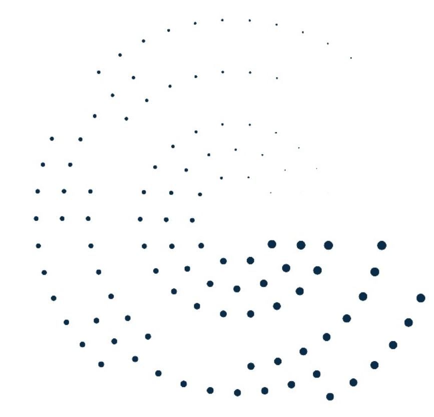
INSTRUCTIONS FOR USE

32 Samples

SOPHiA DDM™

Homologous Recombination Deficiency Solution



Using the SOPHiA GENETICS™ DNA Library Prep Kit III

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SUMMARY INFORMATION

Product Name	SOPHiA DDM™ Homologous Recombination Deficiency Solution
Product Type	Bundle Solution
Product Family	Molecular kit + analytics
Algorithm ID	ILL1XG1S7_FFPE_CNV_NextSeq_1
Gene Panel ID	HRD_v1
Product Version	v2.0
Release Version	v5.10.33 - p5.5.75
Sample Type	Somatic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens
Sequencer	Illumina® NextSeq® 500/550
Document ID	SG-00231
Document Version	v4.2
Revision Date	Dec 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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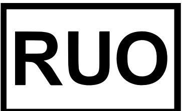
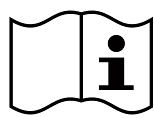
BS0121ILLRSMY08-32





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PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	LIBRARY PREPARATION KIT
REF	BS0121ILLRSMY08-32	B1.H1.0021.R-32	B2.0000.R-32	500232 (x2)

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REVISION HISTORY

DOCUMENT ID / VERSION	DATE	DESCRIPTION OF CHANGE

SG-00231 – v4.2	Dec 2023	• Section 4.2.1 *Input Material Preparation – Input Recommendations*:
		  • Changed DQN fragment length threshold from "300" to "375" bp
		  • Added part number "DNF-464-0500" for HS Large Fragment 50kb kit on Agilent Fragment Analyzer™
		• Minor formatting and typo corrections

SG-00231 – v4.1	Jun 2023	• Added a new limitation (2.) to section 7.2.1 regarding the rounding of QA and GI status for obtaining GI QA metrics and GI index • Removed third-party provider's intellectual property from material descriptions and trademarks • Replaced SOPHiA DDM™ Desktop App Installation instructions in section 5.1.1 with a reference to the SOPHiA DDM™ core platform User Manual_EN • Corrected typos and formatting errors

SG-00231 – v4.0	Mar 2023	• Product version increased to v2.0
		• Release version increased to v5.10.33 - p5.5.75
		• Update of trademark symbols and product names
		• Cosmetic text and formatting changes and minor document restructuring (including updates to figure and table numbering and cross-references)
		• Corrected reagent naming:
		  • "PCR Enhancer" → "Post Capture PCR Enhancer"
		  • "Post Capture PCR Master Mix" → "Post Capture PCR Master Mix 2x"
		  • "Stubby Universal Adapter" → "SU (Stubby Universal Adapter)"
		• Clarifications of DNA input recommendations (section 4.2.1)
		• Increased incubation time on the magnetic rack during Post-Capture Amplification Clean Up from 3 min to 5 min (section 4.4.6; step 11)
		• Information added regarding NGS data demultiplexing (section 5.1.2)
		• Warnings added regarding Kit Selection (section 5.1.3)
		• Updated content to align with IVD version of this IFU:
		  • Table *BRCA status translation* (section 5.2.4)
		  • Section 5.2.10 *Considerations Regarding Inconclusive Results*
		  • Section 5.2.11 *Considerations Regarding GI Negative* Statuses*
		  • Table and caption for *Proposed HRD status computation*(section

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DOCUMENT ID / VERSION	DATE	DESCRIPTION OF CHANGE
		5.3.4) • Section 5.3.4 *Included Analysis Modules – Proposed BRCA Status* • Section 5.3.4 *Included Analysis Modules – Proposed Genomic Integrity Status* • Modified limitation 2 in section 7.2.2 *Module-Specific Limitations – Proposed Genomic Integrity Status* • Added section 7.3 *Precautions* • Updated workflow illustrations in section 10.3 *Appendix III* • Updated support information (section 9)
SG-00231 – v3.0		• Incorporation of SOPHiA GENETICS™ • Incorporation of SOPHiA DDM™ throughout • Cosmetic minor changes
SG-00231 – v2.0	Jun 2022	• Document ID Change • Cosmetic changes to text and graphs • SOPHiA DDM™ (desktop app) name change • Hazards – Components, Warnings adjusted • Symbols list updated
ID-60101-142 – v1.3	25.03.2022	• Updated Table 7 with numbered tabs
ID-60101-142 – v1.2	11.03.2022	• Updated Symbols and Trademarks • Corrected issue with images on page 48 to 50
ID-60101-142 – v1.1	03.03.2022	• Change in number of samples (Section 5.4.1) • Change of typo GIS to GII (Section 8.2.7) • Inserted LM and UM full forms • Table 1 annotated • Changes in time in section Amplification Cleanup • Cosmetic changes • Addition of Document ID to all pages
ID-60101-142 – v1.0	21.02.2022	• Initial Release

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1 PRODUCT INTRODUCTION

The intended use of the SOPHiA DDM™ Homologous Recombination Deficiency Solution is to facilitate research in solid tumor samples from Ovarian Cancers. Users will be able to detect Homologous Recombination Deficiency (HRD) through both: i) the direct observation of genomic aberrations, which are known or suspected causes of HRD, ii) the indirect detection of HRD, via the observation of genomic integrity loss.

The product includes a Next Generation Sequencing (NGS) kit and protocol to allow the user to perform, using a single workflow, targeted sequencing, and low-pass Whole Genome Sequencing (lpWGS) on DNA extracted from FFPE tissue.

The product includes a bioinformatics pipeline for: i) the detection of somatic and germline mutations in *BRCA1*, *BRCA2*, as well as in other genes involved in Homologous Recombination Repair (HRR), ii) the calculation of a genomic integrity (GI) index and determination of a GI status, known to be impaired in HRD samples, and iii) the detection of gene amplification events. After performing tertiary annotation, the bioinformatics pipeline computes and proposes to the end-user a sample BRCA status, GI status and HRD status.

The product includes SOPHiA DDM™ desktop app to allow the user to visualize the bioinformatics pipeline results, review and, if needed, overwrite, the proposed BRCA, GI and HRD statuses. DDM also allows the user to assess and report the status of non-BRCA genes involved in HRR as well as the *CCNE1* gene amplification status, which have been statistically associated with HRD. These two additional biomarkers have been shown in the literature to be associated with HRD. For these two biomarkers, the bioinformatics pipeline will not compute and propose a status.

The HRD product shall be used with FFPE samples from Ovarian cancers and in combination with the Illumina® NextSeq® 500/550 sequencer. The product shall be used through the SOPHiA DDM™ desktop app platform to enable data upload, result visualization, as well as reporting of the sample HRD status.

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2 GENERAL STATEMENT OF THE TEST PRINCIPLES AND PROCEDURE

The SOPHiA DDM™ Homologous Recombination Deficiency Solution product is intended for the preparation of DNA libraries to assess the HRD status of an ovarian tumor based on DNA extracted from FFPE tissue. The NGS kit should allow users to perform the following to determine HRD status in their own laboratory with a single workflow:

- Targeted sequencing (TS) of *BRCA1*, *BRCA2*, as well as other genes involved in HRR or linked to HRD.
- Low-pass Whole Genome Sequencing (lpWGS).

The analytical algorithms will:

- Process TS data to identify genomic aberrations which are known or suspected to cause HRD.
- Process lpWGS data via a deep-learning algorithm capable of quantifying genomic integrity.

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3 PRODUCT COMPONENTS

The product consists of three major components: DNA library preparation and capture kit, the bioinformatics pipeline, and the SOPHiA DDM™ desktop app.

The purpose of the DNA library preparation and capture kit is to allow the preparation of libraries from FFPE samples suitable for sequencing on an Illumina sequencing platform.

The purpose of the bioinformatics pipeline is to analyze the sequencing data obtained from an Illumina® NextSeq® 500/550 sequencer and return variant calls, gene amplification calls, genomic integrity analysis results (i.e., secondary analysis), as well as proposed GI, BRCA, and HRD statuses, while the purpose of the SOPHiA DDM™ desktop app is to host the pipeline and serve as the interface for the upload of sequencing data and tertiary analysis.

Sequencing data for analysis with the workflow should be generated starting with tumor FFPE samples by following the instructions for library preparation using the SOPHiA DDM™ HRD library preparation and capture kit and sequencing these samples on an Illumina® NextSeq® 500/550 sequencer.

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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)

- Universal Blockers - TS Mix (12 µl)
- Human Cot DNA (25 µl)
- HRD_v1 Probes by SOPHiA GENETICS (20 µl)
- 2x Hybridization Buffer (50 µl)
- Hybridization Buffer Enhancer (20 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 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)
- Post-Capture PCR Enhancer (20 µl)
- Post-Capture PCR Master Mix 2x (122 µl)
- SU (Stubby Universal) Adapter (220 µl)

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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)

4.1.3 SOPHiA GENETICS™ 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)

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

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

Refer to Warnings and Precautions below for additional details.

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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

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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

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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

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- 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 (e.g., Qubit® or similar)
    - Magnetic separation rack (96-well type)
    - Multichannel pipettes (P10 or P20; P100; P200) with appropriate filter tips
    - Tabletop microcentrifuge (8-tube strips compatible)
    - Thermal cycler (programmable heated lid; 0.2 ml PCR tubes compatible)
    - Vortex mixer
- *Post-PCR zone*
    - Capillary electrophoresis system
    - DNA vacuum concentrator (e.g., SpeedVac™ or similar)
    - Fluorometric quantitation equipment and reagents (e.g., Qubit® or similar)
    - Magnetic separation rack (1.5 ml tube compatible)
    - Magnetic separation rack (96-well type)
    - Multichannel pipettes (P10 or P20; P100; P200) with appropriate filter tips
    - Tabletop microcentrifuge (8-tube strips compatible)
    - Thermal cycler (programmable heated lid; 0.2 ml PCR tubes compatible)
    - Thermoblock or water bath (1.5 ml tube compatible)
    - Vortex mixer

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4.2 Library Preparation

4.2.1 Input Material Preparation

Materials

- DNA extracted from formalin-fixed paraffin-embedded tissue (FFPE DNA) or DNA extracted from fresh frozen material (FF DNA)
- IDTE
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

Input Recommendations

The SOPHiA DDM™ HRD Solution is intended for samples with a tumor content above 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 the integrity of DNA molecules and can lead to DNA fragmentation. It also induces sequencing artifacts due to deamination events. Insufficient DNA quality can thereby confound the data analysis and cause false-positive, false-negative, and uninterpretable results.

DNA integrity of FFPE DNA samples must be assessed prior experiment using high-resolution gel electrophoresis on the Agilent Fragment Analyzer™ system. FF DNA is usually of good quality and DNA integrity does not have to be assessed.

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.

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 *7 Warnings, Limitations, and Precautions*).

Depending on the DNA quality, adjust the protocol according to the following table (if the DQN cannot be assessed, follow the input recommendations for samples with a DQN larger than 5):

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

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*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

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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.

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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

1. 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	∞

2. 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:

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

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.

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4.2.4 Ligation

Materials

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

Preparation

1. During the Fragmentation, prepare new PCR strips with 5 µl of SU 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

2. 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:

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

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 SU Adapters.
3. Mix thoroughly by pipetting up and down 10 times and spin briefly.
4. Using a multichannel pipette, add 45 µl 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.

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.

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4.2.5 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.

4.2.6 Library Amplification

Materials

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

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Preparation

1. 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	8 cycles
Step 3: Annealing	60	30	
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 (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.

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

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- 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 and 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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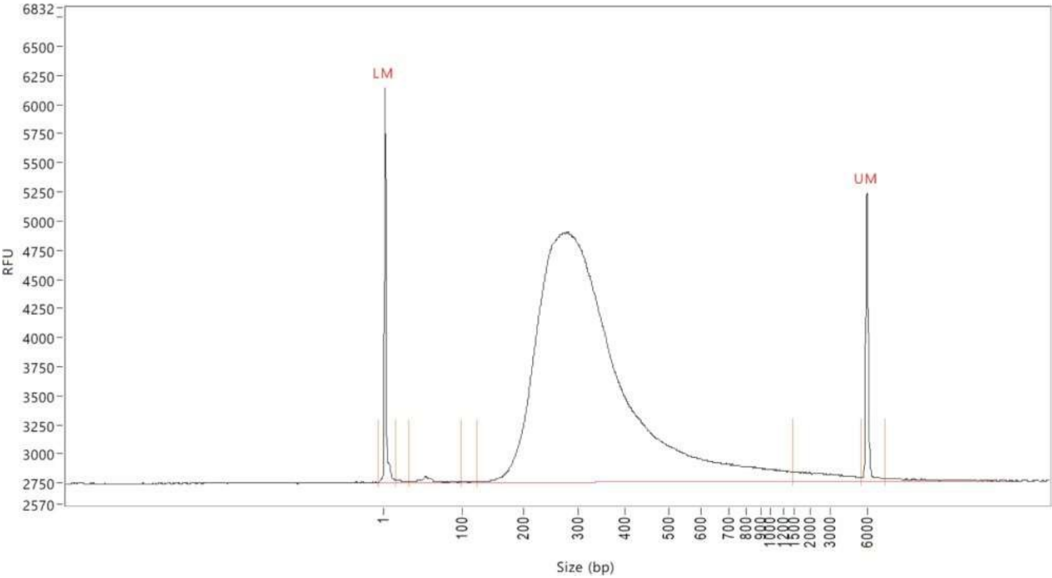
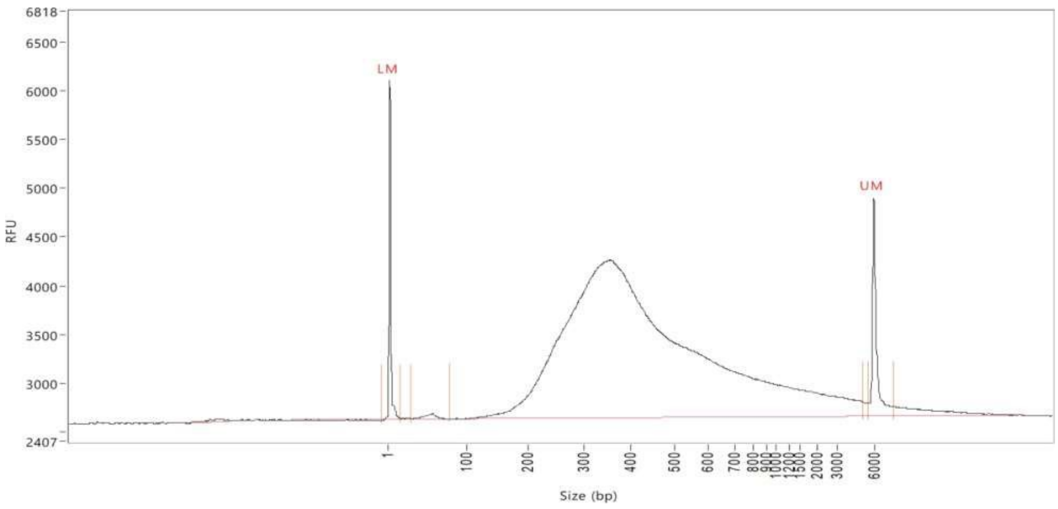
Procedure

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 prepared previously).
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: Lower Marker, UM: Upper Marker.



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

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4.3 Library Pooling

4.3.1 Library Pooling For Low-Pass Whole Genome Sequencing (lpWGS)

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 *4.2.8 Individual 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. 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 Whole Genome Sequencing (WGS) pools as there are capture pools.

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

4.3.2 Library Pooling for Hybridization and Capture

Materials

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

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Procedure

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

CAPTURE PRE-MIX				
Number of captures	**1**	**2**	**3**	**4**
Human Cot DNA (µl)	5	11	16.5	22
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 Capture 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.

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4.4 Capture

4.4.1 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).

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

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Procedure

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

HYBRIDIZATION PRE-MIX				
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 sections 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**

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BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer III	44	396	**440**
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

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- 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

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

Procedure

Work quickly to ensure that the temperature of the sample(s) 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.

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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 immediately 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.

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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

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- Post-Capture Illumina® Primers Mix
- Post-Capture PCR Enhancer
- Nuclease-free water

Preparation

1. 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
Post-Capture 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

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- 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 if required 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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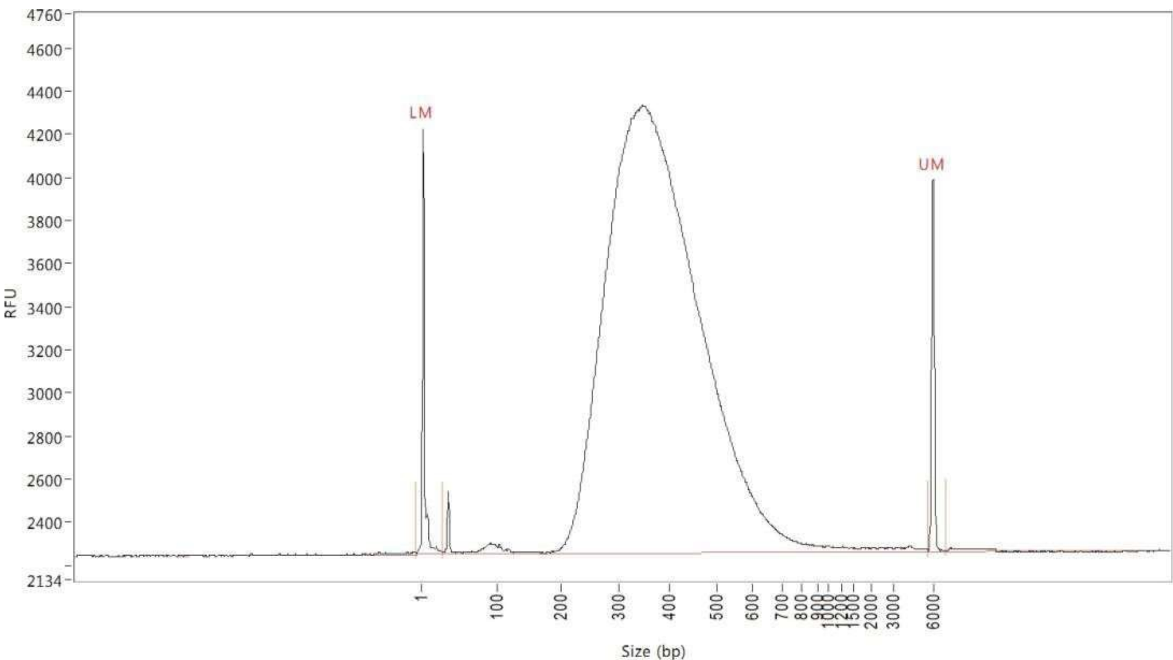
Procedure

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: Lower Marker, UM: Upper Marker.

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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. Thaw the WGS pool generated in *4.3.1 Library Pooling for Low-Pass Whole Genome Sequencing (lpWGS)*.
4. 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.
5. If processing multiple sequencing pools, mix them in equal amounts (e.g., 5 µl of each) following the sequencing recommendations in the table below:

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

6. Mix it well and use this dilution according to Illumina® standard denaturation recommendation.
7. 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]	

8. 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	32 million	16 million

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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 *7 Warnings, Limitations, and Precautions*.

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5 ANALYSIS

5.1 Analysis Prerequisites

5.1.1 SOPHiA DDM™ Desktop App Installation Instructions

For instructions on SOPHiA DDM™, please visit https://www.sophiagenetics.com/docs/ and download the SOPHiA DDM™ core platform User Manual_EN.

5.1.2 NGS Data Demultiplexing Instructions

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).

5.1.3 Data Upload And Kit Selection

Data Upload

Run Composition and Size

For high-quality results based on SOPHiA DDM™ analytics, 16 million fragments or read pairs (i.e., 32 million reads) per sample are recommended. This would be reflected in a total of 24 samples for a NextSeq 500/550 high output run or 8 samples for a NextSeq 500/550 mid output run. If these specifications are not followed, please refer to section *7 Warnings, Limitations, and Precautions* for details on the maximum file size.

Dependencies

The upload will be terminated if any of the following occurs during the upload:

1. The computer or laptop is shut down, or laptop lid is closed.
2. The user logs out of their account.
3. Files are removed from their original location before the upload is completed.

If any of this happens, SOPHiA DDM™ desktop app must be restarted to force the upload to start again. This must be done on the same computer, using the same account, and by the same SOPHiA DDM™ desktop app user.

Usage Of USB Key

When using a USB key to transfer data, it is recommended to copy the files onto the computer first and then start the run using the local copy.

The USB key should not be removed before the copying is complete.

To remove the key, use the "Safely Remove Hardware" option on Windows or "Eject" on Mac. This is also valid when making the copy from the sequencer.

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Network Drives and Shared Folders

It is recommended to copy the data from the network drive to the local computer first and subsequently upload using the local copy to prevent issues due to limited network bandwidth. When using a network drive, it is important not to disconnect from the local network before the run upload is completed.

Naming Convention

SOPHiA DDM™ desktop app uses the Illumina naming convention and folder configuration to find the FASTQ files. Therefore, it is strongly advised NOT to reorganize or rename the FASTQ files after they are copied from the sequencer; otherwise, the run might fail.

Illumina® NextSeq 500/550 File Specifications

A minimum of two fastq files (R1 and R2) per analysis are required to be present in the same folder. More than two files per sample are acceptable, provided the files are always in pairs.

Analysis files should use the standard Illumina naming convention (Sampleidentifier1_S1_L001_R1_001.fastq.gz).

All analysis folders in one run should be in the same folder – the main folder.

Sample identifier name “Sampleidentifier1” should not contain an underscore sign “_”, a point"." or any special characters.

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™ Homologous Recombination Deficiency Solution”—is selected for all samples, as the automatic kit selection may not work optimally. See the SOPHiA 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.

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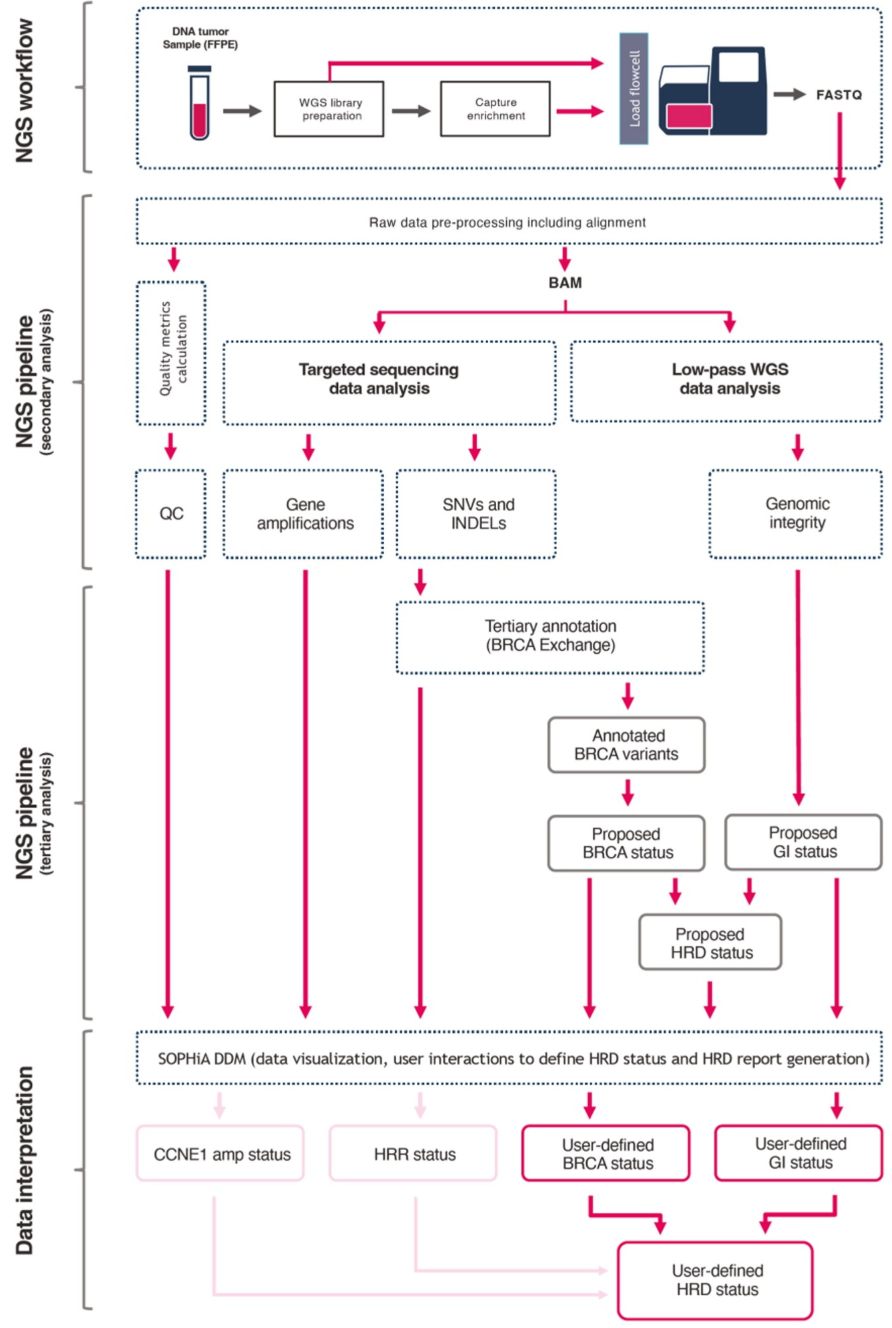
5.2 Analysis Workflow

5.2.1 Workflow

The following schematic illustrates the workflow from the DNA samples isolated from FFPE material through the sequencing steps and finally into the proposed HRD status in SOPHiA DDM™.



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5.2.2 Start An HRD Interpretation

To create an interpretation, please refer to section 3.12 of the SOPHiA DDM™ user manual.

The HRD status is displayed in the lower-left corner of the Overview tab of the interpretation window (see *Figure 1* below). To access the HRD review window, click on the HRD status button.

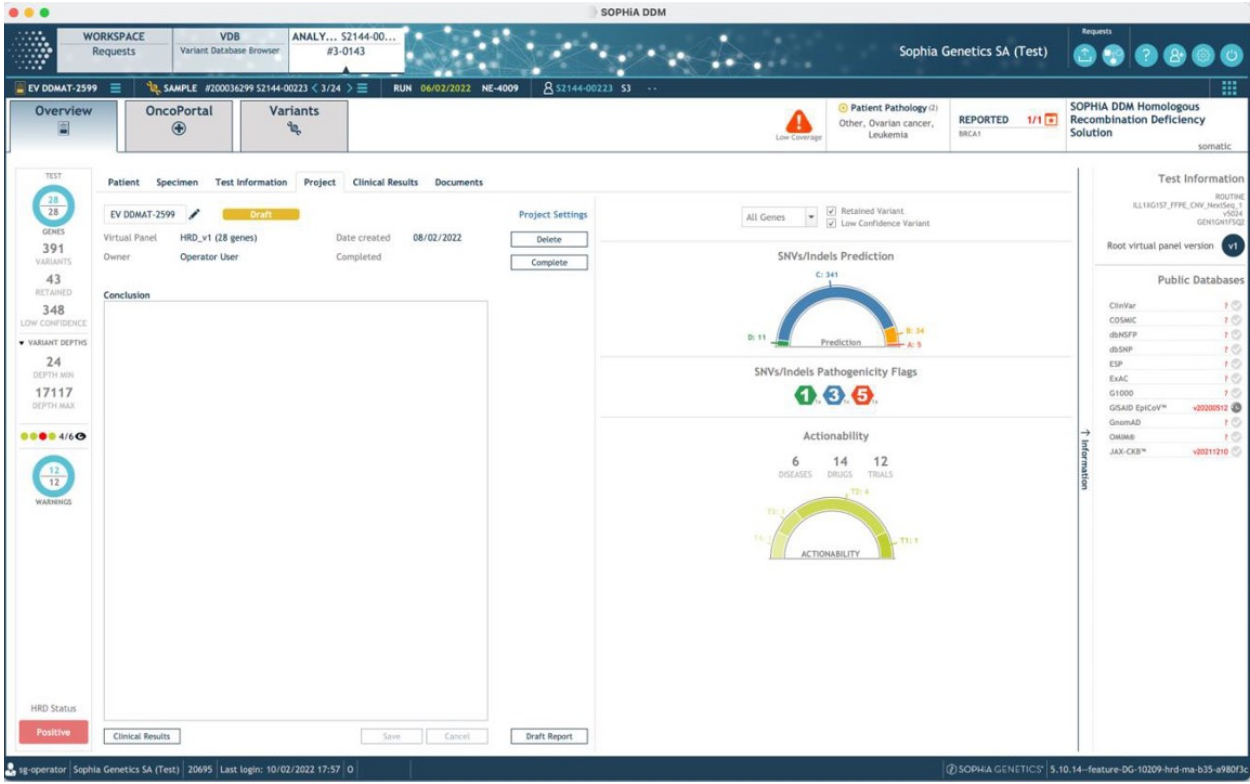

Figure 1: Interpretation overview window with the HRD status indicator

5.2.3 Review And Edit HRD Status

The HRD status edit/review window displays:

- The final HRD status. By default, the final status is based on the Proposed HRD status computed by the pipeline.
- A Proposed HRD status computed by the pipeline following the rules described in *Table 9* on page 55.
- The HRD-supporting Genomic Integrity (GII), BRCA, HRR, and CCNE1 status. By default, only the GII and BRCA statuses are selected to be reported together with HRD.

To edit the final HRD status, click on "edit" and select the status value. To review or edit any of the HRD-supporting statuses, click "view" (*Figure 2* on the next page).

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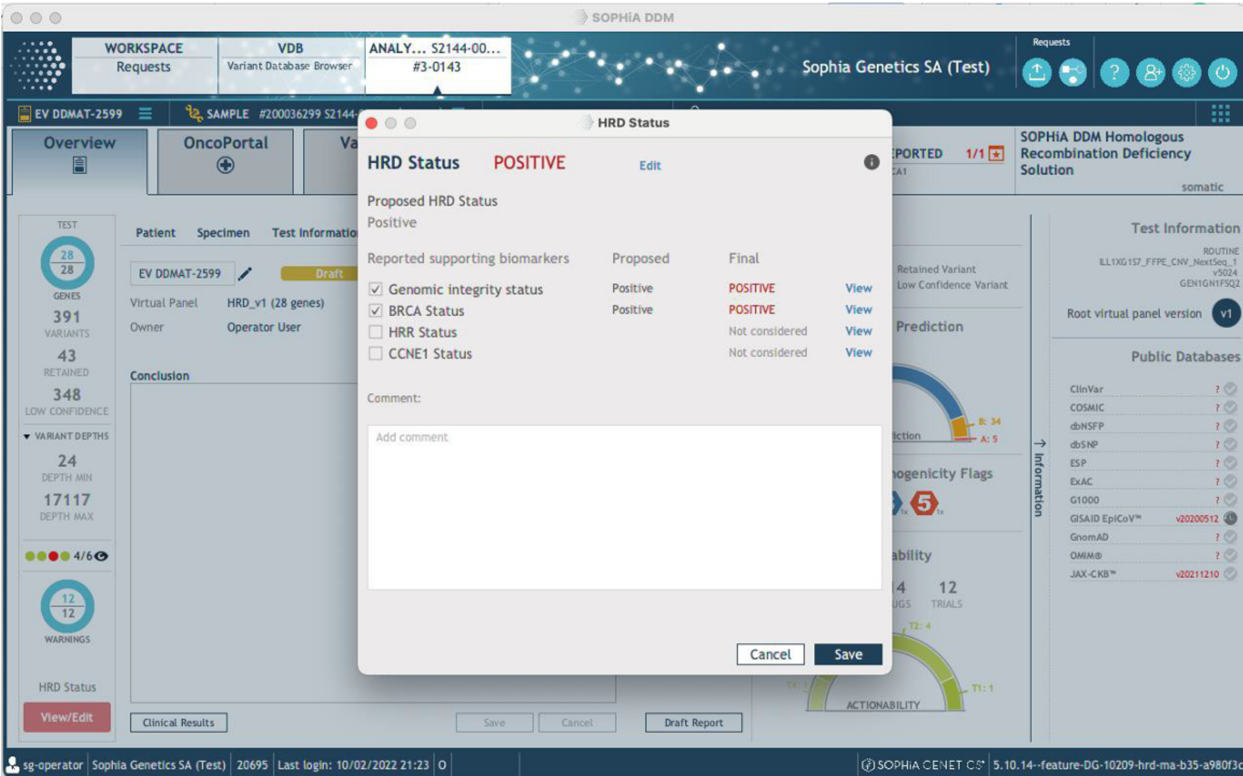

Figure 2: HRD Status edit/review window

5.2.4 Review And Edit BRCA Status

The BRCA status window (*Figure 3* on the next page) displays the following:

- The final BRCA status. By default, the final status is based on the proposed BRCA status following the rules described in *Table 1* on the next page.
- A proposed BRCA status computed by the pipeline.
- The list of BRCA variants supporting the proposed status (only high confidence variants with positive, predicted positive, complex case or undetermined BRCA variant score will be prepopulated; these variants are reported if the BRCA status is selected for report).

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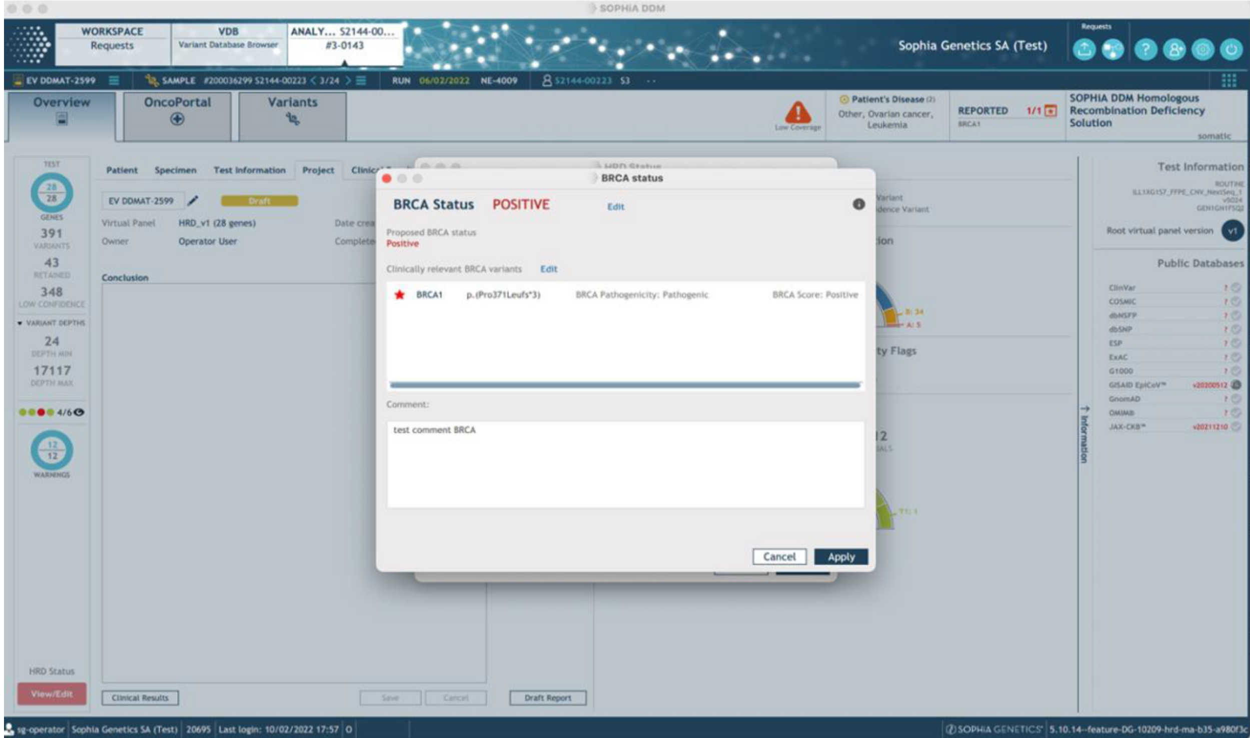

Figure 3: The BRCA Status edit/review window

Table 1: Translation of the proposed BRCA status into the default final BRCA status.

PROPOSED BRCA STATUS	FINAL BRCA STATUS
Positive	Positive
Predicted Positive	Positive
Complex Case	Undetermined
Undetermined	Undetermined
Inconclusive	Undetermined
Predicted Negative	Negative
Negative	Negative

The BRCA window can also display warnings to inform users of (see section *Quality Indicators*):

1. **False negative risk:** Less than 99% of the BRCA1, BRCA2 regions targeted by the panel are covered by >100x unique molecules. In this case, users should check low coverage regions to judge if the data quality is sufficient to confidently report a final BRCA status. Users can quickly check the % of BRCA1, BRCA2 regions covered by >100x unique molecules through the dedicated quality indicator (see *Figure 4* on the next page). For more info about the quality indicator, see section 3.10 of the SOPHiA DDM™ Desktop App Operation Manual.

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Figure 4: BRCA coverage indicator

2. **Review clinical interpretation:** High confidence variants with BRCA score = complex case or undetermined were detected. In this case, users should look for them and, if they want, flag them as in scope for BRCA analysis.

3. **Review low confidence variants:** Some relevant BRCA variants have been detected by the pipeline with a low confidence warning. In this case, users may want to check those and bring them up for BRCA interpretation (see section 4 of the SOPHiA DDM™ Desktop App Operation Manual).

To edit the final BRCA status, click on "edit" and select the status value. To add or remove variants from the list of supporting BRCA variants, click on "edit". The displayed panel (*Figure 5* below) shows all detected BRCA variants and allows users to add or remove them from the report. Alternatively, the variants can also be accessed in the variant table.

> **Note:** If the users choose to restrict the interpretation using a virtual panel that excludes BRCA genes, then the BRCA status is disabled.

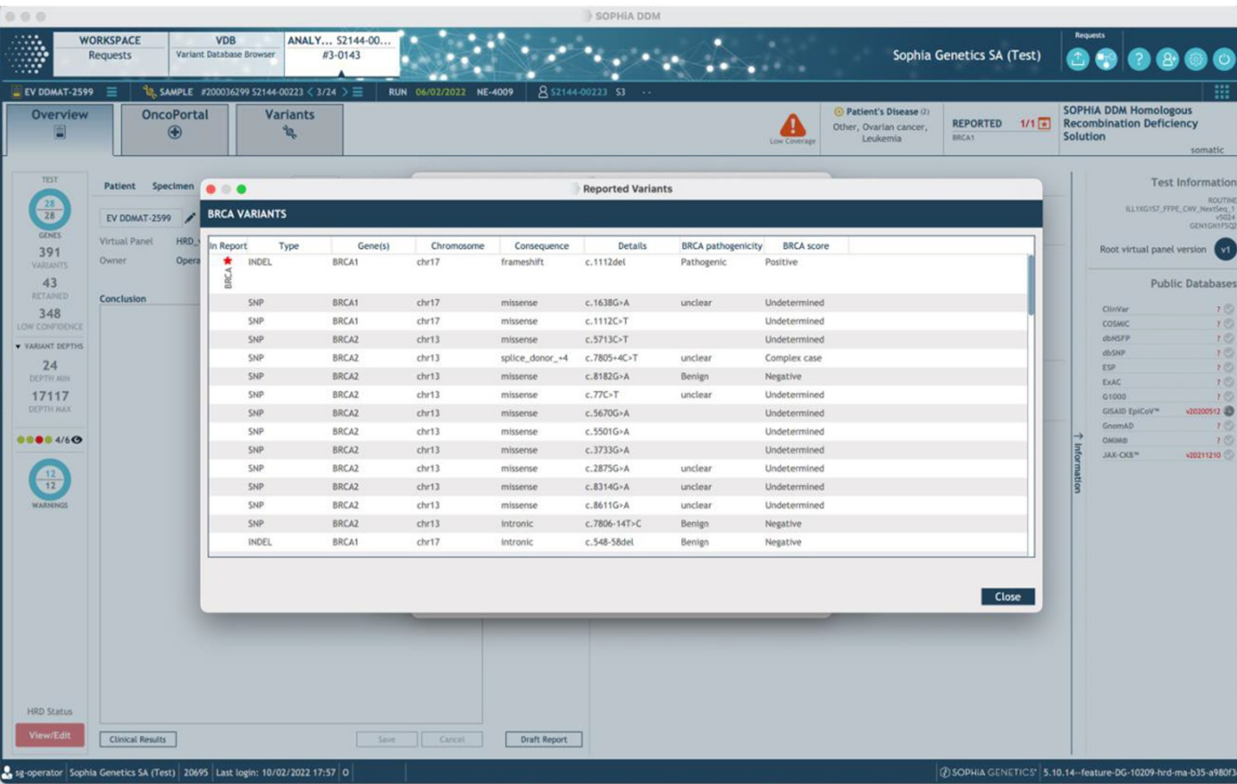

Figure 5: Overview of BRCA variants

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5.2.5 Review And Edit Genomic Integrity Status (GIS)

The GIS window (see *Figure 6* below) displays:

- The final GI status. By default, the final status is based on the proposed GI status following the rules described in *Table 2* below.
- The proposed GI status for Ovarian Cancer (see *5.3.4 Included Analysis Modules*, *Proposed HRD Status*).
- The Genomic integrity index (see *5.3.4 Included Analysis Modules*, *Proposed HRD Status*). The Genomic integrity index is not shown for samples that are "Rejected" or "Inconclusive".
- The Genomic integrity QA status (see *5.3.4 Included Analysis Modules*, *Proposed HRD Status*).
- The low pass WGS (lpWGS) coverage profile, showing the data used to compute the GI index.

Figure 6: Overview of GIS edit/review window

Table 2: Translation of the proposed GI status into the default final GI status

PROPOSED GI STATUS	FINAL GI STATUS
Positive	Positive
Negative	Negative
Negative*	Negative
Inconclusive	Undetermined
Rejected	Undetermined

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Editing the final GI status might be useful in situations where the user wants to consider a different GI index threshold to set the GI status (e.g., situations in which a non-ovarian cancer sample is analyzed).

To edit the final GI status, click the “edit button”.

Click on “Download full report” to access the PDF GI report where additional QC metrics are reported. It is useful to further investigate GI undetermined samples and understand why a sample has been rejected.

5.2.6 Define HRR Status

Using the SOPHiA DDM™ HRD solution, users can report non- BRCA variants that are considered relevant for HRD assessment (e.g., deleterious variant in PALB2).

The HRR edit/review window (*Figure 7* below) displays:

- The final HRR status. By default, the value is set to “Undetermined”; the pipeline does not compute a proposed HRR gene status.
- The list of HRR status supporting variants. This list contains all variants reported by the user within the scope of HRR gene status determination. By default, this list is empty as the system does not pre-populate HRR variants. Section *5.2.8 Report Variants In Scope* describes how to report variants.
- To edit the final HRR gene status, click the “edit” button.

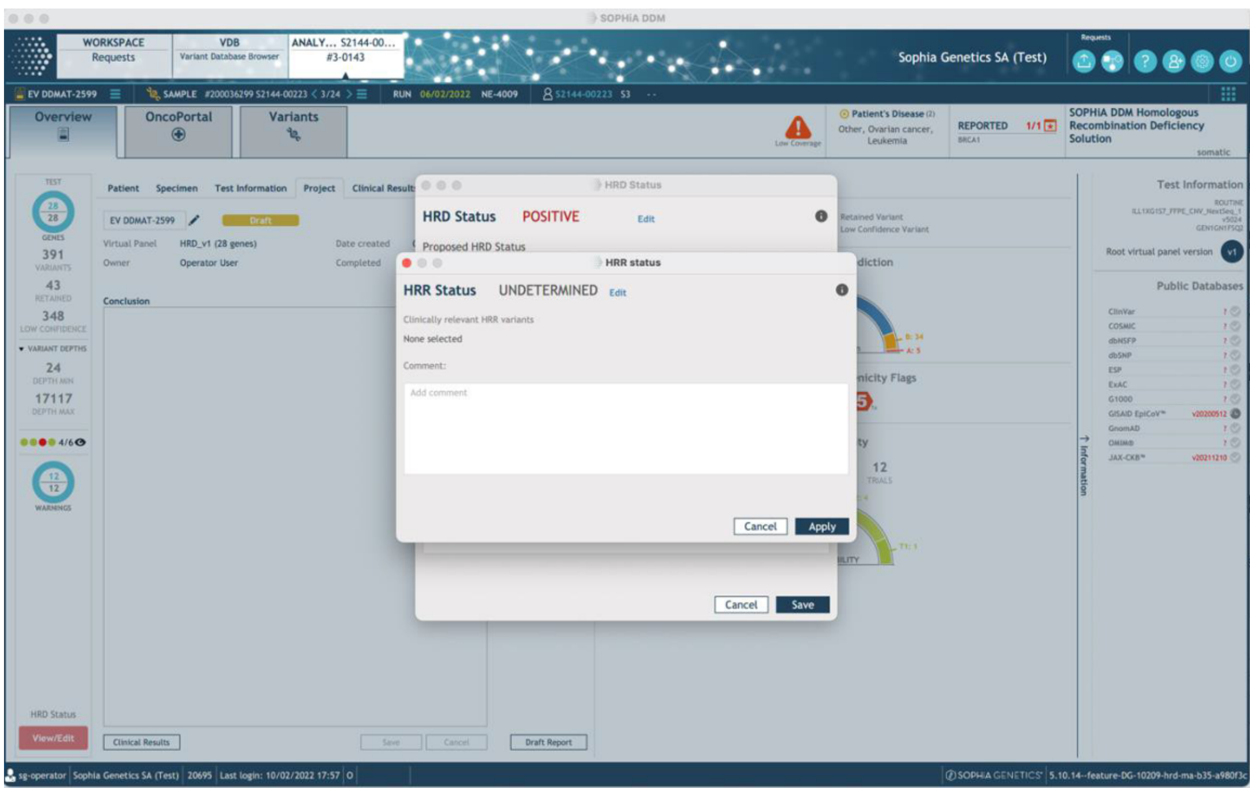

Figure 7: Overview of HRR edit/review window

5.2.7 Define CCNE1 Gene Amplification Status

Using the SOPHiA DDM™ HRD solution, users can also report the presence of CCNE1 amplifications to possibly support HRD negativity.

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The CCNE1 amplification status edit/review window (*Figure 8* below) displays:

- The final CCNE1 status defined by the user. By default, the value is set to "Undetermined". The bioinformatics pipeline does not compute a proposed CCNE1 gene amplification status.
- The CCNE1 copy number detected by the Gene Amplification/CNV module (see section *Gene Amplification/CNVs*; also see section 7 of the SOPHiA DDM™ Desktop App Operation Manual).
- For samples with a CN>3.25 (i.e., if a gene amplification is reported), the user can further interpret the magnitude of the CCNE1 copy number gain by visually inspecting the lpWGS CCNE1 copy number profile plot (see section *6.4 Variant Filtering*). The plot is not available for samples with a GI rejected status.

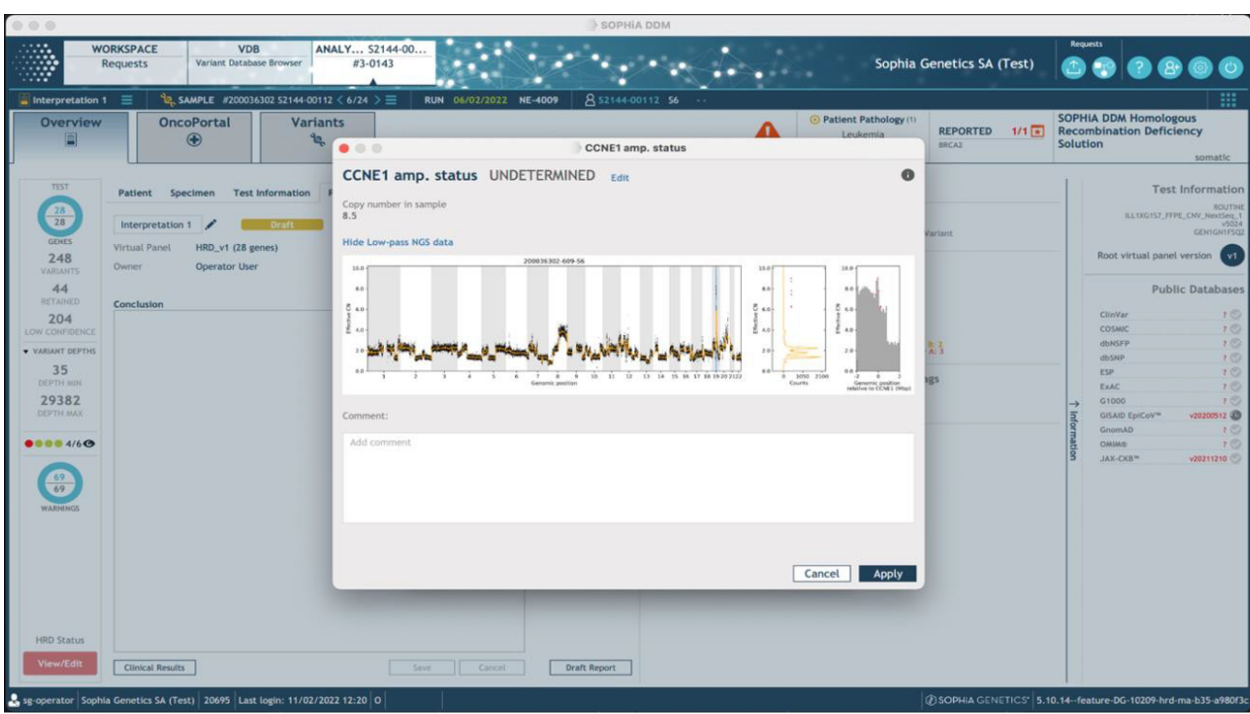

Figure 8: Overview of CCNE1 gene amplification edit/review window

5.2.8 Report Variants In Scope

Using the SOPHiA DDM™ HRD solution, users can report variants as relevant for BRCA or HRR status assessment. In the variant detail view of the variant table, click on "Add to report", then select the scope between BRCA, HRR and Other (*Figure 9* on the next page).

Variants reported in scope of BRCA and HRR will appear in the variant table with the report indicator (star) and a BRCA or HRR label, respectively. Variants reported as "Other" will be displayed in a separate section of the report.

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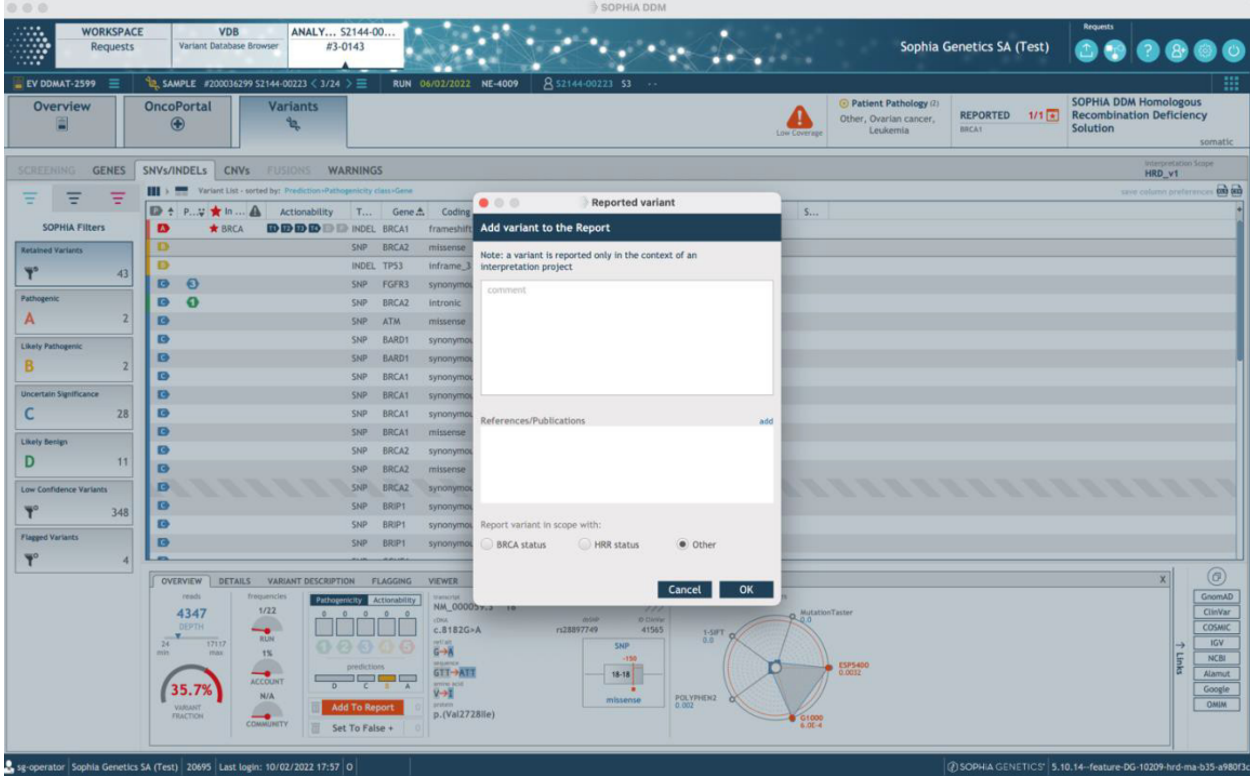

Figure 9: Reporting variants in scope with HRR or BRCA

5.2.9 Generate HRD Report

To generate the HRD report, please refer to the SOPHiA DDM™ Desktop App Operation Manual.

- The first part of the report is dedicated to the display of HRD, GII, and BRCA statuses (if selected).
- The second part of the report is dedicated to HRR and CCNE1 statuses (if selected).
- The third part of the report is dedicated to any additional variants that might be reported out of scope of HRD (see section *5.2.8 Report Variants In Scope*.

5.2.10 Considerations Regarding Inconclusive Results

In Case Of GI Rejection

In case of a GI rejected status, we suggest the user to consult the sample QA metrics (available in the pipeline GI report) as well as the *Table 3* on the next page to identify the underlying cause.

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Table 3: Recommendations in case of GI Rejection

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATION
Insufficient coverage due to issues in balancing WGS with capture.	• Check the Sample QA section of the pipeline GI report. A sample is rejected when the number of WGS fragments is below 4M. • The Sample QA section of the pipeline GI report shows the percentage of WGS fragments. Compare this metric for all samples that were in the same capture pool. Identify if all the samples in the pool have an unusually low value (<50% of WGS fragments).	The WGS pool was not sequenced to the sufficient depth. We recommend re-sequencing the sample pool. If needed adjust the ratio between capture and WGS pool to obtain enough WGS reads.
Insufficient coverage due to issues balancing the samples multiplexed in the same run.	• Check the Sample QA section of the pipeline GI report. A sample is rejected when the number of WGS fragments is below 4M. • Check the Sample QA section of the pipeline GI report. Compare the number of WGS fragments (M) between samples in the same run. Check if the reads distribution in the run is balanced, or if there are outliers with very few or too many reads (3-fold).	If you observe a strong imbalance in the sequencing run: We recommend re-sequencing your samples, ensuring to quantify your libraries carefully and to adjust all samples to the same molarity before pooling.
Insufficient coverage due to poor yield of the sequencing run.	• Check the Sample QA section of the pipeline GI report. A sample is rejected when the number of WGS fragments is below 4M. • Go to the pipeline QA report and check the total number of reads produced by the sequencing run. Compare this with the expected number of reads (~260M reads for a NextSeq Mid Output run, ~800M reads for a NextSeq High Output run).	Repeat the sequencing run adjusting the final sequencing library loading concentration to avoid under- or over-clustering of the run.

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Recommendations in case of GI Rejection (continued)

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATION
Excess of residual noise	• Check the Sample QA section of the pipeline GI report. Residual noise ≥0.17 leads to sample rejection if purity-ploidy ratio is not detected. *Examples of lpWGS coverage profiles with excessive residual noise, in which PPR was not detected.*	High levels of noise can be a sign of low-quality input DNA, possibly resulting from poor DNA extraction To improve the library preparation efficiency, repeat the library preparation, doubling the amount of input DNA and, if possible, starting with a new DNA extraction
High proportion of coverage outliers	• Check the Sample QA section of the pipeline GI report. A sample is rejected if the proportion of coverage outliers is larger or equal to 20%. • A high proportion of coverage outliers affecting the lpWGS coverage profile may result from sub-optimal execution of the capture step or from low DNA quality. • To distinguish the two scenarios, compare the proportion of coverage outliers for all samples that were in the same capture pool. Identify if all samples in the pool have an unusually high value ≥20%).	• In case all samples in the pool are rejected due to coverage outliers we recommend repeating the entire workflow for the sample pool paying close attention to the capture instructions. • In case only a minority of the samples in the pool are rejected due to coverage outliers, we recommend repeating the entire workflow only for the rejected samples, doubling the amount of input DNA.

In Case Of GI Inconclusive Status

In case of a GI inconclusive status, we suggest the user to consult the sample QA metrics (available in the pipeline GI report) as well as *Table 4* on the next page to identify the underlying cause.

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Table 4: Recommendations in case of GI Inconclusive

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATION
The sample does not feature large copy number aberrations	• Check the Sample QA section of the pipeline GI report. A sample is classified GI inconclusive if signal to noise ratio is insufficient (i.e. SNR smaller than 0.55). • Perform a visual inspection of the lpWGS profile in the GI report. The smoothed coverage profile (orange line) should be flat, with no sign of large CN aberrations. The insufficient SNR is due to an absence of signal which could result either from: i) the absence of CN changes in the sample or, ii) from extremely low tumor content. *Examples of lpWGS coverage profiles without large CN aberrations*	• If the sample tumor content is larger than 30%, it is likely that the sample does not feature large copy number aberrations. Repeating the experiment will not affect the results of the GI analysis. • If there are doubts about the sample tumor content, we recommend repeating the entire wetlab workflow, starting with a sample with higher tumor content.

5.2.11 Considerations Regarding GI Negative* Statuses

GI Negative* statuses are medium confidence negative calls with an increased false negative risk. GI Negative* calls result from low signal-to-noise ratio in the NGS data, possibly reflecting insufficient sample tumor content. *Table 5* on the next page illustrates how insufficient tumor content impacts SNR.

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Table 5: Considerations regarding case of GI Negative* statuses

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATION
Insufficient tumor content	• Verify that sample tumor content estimated before processing the DNA sample is larger than 30%. • Check the GI report and confirm that the PPR was either lower than 0.1 or not detected. • Note that GI analysis does not perform tumor content estimation. *Illustration of lpWGS profile obtained by decreasing sample tumor content. The magnitude of the coverage changes between segments decreases, with decreasing tumor content.* Tumor Content = 100% Tumor Content = 66% Tumor Content = 33% *Examples of lpWGS coverage profiles for insufficient tumor content samples in which PPR was not detected.*	If there are doubts about the sample tumor content, we recommend repeating the entire wetlab workflow, starting with a sample with higher tumor content.

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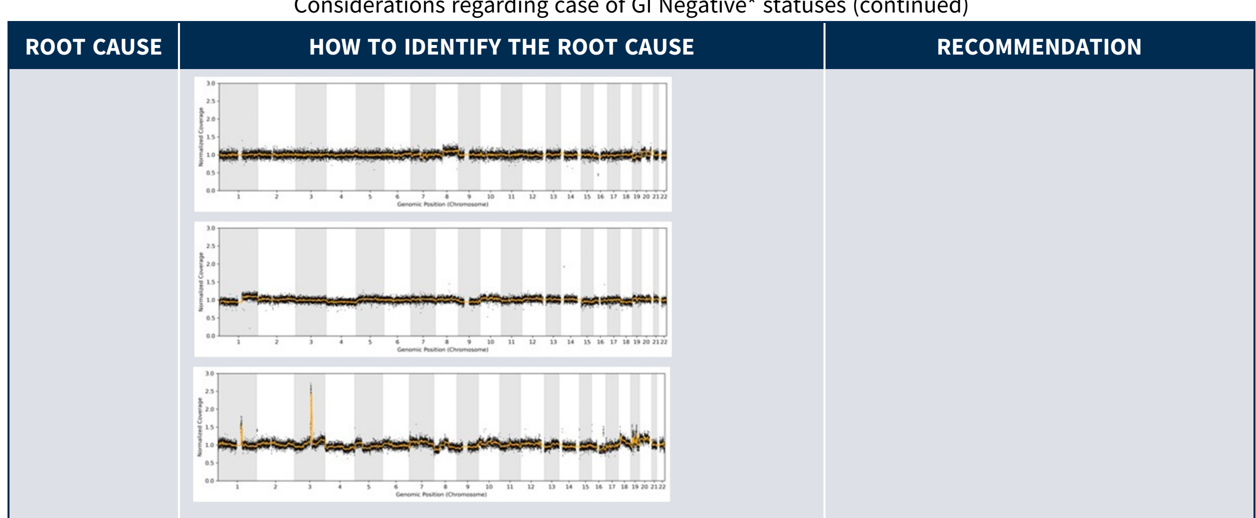
Considerations regarding case of GI Negative* statuses (continued)

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATION
		

In Case Of BRCA Inconclusive And/or Presence Of BRCA FN Risk

Assess the severity of the FN risk by checking the % of the BRCA1/2 regions covered with >100x unique molecules. This can be done by looking at the quality indicator “BRCA Cov Percentile 100” that is showed as a traffic light in SOPHiA DDM™ (see description in the quality indicators section, as well as *Figure 4* on page 38). A good sample is expected to have a score of at least 99%.

Note: When reading the troubleshooting guidelines in *Table 6* below, multiple root causes can apply. Make sure to read the entire guide and identify the most relevant cause.

Table 6: Recommendations in case of BRCA undetermined associated with a warning of FN risk

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATION
Poor coverage uniformity	• Check the coverage heterogeneity (<0.01) in the pipeline QA report • Identify if the coverage uniformity is low in all samples of the capture pool or just in individual samples.	• If you observe a pool effect: The capture efficiency is low, we recommend repeating the entire batch of samples, and pay close attention to the IFU section describing the hybridization and capture procedure. • If you observed the effect in an individual sample: The library preparation efficiency and/or sample quality is low. To improve the library quality, we recommend doubling the amount of input DNA and repeating the entire workflow, starting with a new DNA extraction, if possible.
Insufficient number of fragments mapping to the target regions of the panel	• Check the Sample QA section of the pipeline GI report. Calculate the number of fragments (M) on the target regions of the panel by subtracting the number of WGS fragments from the total number of fragments. Each sample should have at least 2M fragments mapping to the target regions of the panel.	• If you observe a pool effect: The capture pool was not sequenced to the sufficient depth, we recommend resequencing the sample pool. If needed, adjust the ratio between capture and WGS pool to obtain enough reads mapping to the target regions.

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Recommendations in case of BRCA undetermined associated with a warning of FN risk (continued)

ROOT CAUSE	HOW TO IDENTIFY THE ROOT CAUSE	RECOMMENDATION
	• Identify if the number of fragments mapping to the target regions of the panel is low in all samples of the capture pool or just in individual samples. • Check if the reads distribution in the capture is balanced, or if there are outliers with very few or too many reads (3-fold difference).	• If you observed the effect in an individual sample: Repeat the entire workflow for the sample and ensure to quantify your libraries carefully before pooling and capture. • If you observe a strong imbalance in the capture pool: Ensure to quantify your libraries carefully before pooling and capture. If you know the quality of your input DNA, you can improve the reads balance by pooling similar quality samples in the same pool. ***Warning:*** *The first two scenarios only apply if the coverage uniformity of the samples was of sufficient quality.*
Poor library diversity	• Check the Mapping Statistics in the pipeline QA report to determine the duplicate fraction in the sample. PCR duplicates should be similar for all your samples.	• High percentages of PCR duplicates suggest low library preparation efficiencies. We recommend repeating the entire workflow and, if possible, by doubling the amount of input DNA.
Small library fragment size	• Check if the library size is unusually low in comparison with the other samples processed. You can obtain the average library fragment size via high-resolution capillary electrophoresis, which is used to check the library quality at the end of the library preparation workflow.	• Small library sizes of individual samples can be a sign of low- quality input DNA (assuming multichannel pipettes were used to process samples according to good laboratory practice). To improve the library preparation efficiency, we recommend doubling the amount of input DNA.

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5.3 Analysis Description And Parameters

5.3.1 Resource Files

Alignment and variant calling are performed against the GRCh37 reference genome (also referred to as hg19). Variant coordinates are also provided against GRCh38 – the latest major release of the human reference genome, (referred to as hg38), via a conversion operation termed liftOver.

The liftOver is performed using Picard and relies on the mapping of regions between the two assemblies (i.e., chain file) provided by UCSC.

Publicly available sources for all these requirements can be found at:

GRCh38 – hg38 reference genome:
https://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/seqs_for_alignment_pipelines.ucsc_ids/GCA_000001405.15_GRCh38_no_alt_plus_hs38d1_analysis_set.fna.gz.

GRCh37 – hg19 reference genome:
https://storage.googleapis.com/genomics-public-data/references/b37/Homo_sapiens_assembly19.fasta.gz

Chain files:
http://hgdownload.soe.ucsc.edu/goldenPath/hg38/liftOver/hg38ToHg19.over.chain.gz
ftp://ftp.ensembl.org/pub/assembly_mapping/homo_sapiens/GRCh37_to_GRCh38.chain.gz

Picard:
https://github.com/broadinstitute/picard, version 2.23.8

5.3.2 Target Regions

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

GENE	BASES COVERED BY HRD_V1	TOTAL BASES ON CODING REGION	% OF GENE COVERED BY HRD_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

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GENE	BASES COVERED BY HRD_V1	TOTAL BASES ON CODING REGION	% OF GENE COVERED BY HRD_V1
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
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

Table 7: Target regions of SOPHiA DDM™ HRD_v1

5.3.3 Raw Data Pre-Processing

The following is a brief description of the steps taken in the pipeline, up to and including alignment.

Pre-processing

- Collect quality metrics based on the raw FASTQ files.
- Truncate the FASTQ files to a maximum size of 2.5 GB.

Alignment

- Cut adapters and trim low-quality ends from reads (base quality below Q20).
- Align reads to the hg19 human 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.

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- 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 for better detections of long INDELs.
- 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.

5.3.4 Included Analysis Modules

The different results that this pipeline returns are: Quality Indicators, SNVs/INDELs, Gene amplification, and GI score.

Quality Indicators

For each analyzed sample, a panel of traffic-light-like quality indicators are displayed in SOPHiA 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. For information on how to interpret the color codes of the quality indicator dots, please refer to Section 3.10 of SOPHiA DDM™ Desktop App Operation Manual. 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 (i.e., 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 and variant calling software that apply statistical tests to identified mismatches versus the reference genome, variant regularization functions, variant quantification functions, and variant filtering functions.

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Variant Calling

- Identify variants (SNVs and INDELs up to 10,000 bp) 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).
- Re-quantify the variant fraction considering all the haplotypes in the neighboring region.
- Unify homopolymer annotation to long anchor standards.

Variant Filtering

1. Filter variants below the background noise level of the panel and sequencer (filter = high_background_noise).
2. Filter variants with variant fraction below 4% (filter = low_variant_fraction).
3. Filter variants with read coverage below 30 (filter = low_coverage).
4. Filter variants outside of the target regions (filter = off_target).
5. Filter INDELs in homopolymers if the homopolymer length is ≥ 10 bp (filter = homopolymer_region).
6. 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).
7. Remove variants beyond the target regions with a padding of 500 bp.
8. Remove duplications longer than 500 bp.
9. 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 the closest exon, ref and alt codon sequence, and 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 identifiers, allele frequencies from GnomAD, 1000 genomes 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)
- The BRCA Exchange databases

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> **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 *Table 8* 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”
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).

Table 8: Rules followed for aggregating BRCA_Exchange information into the “BRCA_Pathogenicity” displayed in the variant table

Gene Amplification/CNVs

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 HRD panel.

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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.

lpWGS CCNE1 Gene Amplification Plot

The copy number levels reported by the CNV module quantify the copy number in the DNA sample (effective CN), not the absolute copy number of the tumoral DNA present in the sample. The lpWGS CCNE1 amplification plots are designed to help the users estimate the absolute copy number of the tumoral DNA present in the sample upon visual inspection (see *Figure 10* below and *Figure 11* below).

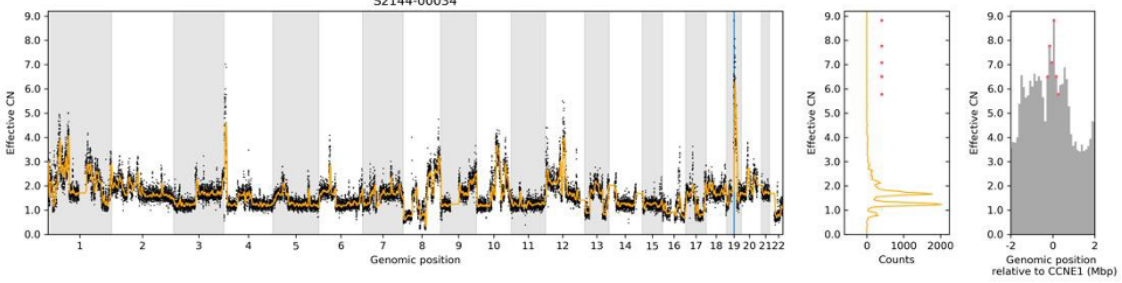

Figure 10: Example of CCNE1 amplification plot with a strong amplification

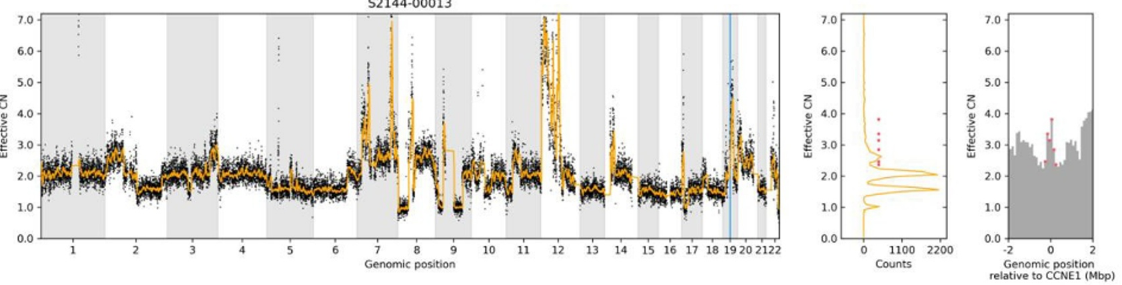

Figure 11: Example of CCNE1 amplification plot with a weak copy number gain

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The left panel shows the lpWGS coverage profile after normalization (black: raw data at 100 kb resolution, orange: smoothed version of raw data), with the vertical blue line showing the genomic position of CCNE1.

The middle panel shows: i) Orange: the histogram of the smoothed lpWGS coverage profile shown in the left panel; ii) Red dots: the value of the normalized lpWGS coverage profile measured around the CCNE1 gene (±300 Kbp of CCNE1 genomic position). Distinct peaks in the histogram correspond to distinct copy number levels present in the tumor DNA. The position of the red dots with respect to the histogram peaks allows the user to interpret the magnitude of the CCNE1 CN gain present in the tumor DNA. In *Figure 10* on the previous page, the red dots are far away from all histogram peaks, suggesting the presence of a strong copy number gain in CCNE1. In *Figure 11* on the previous page, the red dots overlap with some histogram peaks, suggesting the presence of a weak copy number gain in CCNE1.

The right panel provides a zoomed-in view of the lpWGS coverage profile after normalization (same data as in left panel) around the CCNE1 genomic location (±2 Mbp). The regions within ±300 Kbp of CCNE1 are highlighted by red dots (same red dots as shown in the middle panel).

Proposed HRD Status

The proposed HRD status is computed from the aggregation of the “Proposed GI status” and the “Proposed BRCA status”. The set of rules that determine the “Proposed HRD status”, represented in the table cells, can be inferred from *Table 9* below, where the “Proposed GI status” is represented along the input row and the “Proposed BRCA status” is represented along the input column.

HRD negativity is constrained by the type of genomic aberrations that are covered by the product. See limitations of proposed BRCA status determination for more information.

Table 9: Proposed HRD status computation

PROPOSED GI STATUS	PROPOSED BRCA STATUS						
	POSITIVE	PREDICTED POSITIVE	COMPLEX CASE	UNDETERMINED	PREDICTED NEGATIVE	NEGATIVE	INCONCLUSIVE
Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Inconclusive	Positive	Positive	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined
Rejected	Positive	Positive	Undetermined	Undetermined	Undetermined	Undetermined	Undetermined
Negative*	Positive	Positive	Undetermined	Negative	Negative	Negative	Undetermined
Negative	Positive	Positive	Undetermined	Negative	Negative	Negative	Undetermined

Proposed BRCA Status

The “Proposed BRCA status” exposes the user to state-of-the-art clinical knowledge for BRCA1/2 variants while transparently conveying uncertainty in the variant detection and calling. The Proposed BRCA status thus combines clinical knowledge and variant detection metrics gathered at the variant and sample levels.

The system proceeds sequentially by:

1. Establishing the functional impact and clinical relevance of each BRCA1/2 variant detected in the sample.
2. Establishing the functional status of BRCA1/2, at the sample level, via a “Proposed BRCA status”.

The present section exposes the data flow, rules and intermediate steps involved in establishing the “Proposed BRCA status”. The nomenclatures and definitions of labels emitted by the pipeline are detailed in *Table 10* on page 57, *Table 11* on page 58, *Table 12* on page 60, and *Table 13* on page 60.

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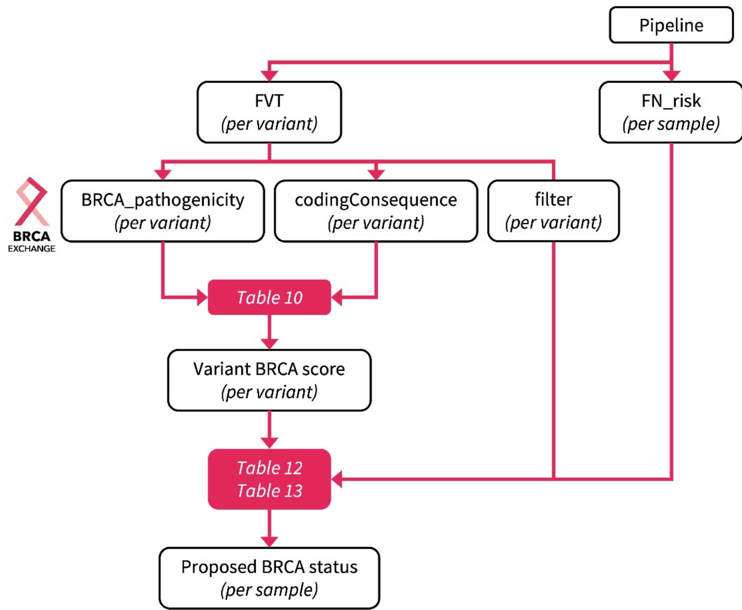

The analysis starts with the Full Variant Table (columns “BRCA_pathogenicity” and “codingConsequence”). The system establishes the functional impact (i.e., coding consequence) and clinical relevance (i.e., BRCA pathogenicity) of each variant detected in the sample. As a result, each BRCA variant gets assigned a “variant BRCA score” as per rules detailed in *Table 10* on the next page (with nomenclature and conventions listed in *Table 11* on page 58). Those rules classify each BRCA1/2 variant into:

- **Positive:** variant known as pathogenic as per clinical knowledge
- **Predicted positive:** variant with coding consequence predictive of loss-of-function
- **Complex case:** VUS with coding consequence requiring further inspection by end user
- **Predicted Negative:** variant with coding consequence predictive of no functional alteration
- **Negative:** variant known as benign as per clinical knowledge
- **Undetermined:** otherwise.

The system reports an “NA” category in case of absence of detected variant in BRCA.

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Table 10: Rules used to establish the variant BRCA score

CODING CONSEQUENCE	BRCA PATHOGENICITY FLAGS			
	EX:PATHOGENIC OR AG:PATHOGENIC	EX:BENIGN OR AG:BENIGN	UNCLEAR	NA
3'UTR	Positive	Negative	Undetermined	Predicted negative
5'UTR	Positive	Negative	Undetermined	Predicted negative
intronic	Positive	Negative	Undetermined	Predicted negative
synonymous	Positive	Negative	Undetermined	Predicted negative
splice_*	Positive	Negative	Complex case	Complex case
inframe*	Positive	Negative	Complex case	Complex case
missense	Positive	Negative	Undetermined	Undetermined
no_stop	Positive	Negative	Undetermined	Undetermined
frameshift_ 5'	Positive	Negative	Predicted positive	Predicted positive
frameshift_ 3'	Positive	Negative	Undetermined	Undetermined
nonsense_5'	Positive	Negative	Predicted positive	Predicted positive
nonsense_3'	Positive	Negative	Undetermined	Undetermined
no variant detected for BRCA 1/2	NA			

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Table 11: Nomenclature and conventions

TERM	DESCRIPTION
AA_position	Amino acid position of the variant along the BRCA1_refTranscript or BRCA2_refTranscript reference.
BRCA1_refTranscript	NM_007294.3
BRCA2_refTranscript	NM_000059.3
AA_threshold_BRCA1	AA_threshold_BRCA1 = most 3' AA_position of pathogenic variants as per the BRCAexchange catalog (version in production). Given that the most 3' pathogenic variant for BRCA1 according to BRCA_Exchange is p.(Ala1823_*1864del), this places AA_threshold_BRCA1 value that is aminoacid 1863, which corresponds to the full length of the protein.
AA_threshold_BRCA2	AA_cutoff = 3326 (AA coordinates), as per Mazoyer S et al., Nature Genetics 1996, 14:253-254. This is equivalent to genomic position hg19:13:32972627
codingConsequence	As per codingConsequence column of the Full Variant Table file produced by the annotation system.
BRCA_pathogenicity flags	As per BRCA_pathogenicity column of the Full Variant Table file produced by the annotation system. Pathogenicity assertions provided per variant, as retrieved from BRCA exchange after label recasting and error checking exposed in the annotation sections.
splice_*	any codingConsequence matching with the label "splice"
inframe*	any codingConsequence matching with the label "inframe"
frameshift_5'	BRCA1 : codingConsequence is "frameshift" and AA_position ≤ AA_threshold_BRCA1
	BRCA2 : codingConsequence IS "frameshift" AND AA_position ≤ AA_threshold_BRCA2
frameshift_3'	BRCA1 : codingConsequence IS "frameshift" AND AA_position > AA_threshold_BRCA1
	BRCA2 : codingConsequence IS "frameshift" AND AA_position > AA_threshold_BRCA2
nonsense_5'	BRCA1 : codingConsequence IS "nonsense" AND AA_position ≤ AA_threshold_BRCA1
	BRCA2 : codingConsequence IS "nonsense" AND AA_position ≤ AA_threshold_BRCA2
nonsense_3'	BRCA1 : codingConsequence IS "nonsense" AND AA_position > AA_threshold_BRCA1
	BRCA2 : codingConsequence IS "nonsense" AND AA_position > AA_threshold_BRCA2

The next step calls the “Proposed BRCA status”, reports the set of supporting variants and emits user warnings. The rule set is detailed in *Table 12* on page 60 and *Table 13* on page 60. For that, the bioinformatics pipeline takes into consideration:

1. The variant BRCA score (established in step 1), and ranks for all BRCA variants found in a sample according to their clinical relevance: “Positive” > “Predicted positive” > “Complex case” > “Undetermined” > ”Predicted negative” > “Negative” > “NA”.
2. The uncertainty in variant detection, considering separately high- and low-confidence variant calls.
3. The presence or absence of False Negative risk (see section *5.3.4 Included Analysis Modules Quality Indicators*: BRCA_cov_percentile_100).

Variants gathered from high-confidence calls are used to establish the proposed BRCA status. The sample is called as either BRCA:

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1. Positive: score of the most relevant high-confidence variant is “Positive”.
2. Predicted positive: score of the most relevant high-confidence variant is “Predicted positive”.
3. Complex case: score of the most relevant high-confidence variant is “Complex case”.
4. Predicted negative: score of the most relevant high-confidence variant is “Predicted negative” and there is no FN risk.
5. Negative: score of all variants is “Negative” (or no variants are detected) and there is no FN risk.
6. Inconclusive: score of the most relevant high-confidence variant is “Predicted negative” or “Negative” and there is a FN risk.
7. Undetermined: none of the previous cases. Is a result of insufficient clinical knowledge regarding the reported BRCA1/2 variants (BRCA_Pathogenicity is “unclear”).

The warnings signal three possible situations to the user:

1. “Review low-confidence variants with relevant BRCA score”: One or more low-confidence variants with a relevant BRCA score have been detected.
2. “Review clinical interpretation”: the most relevant evidence found is a “Complex case” or “Undetermined” high-confidence variant.
3. “False negative risk”: no relevant evidence is found, but a False-Negative risk cannot be excluded for the sample.

The obtained “proposed BRCA status”, the list of supporting evidence, and the warnings are then used to initiate the user interfaces are detailed in section *6 Pipeline Deliverables*.

Table 12 on the next page and *Table 13* on the next page: Proposed BRCA status. The following set of rules are applied to aggregate *variant BRCA scores* into a sample-level *proposed BRCA status*. The *variant BRCA scores* of the obtained variants are combined into a sample-level *proposed BRCA status* according to rules that apply either to:

a) samples for risk of False-Negatives has not been identified (i.e., FN_risk is PASS) or

b) samples with False-negatives risk (i.e., FN_risk is FAIL).

The tables are formatted to expose rule inputs as rows (*BRCA score* of most clinically relevant variant in the high confidence calls) and columns (*BRCA score* of most clinically relevant variant in low confidence calls). Values in the table represent sample-level proposed BRCA status. Finally, the rule system emits a series of user warnings, listed at the bottom of *Table 13* on the next page.

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Table 12: Sample FN risk: FAIL

BRCA SCORE OF THE MOST RELEVANT VARIANT CALLED WITH HIGH CONFIDENCE	BRCA SCORE OF THE MOST RELEVANT BRCA VARIANT CALLED WITH LOW CONFIDENCE						
	POSITIVE	PREDICTED POSITIVE	COMPLEX CASE	UNDETERMINED	PREDICTED NEGATIVE	NEGATIVE	NA
Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive
Complex case	Complex case[1,2,3]	Complex case[1,2,3]	Complex case[2,3]	Complex case[2,3]	Complex case[2,3]	Complex case[2,3]	Complex case[2,3]
Undetermined	Undetermined [1,2,3]	Undetermined [1,2,3]	Undetermined [1,2,3]	Undetermined [2,3]	Undetermined [2,3]	Undetermined [2,3]	Undetermined [2,3]
Predicted negative	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]
Negative	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]
NA	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]	Inconclusive [1,3]

Table 13: Sample FN risk: PASS

BRCA SCORE OF THE MOST RELEVANT VARIANT CALLED WITH HIGH CONFIDENCE	BRCA SCORE OF THE MOST RELEVANT BRCA VARIANT CALLED WITH LOW CONFIDENCE						
	POSITIVE	PREDICTED POSITIVE	COMPLEX CASE	UNDETEMINED	PREDICTED NEGATIVE	NEGATIVE	NA
Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive	Predicted positive
Complex case	Complex case [1,2]	Complex case [1,2]	Complex case [2]	Complex case [2]	Complex case [2]	Complex case [2]	Complex case [2]
Undetermined	Undetermined [1,2]	Undetermined [1,2]	Undetermined [1,2]	Undetermined[2]	Undetermined [2]	Undetermined [2]	Undetermined [2]
Predicted negative	Predicted negative[1]	Predicted negative[1]	Predicted negative[1]	Predicted negative[1]	Predicted negative	Predicted negative	Predicted negative
Negative	Negative[1]	Negative[1]	Negative[1]	Negative[1]	Negative	Negative	Negative
NA	Negative[1]	Negative[1]	Negative[1]	Negative[1]	Negative	Negative	Negative

User warnings: [1]Review low-confidence variants with relevant *BRCA score*, [2]Review clinical interpretation, [3]False negative risk

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

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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 proposed 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 proposed 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: Proposed GI Status determination:

A proposed 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.

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- *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 proposed genomic integrity status of a sample.

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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 (e.g., QA report, CNV summary report).

An example is shown in *Figure 12* below:

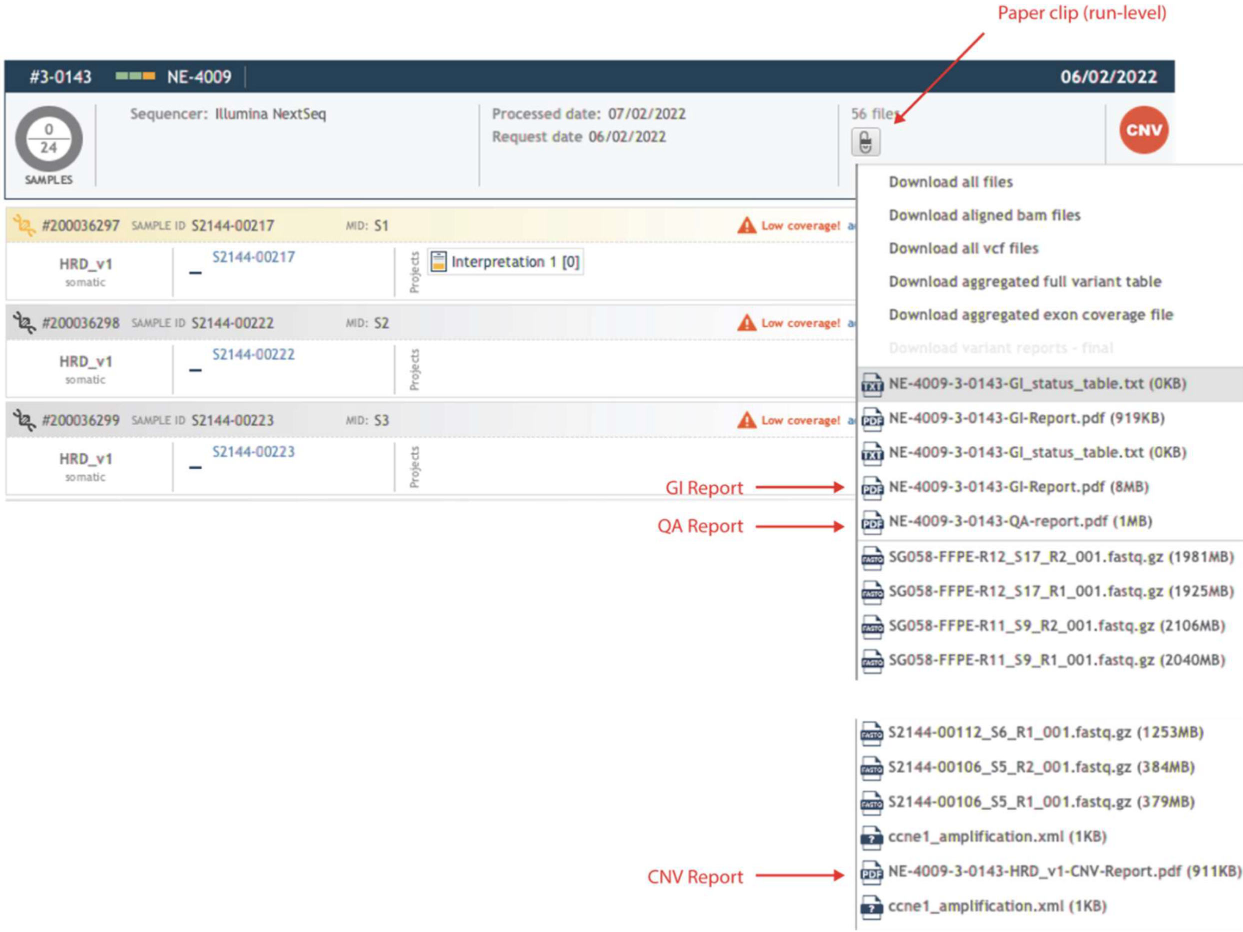

Figure 12: Schematic representation of the available files on the SOPHiA DDM™ Desktop App.

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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 statistics (computed in molecules) at the exon level.
- **QA-report.pdf:** The pipeline QA report in PDF format at the sample and run level.
- **CNV-report.pdf:** The report for gene amplifications in PDF format.
- **GI-report.pdf:** The report for the genome integrity analysis in PDF format.
- **GI_status_table.txt:** A text file summarizing the GI results.

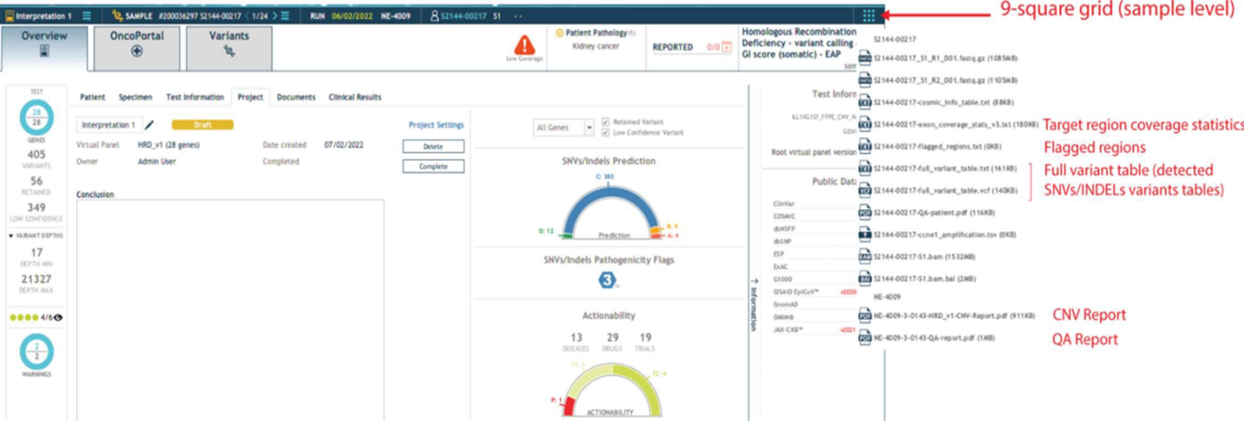

Figure 13: List of output flies available for download.

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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 User 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.

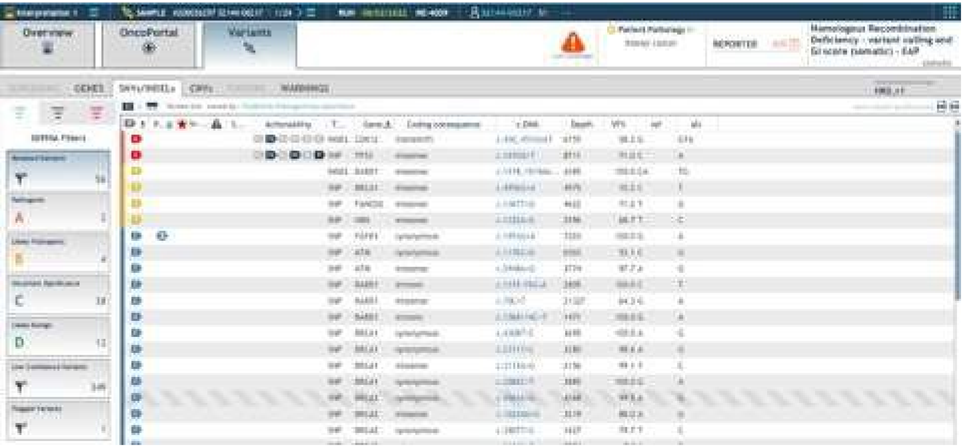

Figure 14: The Results Visualization panel on the SOPHiA DDM™ Desktop App

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.

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6.4 Variant Filtering

A list of reasons for variants being in the low confidence (i.e., being considered as low-confidence) tab is given in the table below.

FILTER TYPE	DESCRIPTION
off_target	The variant is placed outside of the panel target region.
low_variant_fraction	The variant has a variant fraction value below cutoff (cutoff can be different for different variant types: SNVs/INDEL, INDELs in homopolymers, etc.).
homopolymer_region	The variant is in a homopolymer region with a length higher than the cutoff.
low_coverage	The variant is supported by insufficient number of high quality read position (phred score >20).
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 sequencer-specific noise.

Table 14: Reasons for variants being in the low confidence tab

Please refer to section *5.3.4 Included Analysis Modules - SNVs/INDELS* for more details.

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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.

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

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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

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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

Table 15: Example of a full variant table (FVT)

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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 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. HRD solution has been fully validated for DNA FFPE samples from Ovarian Cancer with a DQN>3 (measured using Fragment Analyzer) and sequenced on Illumina NextSeq500/550.

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7.2 Limitations

7.2.1 General Limitations

1. The proposed GI status and the proposed HRD status computed by the pipeline only apply to Ovarian Cancer samples
2. 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).
3. The maximal run size that can be uploaded is 200 GB. Uploading higher volumes in a single batch is not allowed. The pipeline has been shown to successfully process individual samples with up to 50 GB of data. Higher volumes per sample might cause the pipeline to fail.
4. Even when sample multiplexing recommendations are followed, and the recommended average number of reads per sample (~32M) 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).
5. 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.
6. 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 insignificantly low variant fraction, i.e., below the variant calling threshold; nevertheless, variant cross-talk 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 an Illumina® NextSeq 500/550 sequencer (Mid and High Output flow cells).

SNVs/INDELs

1. Variant detection in this product has been optimized for SNVs and short INDELs (up to 50 bp).
   Please note that any other type of alteration that can have an impact in the BRCA status can be missed by our algorithm.
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:

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1. Multiple nucleotide variants (MNVs)
2. Delins
3. INDELs that are larger than 50 bp
4. DNA fragments carrying multiple variants.

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.

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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.

Proposed HRD Status

Proposed BRCA Status

1. Structural variants, including CN gains and losses affecting one or multiple exons of BRCA1 and BRCA2, are NOT within the scope of the Proposed BRCA status provided by this product.
2. Mobile element insertions are NOT within the scope of the Proposed BRCA status provided by this product.
3. The proposed BRCA status is computed based on the SNVs and short INDELs detected by the pipeline.
4. Other types of pathogenic genomic aberrations are not considered for the proposed BRCA status calculation.
5. The present product relies on data provided by “BRCA exchange” which is a third-party provider. The product is thereby exposed to licensing and data sharing constraints that are independent ofSOPHiA GENETICS's control. Such constraints can introduce deviations between the observations reported in the product and those exposed via the website of “BRCA exchange”.

Proposed Genomic Integrity Status

1. The limit of detection for HRD status and Genomic Integrity status determination is a tumor content of 30%.
2. The limit of detection for SOPHiA DDM™ 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.
3. Low-pass WGS data do not allow to reliably distinguish samples lacking large copy number aberrations from samples having insufficient tumor content. Processing samples that do not feature large copy number alterations will likely produce GI inconclusive calls.
4. The repeatability of the GI index observed by processing the same samples multiple times is characterized by an average standard deviation of 0.26.

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7.3 Precautions

7.3.1 General Precautions

1. 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.
2. Physically separated pre- and post- PCR rooms should be used to prevent DNA sample contamination.
3. Correctly calibrated pipettes and proper lab equipment should be used to perform the experiment.
4. Different lot numbers of reagents should not be mixed.
5. Store and handle reagents according to instructions on the kit boxes, and do not use if expired.
6. 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.
7. The recommended EDTA concentration in DNA storage buffer is 0.1 mM. An excess of EDTA in sample storage buffers could impair sample processing.
8. For detailed instructions on the software, refer to the SOPHiA DDM™ User Manual.

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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
Σ	Contains sufficient for <n> tests
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.

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SYMBOL	TITLE
1	Box 1
2	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-mail support@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 is established.

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

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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™ 500/550	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

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INDEX	i5 SEQUENCES FOR SAMPLE SHEET NextSeq™ 500/550	i7
sgUDI-62	CTCGACTA	TACTAGCA
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

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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

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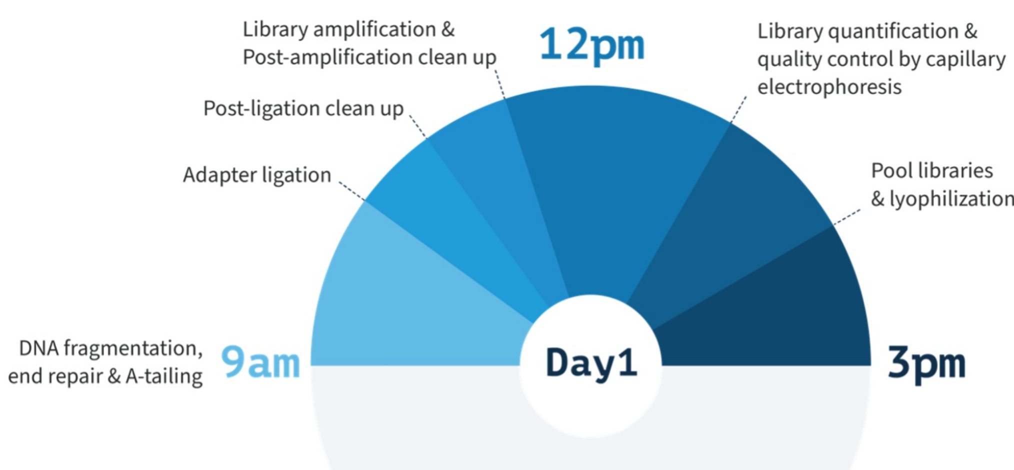
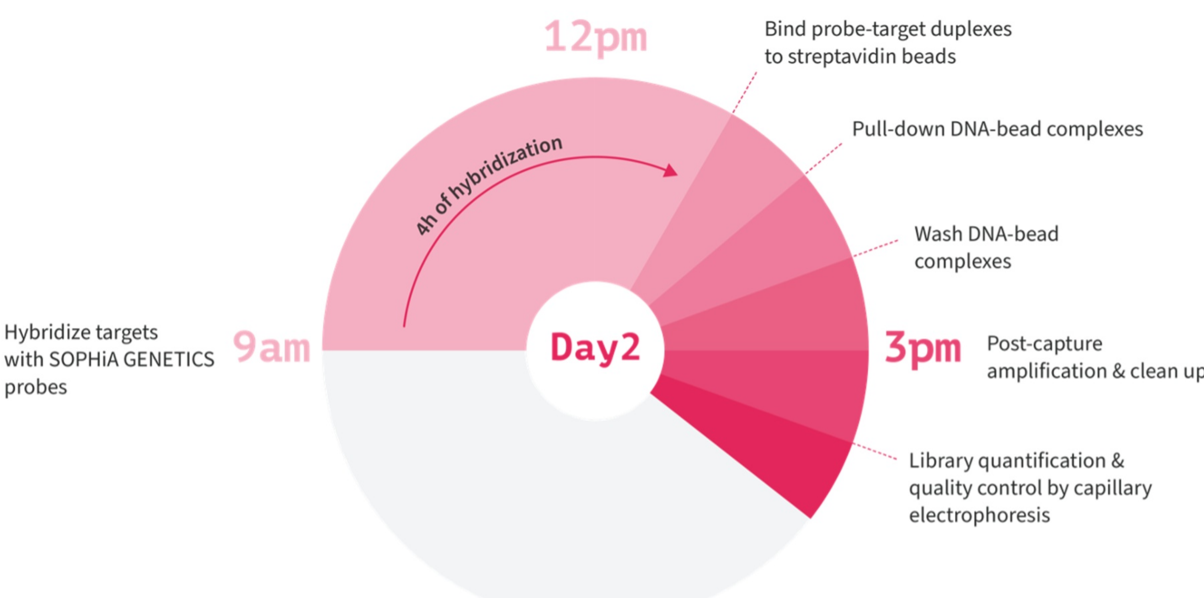
10.3 Appendix III: General Workflow–SOPHiA DDM™ Capture Solution

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.

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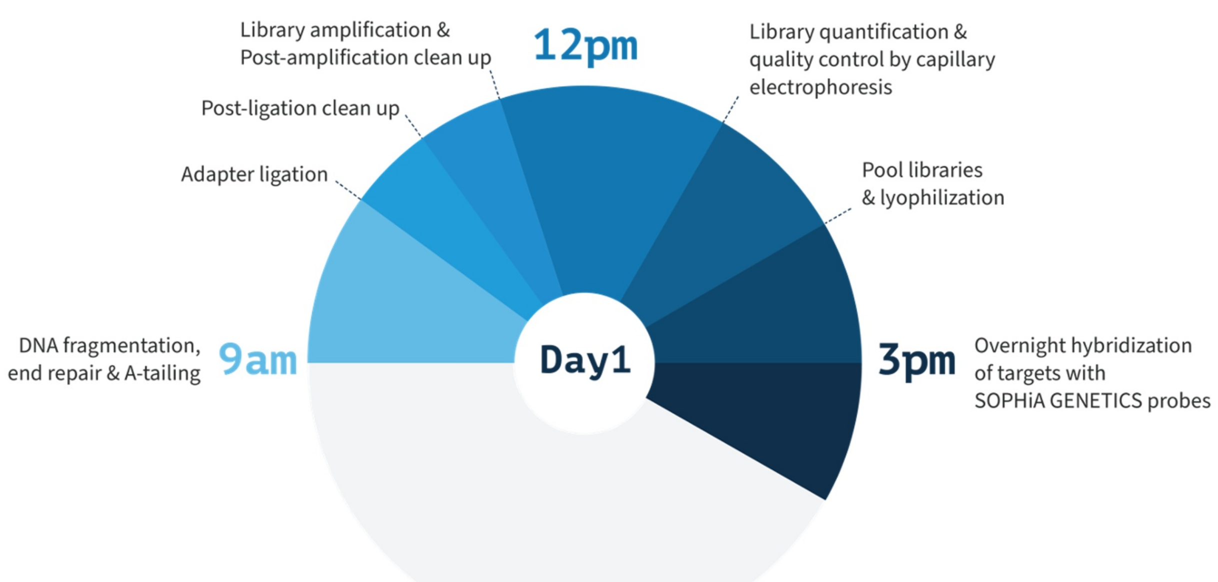
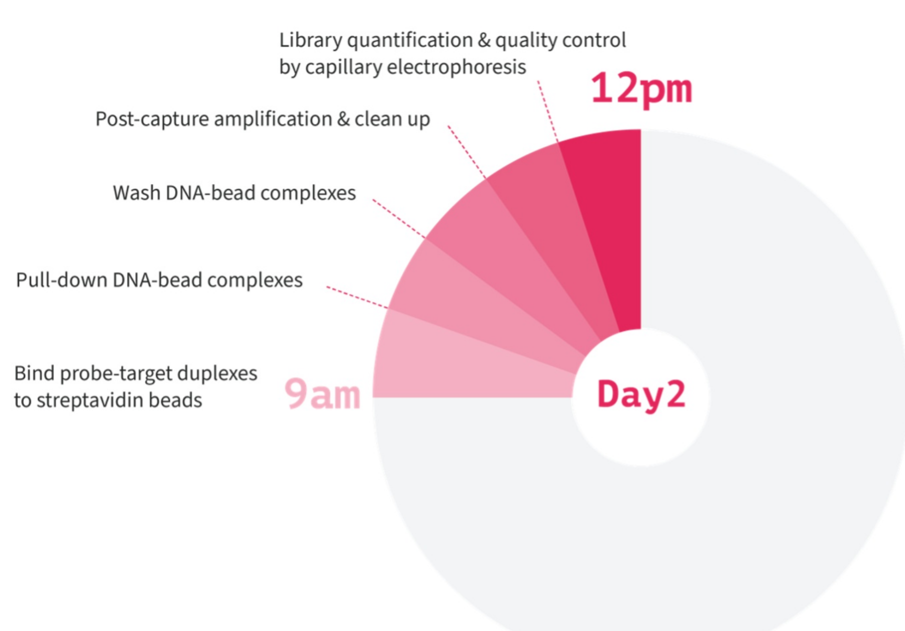
10.3.2 Overnight Hybridization





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Document Approvals

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