Materials

- Individual sequencing libraries

- Human Cot DNA

- xGen® Universal Blockers - TS Mix

- DNA low-binding 1.5 ml tubes

Procedure

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

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

2. Prepare one DNA low-binding 1.5 ml tube per capture.

3. Pipette 7 µl of the above pre-mix into individual DNA low-binding tubes for each capture.

4. 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.

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

6. Dry each mix using a vacuum DNA concentrator until mix is completely lyophilized. Use mild heating (45–50 °C) to speed up the lyophilization.

    Safe stopping point overnight at -20 °C.

4.4 Capture

4.4.1 Hybridization

Materials

- Lyophilized libraries

- 2x Hybridization Buffer

- Hybridization Buffer Enhancer

- HRD_v1 xGen® Lockdown® Probes by SOPHiA GENETICS (20 µl)

    - *Note:* The product contains ExtHRS_v1 probes, although being labeled as HRD_v1.

- 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).

⚠️ **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 (µl)	8.5	18.7	28.05	37.4
Hybridization Buffer Enhancer (µl)	3.4	7.48	11.22	14.96
Nuclease-free Water (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.

> ⚠️ **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**

> ⚠️ **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**

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Stringent Wash Buffer	88	792	**880**
2x Bead Wash Buffer	550	550	**1100**

⚠️ **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**

⚠️ **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**

⚠️ **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.**

4.4.2 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.

⚠ **Do not allow the beads to dry.**

Proceed immediately to Binding of Hybridized Targets to the Beads.

4.4.3 Binding Of Hybridized Targets To The Beads

Materials

1. Cleaned Streptavidin beads in PCR tube(s)

2. Hybridization reaction(s)

Procedure

⚠ **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.

4.4.4 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 (⅓ at 65 °C and ⅔ 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.

   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.

4.4.5 Post-Capture Amplification

Materials

- Streptavidin beads/nuclease-free water suspension (20 µl)

- Post-Capture PCR Master Mix 2X

- Post-Capture Illumina® Primers Mix

- PCR Enhancer

- Nuclease-free water

Preparation

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

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	
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 (µl)	25	55	82.5	110
Post-Capture Illumina® Primers Mix (µl)	2.5	5.5	8.25	11
PCR Enhancer (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.

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

4.4.6 Post-Capture Amplification Clean Up

Materials

- PCR reaction products in 50 µl each

- AMPure® XP beads equilibrated at room temperature

- Freshly prepared ethanol 80%

- IDTE

- DNA low-binding tubes for library storage

Procedure

1. Add 50 µl of AMPure® XP beads to each of the 50 µl PCR reaction products. Mix thoroughly by pipetting up and down 10 times.

2. Incubate at room temperature for 5 minutes and spin briefly 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.

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

## 4.4.7 Final Library Quantification And Quality Control

Materials

- Fluorometric quantitation equipment and reagents

- Capillary electrophoresis system

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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 pools of libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 200 bp and 800 bp.
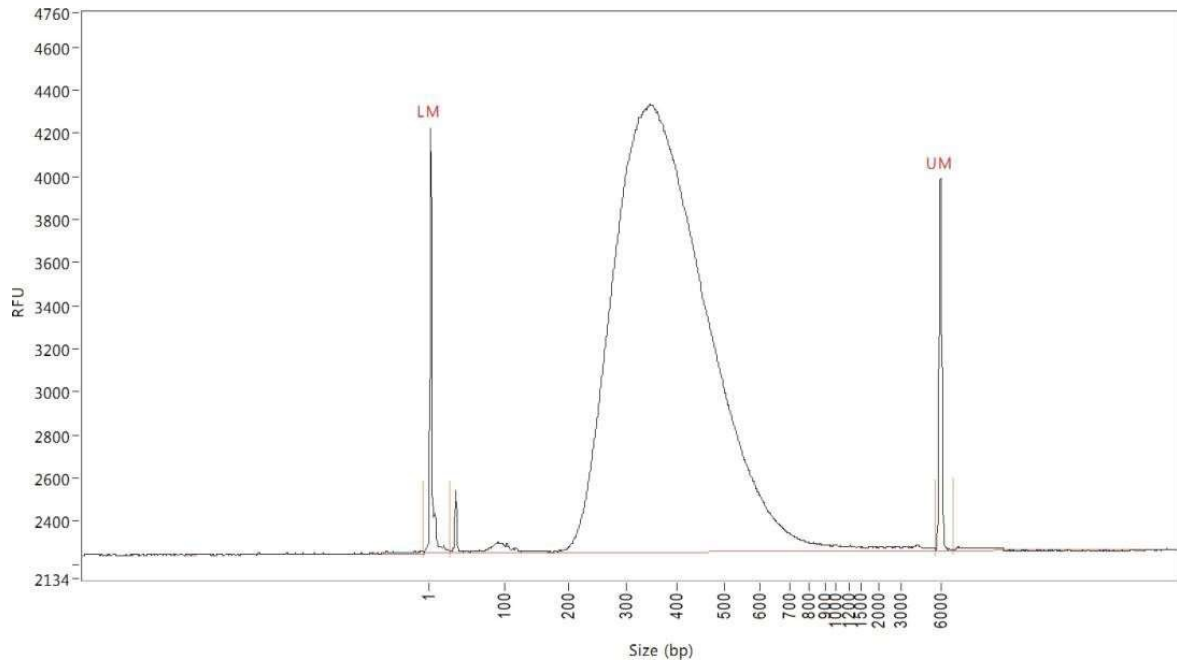
Example of post-capture library pool size distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM refers to Lower Marker, UM refers to Upper Marker.

4.5 Sequencing

4.5.1 Sequencing Preparations

Materials

- Illumina® NextSeq® 500/550 Sequencing Kit

- Final captured libraries

- EBT Buffer or similar

Procedure

1. Determine the molarity of each captured pool with the average size of the library (peak size in base pairs) and concentration (ng/µl) obtained during step *4.4.7 Final Library Quantification and Quality Control* as follows:

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

2. Dilute each captured pool to 10 nM.

3. If processing multiple sequencing pools, mix them in equal amounts (e.g., 5 µl of each) following the sequencing recommendation table below:

NEXTSEQ 500/550 SYSTEM KIT TYPE	SAMPLES PER RUN
Mid-Output	32 samples (4 pools)

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

5. For loading dilution, see the table below:

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

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

READ LENGTH (BP)	RECOMMENDED TOTAL READS PER SAMPLE	RECOMMENDED READ-PAIRS (FRAGMENTS) PER SAMPLE
2 x 150	8 million	4 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 the "Warnings, Limitations and Precautions" section.

Note: The extended Homologous Recombination Solution product is also compatible with sequencing data generated by Illumina® NovaSeq™ 6000 instruments, i.e., at the end of the analysis, the user will have access to the same type of results. However, please note that the analytical performance of this product has only been verified with Illumina® NextSeq 500/550 instruments.

5 ANALYSIS

5.1 Analysis Prerequisites

5.1.1 SOPHiA DDM™ Desktop App Installation Instructions

I. Once access to the SOPHiA DDM™ platform is granted, the user will receive two email communications:

    1. The first email contains the link to download the application and the User's Login credentials.

        a. The application is to be downloaded via the link https://www.sophiagenetics.com/downloads/.

        b. User may log in using the Login credentials.

    2. The second email contains a grid of token numbers that are required for Login.

II. After the installation of the SOPHiA DDM™ desktop app, the following steps are to be carried out by the user:

    1. Launch the application and enter the login credentials provided in the first email.

    2. Enter a token number from a given location on the grid provided in the second email. For example, for the token number in location 8A, the user needs to pick the token number mentioned in Column 8 and Row A in the grid. The user has 30 seconds to enter the token number before it expires, and then the application prompts the user with a new token location.

III. If applicable, the user may be prompted to read the Release Notes from the newly installed release. This is a mandatory step. The user needs to visit the page provided and tick the box to proceed.

IV. A notification regarding the recommended memory allocation for the SOPHiA DDM™ desktop app might appear. Users may either choose "Maybe Later" or "Take Action". Ideally, it is recommended to choose "Take Action". Note that the change will not take effect until the SOPHiA DDM™ desktop app is restarted, which requires a logout and a fresh login.

V. Once logged in to the SOPHiA DDM™ desktop app and at the Dashboard screen, a Technical Information box can be seen on the right side. The user should update their password and change it from what was provided in the email.

The application is now ready for the user to browse and explore the features and functionality of the platform.

> **Note:** For any help or documentation requests, visit the Support section on the Dashboard screen. The FAQs can be accessed by clicking the link or by directly navigating to https://www.sophiagenetics.com/faq/
>
> The Dashboard screen contains a link to the SOPHiA DDM™ desktop app Operation Manual as well. Either click the "?" at the top of the Dashboard or go to the Support section and click on "Operation Manual". For instructions on how to obtain further support, visit the "Support" section of this document.

5.1.2 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 analyses can be performed with bcl2fastq v2.0 or higher (bcl2fastq2 Conversion Software v2.20 Software Guide, Document #15051736 v03).

⚠️ **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 the SOPHiA DDM™ web app. Please refer to the User Manual of SOPHiA DDM™ web app for instructions.**

5.1.3 Data Upload And Kit Selection

After selecting the FASTQ files to be analyzed, the user is asked to specify/confirm the kit that was used for data generation.

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

⚠️ **Before finalizing the upload request, please ensure that the right kit— "SOPHiA DDM™ extended Homologous Recombination Solution"—is selected for all samples, as the automatic kit selection may not work optimally. See the DDM operational manual (section 2.2) for more information about the kit selection.**

After completion of the analysis, users will receive a notification by email. If a notification is not received within 24h from the initiation of the data upload process, users shall contact support.

5.1.4 Report Generation

Once the analysis is completed and the interpretation part is performed by the client, our platforms offer the option of creating a report document in PDF format. The final report is adaptable to the basic request of the client, who can include information about the person in charge of the analysis, service and center, as information concerning the case. Moreover, if desired, the final document may contain the client's conclusions of the analysis and more precise technical information, such as genes analyzed, variants selected (with detailed information), and actionability-associated data.

Finally, the customized outcome is automatically downloadable for the client's interest.

5.2 Analysis Description And Parameters

5.2.1 Resource Files

Alignment is performed against the GRCh37 reference genome (the previous major release of the human 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

5.2.2 Target Regions

See the table below for the target regions of the SOPHiA DDM™ extended Homologous Recombination Solution gene panel.

GENE	BASES COVERED BY EXTHRS_V1	TOTAL BASES ON CODING REGION	% OF GENE COVERED BY EXTHRS_V1
AKT1	387	4329	8.94
ATM	9171	9171	100
BARD1	7311	7311	100
BRCA1	21078	21078	100
BRCA2	10257	10257	100
BRIP1	3750	3750	100
CCNE1	4605	4605	100
CDK12	8919	8919	100
CHEK1	8250	8250	100
CHEK2	5907	5907	100
ESR1	856	11664	7.34
FANCA	9537	9537	100
FANCD2	13128	13128	100
FANCL	2271	2271	100
FGFR1	5523	21459	25.7
FGFR2	4909	26337	18.6
FGFR3	1531	6933	22.1
MRE11	6294	6294	100
NBN	4284	4284	100
PALB2	3561	3561	100
PIK3CA	1147	3207	35.8

GENE	BASES COVERED BY EXTHRS_V1	TOTAL BASES ON CODING REGION	% OF GENE COVERED BY EXTHRS_V1
PPP2R2 A	2718	2718	100
PTEN	3564	3564	100
RAD51B	12525	12525	100
RAD51C	1539	1539	100
RAD51D	2685	2685	100
RAD54L	4488	4488	100
TP53	13338	13338	100

5.2.3 Raw Data Pre-Processing

Pre-processing

- Collect quality metrics based on the raw fastq files.

- Truncate the compressed fastq files to a maximum size of 750MB per file.

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.

- Remove reads that have low mapping quality or low average base phred scores. A threshold of <30 is used in both cases.

- Local coverage at any position within the target regions is limited to 30,000x and to 10,000 outside target regions. Excessive coverage is removed following a random down sampling.

- Remove chimeric reads with hairpin loops.

- Realign soft clips.

- Assign reads to read groups based on start-end coordinates.

- Annotate low coverage regions based on a threshold of 100 molecules (read groups).

- Calculate statistics and coverage metrics on the processed alignment file.

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5.2.4 Included Analysis Modules

Quality Indicators

For each analyzed sample, a panel of traffic-light-like quality indicators are displayed in DDM™ to inform on specific quality metrics of the sample. Each indicator is colored in green when the corresponding quality metric is within the expected range, or in red vice versa. The following indicators are displayed for each sample:

- **Panel_cov_percentile_100:** The percentage of the gene panel covered at >100x molecules. The expected range for this metric is between 95% and 100%

- **BRCA_cov_percentile_100**: The percentage of the on-target BRCA1 and BRCA2 regions covered at >100x molecules. The expected range for this metric is between 99% and 100%. Whenever this indicator fails (ie., below 99%) a FN risk warning will be triggered in the context of BRCA status analysis.

- **BRCA_cov_unif:** The coverage uniformity in the on-target BRCA1 and BRCA2 regions. Coverage uniformity is defined as the fraction of the genomic regions that have coverage within 5 and 1/5 of the median coverage. The expected range for this metric is between 0.9 and 1.

- **Group_size:** The group size is defined as the median number of reads in each read group. A high group size reflects low library conversion rate and high rate of duplications. The expected range for this metric is between 1 and 10.

- **DeamScore**: The deamination score is a score devised to reflect the rate of artificial deamination in a given FFPE sample. The expected range of this score is between 0 and 0.8.

- **FragLength:** The median DNA fragment length in a sample. The expected range for this metric is between 75 and 250.

SNVs/INDELs

The SNV/INDEL detection modules include local realignment algorithms, variant calling software that apply statistical tests to identified mismatches versus the reference genome, variant regularization functions, variant quantification functions, and variant filtering functions.

Variant Calling

- Identify variants (SNVs and indels) by selecting positions in which the signal supporting the alternative allele is significantly different from background noise.

- Perform dedicated variant calling for long duplications and long insertions that cannot be identified by pileup of reads.

- Merge variants together if they are on the same allele (phasing).

- Quantify the variant fraction considering all the haplotypes in the neighboring region.

- Unify homopolymer annotation to long anchor standards.

Variant Filtering

- Filter variants below the background noise level of the panel and sequencer (filter = high_background_noise).

- Filter variants with variant fraction below 4 % (filter = low_variant_fraction).

- Filter variants with read coverage below 30 (filter = low_coverage).

- Filter variants outside of the target regions (filter = off_target).

- Filter Indels in homopolymers of length equal or higher than 10 (filter = homopolymer_region).

- Calculate a score based on the fraction of C:G>T:A variants with low molecular support and apply a differentiated threshold depending on the sample specific low/high score (filter = low_molecular_support).

- Remove variants beyond the target regions with a padding of 500bp.

- Remove duplications longer than 500bp.

- Filter variants with confidence score below 0 (filter = low_quality).

Variants without any filter associated to them are considered as "high confidence" calls and are the ones used for the assessment of analytical performance. Variants labeled with any of the filters mentioned above are considered "low confidence" and are shown with the sole purpose of helping the interpretation of all the weak signals present in the bam file.

Variant Annotation

The annotation system computes transcript-specific annotations following HGVS coordinate normalizations and notation guidelines (c.DNA and protein notation). This module also provides functional information for the variant's coding consequence; along with positional and contextual information such as rank and distance to of the closest exon, ref and alt codon sequence, ref and alt amino acid sequence. Transcript- (RefSeq identifiers) and gene-level (HGNC symbol, OMIM gene number) information is also provided at that stage. The annotation system then queries external databases, via genomic-coordinates matches, to retrieve variant-level information including dbSNP identifier, allele frequencies from GnomAD, 1000 genome project, ExAC, ESP5400, CG69, prediction scores from dbNSFP (SIFT, PolyPhen2, MutationTaster) and clinical significance assertions from ClinVar. The system finally annotates variants with licensed catalogs such as OMIM, CKB (actionable evidence displayed in OncoPortal) and the BRCA Exchange databases.

> **Note:** the BRCA1 and BRCA2 variants are annotated as per "BRCA_pathogenicity"; this score is obtained by aggregating the BRCA exchange's "Pathogenicity_expert" and "Pathogenicity_all" fields, following the set of rules detailed in the table below.

RULES FOLLOWED FOR AGGREGATING BRCA_EXHCANGE INFORMATION INTO THE "BRCA_PATHOGENICITY" DISPLAYED IN THE VARIANT TABLE
The BRCA_pathogenicity is assigned per variant, via classification rules consuming the "Pathogenicity_expert" and "Pathogenicity_all" attributes retrieved from BRCA exchange and applying rules according to the following precedence order:
1: Any variant annotated with 'pathogenic', 'likely pathogenic', 'risk factor', 'probable pathogenic' in **Pathogenicity_expert** gets a "BRCA_pathogenicity" as "pathogenic".
2: Any variant annotated with 'likely benign', 'benign', 'probably not pathogenic', 'benign / little clinical significance' in **Pathogenicity_expert** gets a "BRCA_pathogenicity" as "benign".
3: Any variant annotated with 'pathogenic', 'likely pathogenic', 'risk factor', 'probable pathogenic' in **Pathogenicity_all** gets a "BRCA_pathogenicity" as "pathogenic"

RULES FOLLOWED FOR AGGREGATING BRCA_EXHCANGE INFORMATION INTO THE "BRCA_PATHOGENICITY" DISPLAYED IN THE VARIANT TABLE
4: Any variant annotated with 'likely benign', 'benign', 'probably not pathogenic', 'benign / little clinical significance' in **Pathogenicity_all** gets a "BRCA_pathogenicity" as "benign".
5: Any variant with conflict between rules 3 and 4 gets a "BRCA_pathogenicity" as "unclear".
6: Any variant without any "BRCA_pathogenicity" assigned as per rules 1 to 5; and annotated with 'uncertain significance', 'no known pathogenicity', 'variant of unknown significance' in **Pathogenicity_all** gets a "BRCA_pathogenicity" as "unclear".
Rule traceability: The "pathogenic" and "benign" values, are further prefixed either with 'ex:' (expert) when they originate from priority rules 1 and 2 or with 'ag:' (aggregated) when they are generated by rules 3 and 4. As a result, the final available values for the "BRCA_pathogenicity" field are: - ex:pathogenic   - ag:pathogenic   - ex:benign   - ag:benign   - unclear   "" (in case there is no entry in BRCA exchange).

Gene Amplification/CNV

Copy-number variations (CNVs) are structural changes in the DNA associated with variations in the number of copies of the affected DNA sections. The CNV analysis in this product is gene amplification calling and is based on the NGS data targeting the extHRS panel.

The gene-amplification version of the CNV module is mainly designed for analyzing FFPE samples. It only detects gene-amplification events: multiple replications of large stretches of DNA typically involving whole genes. Deletions are not detected.

Gene-amplification detection, using targeted sequencing data, is performed, by:

- Normalizing the coverage levels of the target regions per sample and across samples within the same batch (by target region in the context of CNV detection, we understand a continuous region covered by probes).

- The average copy-number levels per gene (or other predefined large regions) are estimated.

- Genes with copy-number levels:

  - larger than 3.25 are reported as amplified.

  - lower than 3.25 are reported as not amplified.

- Samples with high levels of coverage noise (residual noise larger than 0.25) are rejected from the analysis. The gene copy-number levels of rejected samples cannot be confidently estimated, and these samples are reported as having undetermined CNV status.

Note: Please download and refer to the full PDF CNV report for a detailed description of the methods and the results.

6 PIPELINE DELIVERABLES

6.1 Results Location

Output files from SOPHiA DDM™ desktop app can be found at the run level (paper clip) or sample level (9-grid square).

- At the run level, a group of files can be downloaded – all files (including fastq), all vcf, all aligned bam files, aggregated full variant table, aggregated exon coverage and final variant reports.

- The summary pipeline QA report can be downloaded here, as well as other summary reports (e.g., CNV summary report).

- At the sample level, sample-specific files can be downloaded – fastq, aligned bam, target region coverage statistics, flagged regions, full variant table, sample-specific pipeline QA report and run-level summary reports (QA report, CNV summary report).

An Example is shown in the figure below:
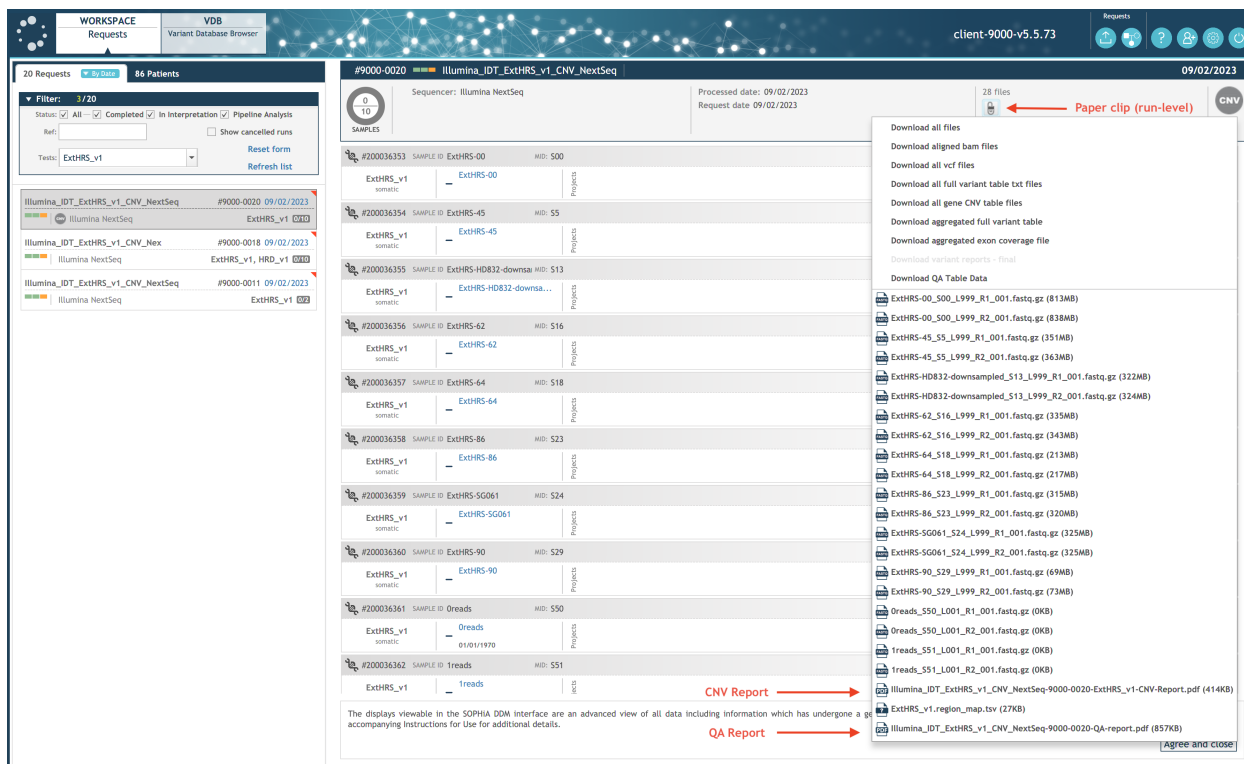
Schematic representation of the available files on the SOPHiA DDM™ desktop app.

6.2 File Composition

- **Fastq.gz files:** The input fastq files zipped.

- **Bam and Bai files:** The alignment file per sample and its index.

- **Full_variant_table.txt/vcf:** The detected variants in text and vcf format.

- **Cosmic_info_table.txt:** A text file containing information from the cosmic database.

- **Exon_coverage_stats_v3.txt:** A text file containing the coverage at the exon level.

- **QA-report:** The pipeline QA report in pdf format at the sample and run level.

- **CNV-Report.pdf:** The report for gene amplifications in pdf format.

List of output flies available for download.

6.3 Results Visualization

For information related to accessing analysis results (Variant display, QA report), please refer to section 4 "Data Analysis" of the SOPHiA DDM™ desktop app Operation Manual. Note that as certain modules are only active for selected pipelines, sections of the platform will be greyed out or bolded.

For example, the 'SNVs and INDELs' tab is activated, while the 'Fusions' tab is inactive in this pipeline.

The Results Visualization panel on the SOPHiA DDM™ desktop app.

Quality Indicators: For information on how to interpret the color codes of the quality indicator dots, please refer to Section 3.10 of the SOPHiA DDM™ desktop app Operation Manual.

6.4 Variant Filtering

List of reasons for variants being in the low confidence (i.e., being considered as low-confidence) tab are listed in the table below.

FILTER TYPE	DESCRIPTION
off_target	The variant is placed outside of the target region.
low_variant_fraction	The variant with variant fraction < 4%.
homopolymer_region	The variant in homopolymer region ≥ 10bp.
low_coverage	The variant is supported by an insufficient number of high quality (phred score > 20) reads
low_molecular_support	The variant is suspected to be a deamination artifact
low_quality	The quality score of the variant is insufficient
high_background_noise	The variant is suspected to be a result of sequencing noise

6.5 Variant Annotation

6.5.1 Description Of FVT Content

See below an example of the Full Variant table (FVT) with a description of each field and an example where possible. Please note that the examples mentioned in the table in italics are not from the same variant.

FIELD	DESCRIPTION	EXAMPLE
id	variant run ID, internal to FVT	1
annotation_id	variant ID in annotation database	60245
gene	HGNC gene symbol	BRCA2
overlapKnown	rsid of pathogenic clinvar entries	rs1555760738
type	variant type	SNP / INDEL
codingConsequence	consequence on protein	5'UTR
refGenome	reference genome	GRCh37/hg19
chromosome	chromosome	13
genome_position	genomic position (from variant caller)	32890572
depth	sequencing depth	12224
var_percent	variant fraction (relative depth of alternative allele)	49.32%
exon_rank	exon identifier	2
c.DNA	HGVS cDNA	c.-26G>A
protein	HGVS protein	
ref	ref in reference genome	G
alt	alternative allele	A
refNum	depth reference allele	6178
altNum	depth alternative allele	6029
refSeq	ref codon	
altSeq	alt codon	
refAA	ref amino-acid	
altAA	alt amino-acid	
tx_id	transcript ID in annotation database	35315
tx_name	transcript symbol in annotation database	NM_000059
refSeqId	transcript symbol in RefSeq	NM_000059
tx_version	transcript version in annotation database	3
refSeqIdVersion	transcript version in RefSeq	3
gene_boundaries	Exome / Intergenic qualifier	within

FIELD	DESCRIPTION	EXAMPLE
exon_id	exon legacy identifier	2
pos_in_exon	position in exon (strand specific)	14
dist2exon	position in intron (distance to closest exon)	0
filter	whether any quality filter should apply	.
dbSNP	dbSNP's rsid	rs1799943
g1000	Allele frequency	0.2093
esp5400	Allele frequency	0.2078
ExAC	Allele frequency	0.243
GnomAD	Allele frequency	0.2427
LJB_PhyloP	dbNSFP's precomputed PhyloP score	
LJB_SIFT	dbNSFP's precomputed SIFT score	
LJB_PolyPhen2	dbNSFP's precomputed PolyPhen2 score	
LJB_PolyPhen2_HumDiv	dbNSFP's precomputed PolyPhen2_HumDiv score	
LJB_LRT	dbNSFP's precomputed LRT score	
LJB_MutationTaster	dbNSFP's precomputed MutationTaster score	
LJB_GERP	dbNSFP's precomputed GERP score	
id_cosmic_coding	COSMIC ID coding variants	COSN20442808
id_cosmic_non_coding	COSMIC ID non-coding variants	
id_clinvar	Clinvar's rsid	rs1799943
CLNSIG	Clinvar's pathogenicity assertion	Benign
CLNREVSTAT	Clinvar's metadata	reviewed_by_expert_panel
gene_strand	strand	+
ref1	normalized ref (3' alnmt, in direction of strand)	G
alt1	normalized alternative allele (3' alnmt, in direction of strand)	A
first1	normalized genome_position (3' alnmt, in direction of strand)	32890572
last1	normalized genome_position(3' alnmt, in direction of strand)	32890572
multiTranscriptId		1
flagged_region_id		
depth_uniq	Number of unique molecular fragments at the position of a variant taking base Phred scores into account	400
refNum_uniq	Number of unique molecular fragments supporting the	203

FIELD	DESCRIPTION	EXAMPLE
	reference allele taking base quality (Phred Score) into account	
altNum_uniq	Number of unique molecular fragments supporting the alternative allele taking base quality (Phred Score) into account	197
matchStatus		exact
OMIM	mim2gene identifier	600185
hg38_chrom	Chromosome in hg38	1
hg38_pos	Genome position in hg38	46259775
hg38_ref	Reference allele in hg38	C
hg38_alt	Alternative allele in hg38	T
lift_diagnostic		PICARD
hg38_refGenome	Reference for hg38	GRCh38/hg38
sgid		00010001c698114b5b53cf6 0b6cdb5d02a62beba
hg38_sgid		00010101651f8aaff0d56960 c3121277b4d53606
BRCA_pathogenicity	"BRCA_pathogenicity"; as obtained by aggregating BRCA exchange's "Pathogenicity_expert" and "Pathogenicity_all" fields, following aggregation rules detailed in section *5.2.4 Included Analysis Module* under *Variant Annotation*	ex:pathogenic

7 WARNINGS, LIMITATIONS, AND PRECAUTIONS

7.1 Warnings

7.1.1 General Warnings

1. This product is for research use only and not for use in diagnostic procedures.

2. For detailed instructions on the software, refer to the SOPHiA DDM™ desktop app Operation Manual.

3. If any part of the handling, protocol, sequencer, multiplexing, etc., is changed, the analyses are not covered by the described user guide parameters.

4. The extended Homologous Recombination Solution has been fully validated for DNA FFPE samples with a DQN>3 (measured using Fragment Analyzer) and sequenced on Illumina NextSeq500/550.

5. Processing a sample without measuring DQN or a DQN lower than 3 might lead to suboptimal library preparation yield and insufficient NGS data quality, possibly resulting in inconclusive results.

6. The analytical performance of the product has only been verified on the Illumina NextSeq 500/550. Analytical performance for data generated using other sequencers is not guaranteed.

7.2 Limitations

7.2.1 General Limitations

1. The maximal run size that can be uploaded is 200Gb. Uploading higher volumes in a single batch is not allowed. The pipeline has been shown to successfully process individual samples with up to 50Gb of data. Higher volumes per sample might cause the pipeline to fail.

2. Even when sample multiplexing recommendations are followed, and the recommended average number of reads per sample (~8M) is achieved for a given sequencing run, read depth may be insufficient to provide sensitive and accurate 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, skewed read depth allocation across the gene panel (e.g., consistently lower read depth in AT-rich regions).

3. Other available devices for high-resolution gel electrophoresis, such as Tapestation, are not suitable for the estimation of DQN on FFPE samples due to interference of the lower marker with highly degraded DNA fragments.

4. Variant crosstalk between samples may occur due to sample-to-sample carryover in the steps before the library amplification. Such variant crosstalk is expected to manifest as potential False Positive variants: in most cases at an insignificantly low variant fraction, i.e., below the variant calling threshold; nevertheless, variant crosstalk between samples at significant variant fraction leading to erroneous variant calls is expected to be rare but cannot be completely ruled out.

7.2.2 Module-Specific Limitations

Data Upload

1. The product was designed to upload and process the volume of data produced by a NextSeq 500/550 (Mid and High Output flow cells).

2. Before finalizing the upload request, please ensure that the right kit—"SOPHiA DDM™ extended Homologous Recombination Solution"—is selected for all samples, as the automatic kit selection may not work optimally. See the DDM operational manual (section 2.2) for more information about the kit selection.

SNVs/INDELs

1. Variant detection in this product has been optimized for SNVs and short INDELs (up to 50bp).

2. 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 negative and/or false positives.

3. DNA fragments carrying SNVs or INDELs may not be efficiently captured by the panel probes, possibly resulting in False Negatives. This may affect:

   1. Multiple nucleotide variants (MNVs)

   2. Delins

   3. INDELs that are larger than 50bp

   4. DNA fragments carrying multiple variants.

   5. Complex variants such as i) delins or ii) INDELs near SNVs

4. Complex variants such as i) delins or ii) INDELs near SNVs or MNVs can create non-trivial soft-clip patterns that cannot be properly processed, resulting in false negatives.

5. Due to the complexity of the genomic context, some variants may be represented in different valid ways at the same time (e.g., different position within a repeated sequence). This would lead to a split assignment of the reads among the different representations of the variant, which would lead to an underestimation of the VAF and possibly to filtering the variant due to low variant fraction.

6. SNVs/INDELs in long repeat/low complexity regions may be missed due to the high levels of noise.

7. For a stable performance of the algorithm, we recommend coverage of unique molecules of at least 100x. Lower coverage will increase the risk of False Negative variant calls significantly.

8. Although we have measured the limit of detection for SNV and short deletions to be 2.2% and 3.1% variant allele fraction (VAF), respectively, variants below 4% VAF are filtered and considered of low confidence to avoid an excess of false positives.

9. SNVs/INDELs in homopolymers of length 10 or higher cannot be called confidently, since their detection is confounded by high background noise for homopolymers of this length.

10. NGS data quality can be affected by the quality and quantity of the input DNA. High levels of DNA degradation reduce the library preparation efficiency and, thus, the library complexity. Further, errors and inaccuracies in the library preparation and capture workflow, as well as sequencing-related errors, can reduce the NGS data quality. Poor NGS data quality can confound the bioinformatics pipeline, possibly resulting in False Positives or False Negatives.

11. Genomic regions of interest with low-complexity nucleotide sequences, nucleotide bias, repeats of any length (e.g., mono-, di- or trinucleotide repeats, transposable elements, Alu repeats, etc.) or with significant sequence similarity to other genomic regions (e.g., pseudogenes and gene families) are at higher risk of variant calling artifacts including False Positive and False Negative variant calls.

Gene Amplification/CNV

1. The copy number levels reported by the CNV module quantify the copy number in the DNA sample, not the absolute copy number of the tumoral DNA present in the sample.

2. The CNV module is designed to detect copy number gains affecting entire genes. The CNV module does not detect copy number losses nor copy number variations that affect only parts of genes.

3. The CNV module was only validated using in-silico data by showing that CNVs with copy number >6 are confidently detected.

4. The gene-amplification module is optimized to reduce the occurrence of false negatives (missed CNVs). Consequently, one should expect a certain number of false positives, particularly when the detected copy number is between 3.25 and 6. We recommend retesting such samples with an independent assay.

5. The CNV modules involve a cross-sample coverage normalization procedure. CN gains that are present in a large fraction of the samples processed in the same batch may be missed.

6. The CNV module is only applied to batches including at least 8 samples. If the total number of non-rejected samples in the batch is below 4, the whole batch is rejected. The performance of the module is expected to improve with an increased number of samples.

7. For optimal performance of the Gene Amplification module, all the samples included in the batch must be processed under the same laboratory condition.

8. The CNV module only considers genomic regions targeted by the ExtHRS panel, with the following regions being excluded: ESR1 (whole gene), AKT1 (whole gene), FANCL_ex1, BARD1_ex1, PPP2R2A_ex1, NBN_ex1, PTEN_ex1, CHEK1_ex1, BRCA2_ex1, PALB2_ex1, FANCA_ex1, RAD51D_ex1, CDK12_ex1, BRCA1_ex1, RAD51C_ex1, CHEK2_ex1; FANCD2 exons 14-23.

8 SYMBOLS

SYMBOL	TITLE
	Consult instructions for use
REF	Catalog number
LOT	Batch code (Lot Number)
	Caution
	Manufacturer
	Temperature Limit
	Use-by date
RUO	Research Use Only
EC REP	Authorized Representative in the European Community
Σ	Contains sufficient for <n> tests
	Importer
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.

SYMBOL	TITLE
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.
	Box 1
	Box 2

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9 SUPPORT

In case of difficulty using the SOPHiA DDM™ Desktop App, please consult the troubleshooting section of the "General information about usage of SOPHiA DDM™" document or contact our support line by telephone at +41 21 694 10 60 or e-mailsupport@sophiagenetics.com. Please visit www.sophiagenetics.com for further details.

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

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

10 APPENDICES

10.1 Appendix I: Unique Dual Index Primer Plates

10.1.1 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					

10.1.2 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

INDEX	I5 SEQUENCES FOR SAMPLE SHEET NEXTSEQ	I7
sgUDI-63	GTGAGGAT	GTAACTGT
sgUDI-64	ACTGCAGC	TGAGTTGA
sgUDI-65	CGATTGCC	CCTTAGAC
sgUDI-66	TAAGATGC	TATCGCCA
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

10.2 Appendix II: 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 programmable heated lid	Biometra	TAdvanced 96	
Fluorometric quantitation equipment and reagents	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

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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

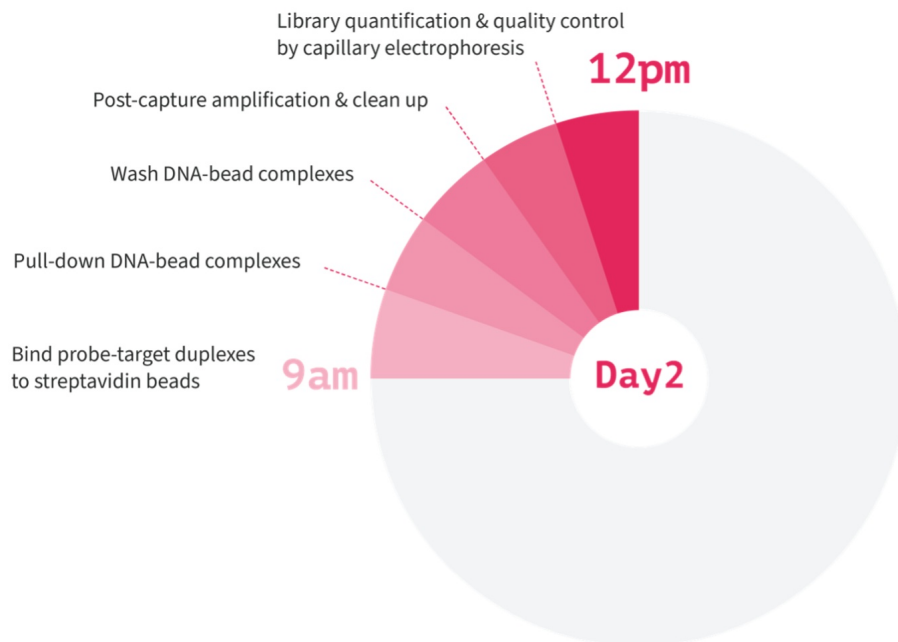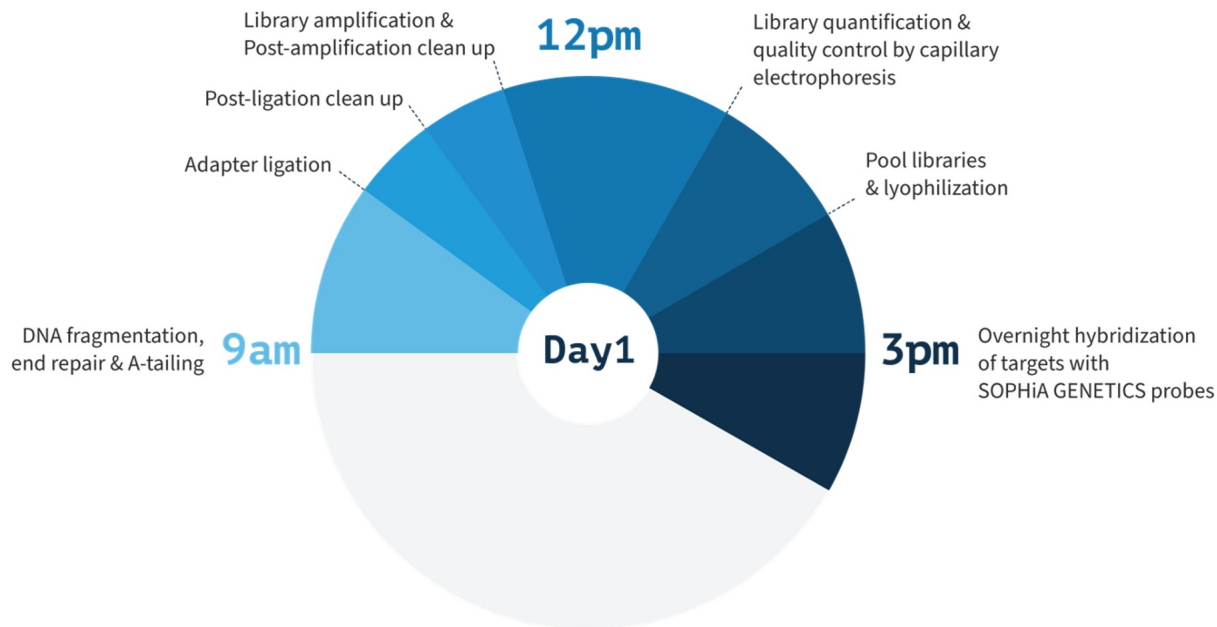# 10.3 Appendix III: General Workflow–SOPHiA Capture Solution

10.3.1 Four Hours Of Hybridization
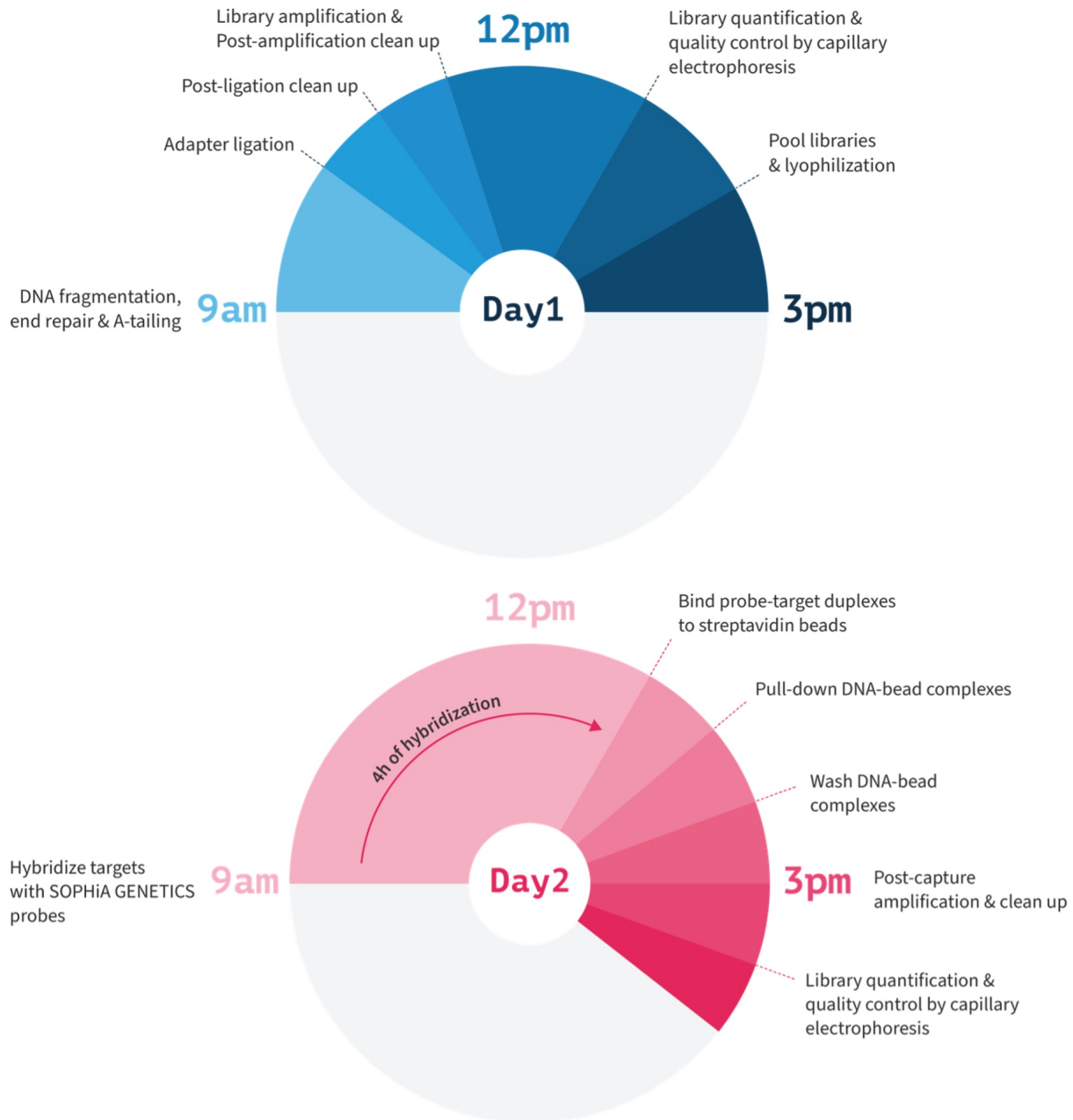
- Lyophilized libraries at the end of Day 1 may be stored at -20 °C before further processing on Day 2.

10.3.2 Overnight Hybridization

Library amplification &
Post-amplification clean up — **12pm**

Post-ligation clean up

Adapter ligation

Library quantification &
quality control by capillary
electrophoresis

Pool libraries
& lyophilization

DNA fragmentation,
end repair & A-tailing — **9am**

Day1

3pm — Overnight hybridization
of targets with
SOPHiA GENETICS probes

Library quantification & quality control
by capillary electrophoresis

Post-capture amplification & clean up

Wash DNA-bead complexes

Pull-down DNA-bead complexes

Bind probe-target duplexes
to streptavidin beads — **9am**

12pm

Day2

SOPHiA GENETICS™

Document Approvals
Approved Date: 01 Mar 2023

Approval Verdict: Approve	Martin Fritzsche, (mfritzsche@sophiagenetics.com) Technical Approval 28-Feb-2023 17:17:48 GMT+0000

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