INSTRUCTIONS FOR USE

16, 32, 48 AND 96 SAMPLES

SOPHiA DDM™ Dx Hereditary Cancer Solution

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

SOPHiA GENETICS™

SUMMARY INFORMATION

Product Name	SOPHiA DDM™ Dx Hereditary Cancer Solution
Product Type	Bundle Solution
Product Family	Molecular diagnostic application (kit + analytics)
Algorithm ID	ILL1XG1G6_CNV
Gene Panel ID	HCS v1.1
Product Version	1.1
Sample Type	Germline DNA isolated from peripheral blood
Sequencer	Illumina - MiSeq
GMDN Description	Reagent kit IVD / Human genomic analysis interpretive software
Document ID	SG-00658
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This Instructions For Use (IFU) is applicable for all SOPHiA DDM™ Dx versions.
Please read the IFU thoroughly before using this product.

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REF
BS0102ILLCGLL01-016
BS0102ILLCGLL01-032
BS0102ILLCGLL01-048
BS0102ILLCGLL01-096

EC REP

CE IVD

PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	LIBRARY PREPARATION KIT
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	BS0102ILLCGLL01-096	B1.01.0002.C-96	B2.0001.C-48	700234

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REVISION HISTORY

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
SG-00658 – 11.0	28. Jan. 26	• Correction to numbering of document sections, minor aesthetic changes document wide.
SG-00658 – 10.0	26. Jan. 26	• Change to version numbering system, no additional versions between 8.5 and 10.0 • Section 2: General statement of test principles and procedure absorbs Summary and Explanation of the Test. • Update of library preparation component name to SOPHiA GENETICS Library Prep Kit I. • Section 4: Removal of the description of non IVD components from device scope. • Warnings and Precautions: Addition of CAS identification and concentration for each listed hazardous component. • Section 6: Removal of SOPHiA DDM Desktop App analysis instructions. • Section 7: Removal of out of scope limitations. • Section 7: Addition of limitation regarding index hopping. • Addition of Figure 1.
SG-00658 - 8.5	23.May.25	• Appendix 1: Removed the table “16 Illumina®-compatible Dual Index Adapters in 96-well plate format (7 µl each)” table from Appendix I due to discontinuation of 16 dual index adapters plate format. • Section 5.1.1 Kit Content – BOX 1: Updated to reflect the change above.
SG-00658 - 8.4	19.Mar.25	• Section 5.1.1 Kit Content – BOX 1: Increased content volume of 2x Hybridization Buffer from 50 µl to 75 µl; increased content volume of Hybridization Buffer Enhancer from 20 µl to 30 µl.
SG-00658 - 8.3	14.Aug.24	• "SOPHiA DDM™ Web App" changed to"SOPHiA DDM™ Dx mode" • Minor rephrasings related to the change above
SG-00658 - 8.2	08.Jul.24	• Updated the EC REP address • Reduced content volume of SOPHiA GENETICS hybridization probes from 20 µl to 18 µl (see section *5.1.1 Kit Content – BOX 1*) • Removed third-party provider's intellectual property from sections *5.1.1 Kit Content*, *5.3.1 Library Pooling*, and *5.3.2 Hybridization*
SG-00658 - 8.1	09.Jan.23	• Correction of formatting errors
SG-00658 - 8.0	05.Dec.22	• Correction of miscellaneous clerical errors
SG-00658 - 7.0	02.Dec.22	• Section 5.1.2 and Section 5.3.6 - Correction to add self-supplied material • Correction of miscellaneous clerical errors
SG-00658 - 6.0	14.Sep.22	• SOPHiA DDM and SOPHiA GENETICS trademark symbol added • Library Prep Kit name amended to SOPHiA GENETICS throughout • Document version upgraded to next full integer without any decimal
SG-00658 - 5.5	19.Apr.22	• SOPHiA DDM™ Web App instructions added
SG-00157 - 5.4	14.Apr.22	• Limitations and Warnings: Modified • Product Versions rectified • SOPHiA GENETICS Office address updated

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
		• Wet Lab changes as recommended globally • Changes in document ID
ID-60101-15 - 5.3	21.Jul.21	• Page 2, 30 - Cosmetic changes. • Page 18 - Step order changed. Page 27 - "48-sample" PCR pre-mix volume corrected.
ID-60101-15 - 5.2	16.Jun.21	• Page 3 - Trademark modified. • Page 45 - Minor addition in file specifications. • Page 55, 56 - Heading typo 'Unique' removed.
ID-60101-15 - 5.1	28.May.21	• Page 2 - Summary information table modified. • Page 3 - Disclaimer modified. • Page 12, 14, 16, 30 - Table header modified. • Page 17 - Step 1 and 2 sequence exchanged. • Page 18 - Ethanol preparation moved under 'Preparation'. • Page 13, 19, 20, 22, 23, 24, 28, 36, 37, 38, 39, 44 - Cosmetic changes and typos. • Page 21 - Point 1, Bullet point 2 - changed "FX Enhancer" to "FX Reaction pre-mix". • Page 47 - Bullet point 2 added.
ID-60101-15 - 5.0	30.Mar.21	• Title page, Company logo, Header, Footer, Last page. • Reorganized the topics. • Included SOPHiA DDM™ Web App installation, upload and naming convention instructions. • Combined four kit size "Instructions For Use" together to include different sample numbers. Included tables and made appropriate changes as and when necessary for this purpose. • Following Kit "Instructions For Use" documents were combined: PM_CEIVD_B.2.1.1.19_r3en M_CEIVD_B.2.1.1.17_r3en PM_CEIVD_B.2.1.1.15_r3en PM_CEIVD_B.2.1.1.26_r2en • Minor changes for clarity in the following sections: • Section 5.3.1 Library Pooling • Section 5.3.5 Wash Streptavidin Beads to Remove Unbound DNA

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1. INTENDED USE/PURPOSE

The SOPHiA DDM™ Dx Hereditary Cancer Solution (HCS) is an in vitro diagnostic test intended for the identification of germline variants occurring in 27 most common genes involved in predispositions to breast and ovarian cancers as well as intestinal cancers in germline DNA samples isolated from peripheral blood (Table 1). The SOPHiA DDM™ Dx HCS is a next-generation sequencing (NGS) assay on the Illumina MiSeq® instrument and is intended to be used as an aid to healthcare professionals and to provide molecular rationale to make a clinical decision related to germline mutations associated with the diseases listed in Table

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

The validated function of the SOPHiA DDM™ Dx Hereditary Cancer Solution (HCS) analytics is to analyze raw NGS data generated by an Illumina® MiSeq® instrument with MiSeq® Reagent Kit v3, on germline samples with SOPHiA GENETICS™ DNA Library Prep Kit I.

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

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

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

The following table shows genes that are targeted by the SOPHiA DDM™ Dx Hereditary Cancer Solution (HCS), which are often found in various hereditary cancer syndromes.

Table 1: Genes targeted by SOPHiA DDM™ Dx Hereditary Cancer Solution (HCS)

ABRAXAS1	EPCAM	PMS2
APC	MLH1	PMS2CL
ATM	MRE11A	PTEN
BARD1	MSH2	RAD50
BRCA1	MSH6	RAD51C
BRCA2	MUTYH	RAD51D
BRIP1	NBN	STK11
CDH1	PALB2	TP53
CHEK2	PIK3CA	XRCC2

3. PRODUCT COMPONENTS

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

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

- The bioinformatics pipeline ("HCS pipeline") processes the raw NGS data via algorithms capable of assessing genomic integrity.

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

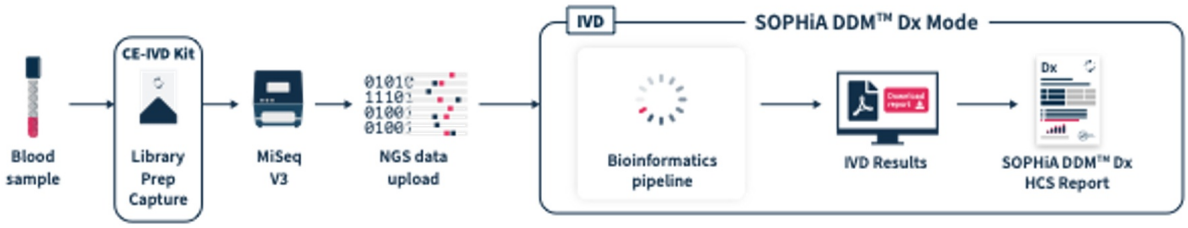


Figure 1: Overview of components of SOPHiA DDM Dx HCS

4. KIT MATERIALS AND METHODS

4.1. Initial Considerations

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

4.1.1. Kit Content (16, 32, 48 or 96 samples)

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

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

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

BOX 1 (STORE AT -25°C TO -15°C)

- Universal Blockers - TS Mix (12 µl)
- Human Cot DNA (25 µl)
- Hereditary Cancer Solution probes by SOPHiA GENETICS (18 µl)
- 2x Hybridization Buffer (75 µl)
- Hybridization Buffer Enhancer (30 µl)
- 2x Bead Wash Buffer (1250 µl)
- 10x Stringent Wash Buffer (200 µl)
- 10x Wash Buffer I (160 µl)
- 10x Wash Buffer II (110 µl)
- 10x Wash Buffer III (110 µl)

- Depending on the kit format: 32, 48 or 96 Illumina®-compatible Adapters with Dual Index in a 96-well plate format (7 µl each): see Appendix 1 for adapters display and sequences.

BOX 2 (STORE AT +2°C TO +8°C)

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

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

Important: Refer to Warnings and Precautions below for additional details.

SOPHiA GENETICS™ DNA LIBRARY PREP KIT I* (STORE AT -25°C TO -15°C)

- For 32 samples, two 16 sample kits are provided.
- For 96 samples, two 48 sample kits are provided.

COMPONENTS	KIT FORMAT	
	16 samples kit	48 samples kit
HiFi PCR Master Mix 2x (in µl)	500	1560
Primer Mix Illumina® Library Amp (in µl)	30	95
FX Enzyme Mix (in µl)	200	625
FX Buffer 10x (in µl)	100	315
FX Enhancer (in µl)	100	315
DNA Ligase (in µl)	200	625
DNA Ligase Buffer 5x (in µl)	400	1250

Important: Refer to Warnings and Precautions below for additional details.

WARNINGS AND PRECAUTIONS

Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
2X Hybridization Buffer		• H300 Fatal if swallowed. • H311 Toxic in contact with skin. • H315 Causes skin irritation. • H370 Causes damage to organs. • H370 Causes damage to organs (Central nervous system). • H411 Toxic to aquatic life with long lasting effects. • P260 Do not breathe vapor/ spray. • P264 Wash contaminated skin thoroughly after handling. • P270 Do not eat, drink or smoke when using this product. • P273 Avoid release to the environment. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P301+P310 If swallowed: Immediately call a poison center/ doctor. • P302+P352 If on skin: Wash with plenty of water. • P308+P311 If exposed or concerned: Call a poison center or doctor. • P321 Specific treatment (see medical advice on this label). • P330 Rinse mouth. • P332+P313 If skin irritation occurs: Get medical advice/ attention. • P362+P364 Take off contaminated clothing and wash it before reuse. • P391 Collect spillage. • P405 Store locked up. • P501 Dispose of contents/ container in accordance with national regulations.	Danger	**Tetramethyl-ammonium chloride** **Concentration:** 49% **CAS:** 75-57-0
Hybridization Buffer Enhancer		• H351 Suspected of causing cancer. • H360 May damage fertility or the unborn child. • H373 May cause damage to organs through prolonged or repeated exposure. • P201 Obtain special instructions before use. • P202 Do not handle until all safety precautions have been read and understood. • P260 Do not breathe vapour/ spray. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P308+P313 IF exposed or concerned: Get medical advice/ attention.	Danger	**Formamide** **Concentration**: 100% **CAS**: 75-12-7

Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
		• 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	**Ethylenedi-aminetetra- acetic acid disodium salt** **Concentration**: 2.5% **CAS**: 6381-92-6
10x Wash Buffer I		• H228 Flammable solid. • H302 Harmful if swallowed. • H315 Causes skin irritation. • H318 Causes serious eye damage. • H332 Harmful if inhaled. • H401 Toxic to aquatic life. • H402 Harmful to aquatic life. • H412 Harmful to aquatic life with long lasting effects. • P273 Avoid release to the environment. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. • P310 Immediately call a poison center/ doctor. • P501 Dispose of contents/ container in accordance with national regulations.	Danger	**Sodium dodecyl sulfate** **Concentration**: 4.9% **CAS**: 151-21-3
DNA Ligase Buffer 5x		• H335 May cause respiratory irritation. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.	Warning	**poly(ethylene glycol)** **Concentration**: 1-5% **CAS**: 9002-93-1

Please use  and  as personal protective equipment.

4.1.2. Material Required (not provided)

USER-SUPPLIED MATERIALS (TO BE PURCHASED SEPARATELY)

- KAPA™ Library Amplification kit KK2620 (Roche Cat. No: 07958978001)
- RNase/DNase-free 0.2 ml 8-tube strips
- DNA low binding 1.5 ml tubes
- 1.5 ml tubes
- 50 ml conical tubes
- Filter tips
- Ethanol (molecular biology grade)
- Illumina® sequencing reagents

LABORATORY EQUIPMENT

To avoid sample contamination:

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

4.2. Library Preparation

Genomic 5.2.1. DNA Preparation

MATERIALS

- Double-stranded high quality genomic DNA (gDNA)
- FX Enhancer
- IDTE
- RNase/DNase-free 0.2 ml 8-tube strips

IMPORTANT

DNA integrity, concentration and purity are critical during this step. The purity of the DNA can be assessed using a UV spectrophotometer. Recommended absorbance ratios are between 1.8-2.0 for 260/280 ratio, and within 1.6-2.4 for 260/230. We recommend confirmation of the sample integrity by capillary electrophoresis or an equivalent technique. In order to avoid mistakes with DNA input, an initial dilution to obtain a concentration in the 50 to 100 ng/µl range is recommended. The DNA concentration should be confirmed by a fluorometric quantitation (e.g., Qubit®, Thermo Fisher) and the obtained value used to calculate the final dilution.

PREPARATION

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

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

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

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

PROCEDURE

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

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

2. Prepare a dilution for each high-quality genomic DNA (gDNA) sample into the appropriate number of PCR strips, in the following manner:

gDNA DILUTION	
gDNA	200 ng
IDTE	Complete to 30 µl

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

  Tip: Safe stopping point overnight at 4°C.

- Depending on the number of samples, proceed as follows:
  - If processing **4 samples**, add 5 µl FX enhancer to each tube of the 4-tube strip containing 30 µl gDNA samples (total of 35 µl in each tube of the 4-tube strip).
  - If processing **8 or more samples**, proceed as follows:
    a. To facilitate pipetting, create a reservoir of FX Enhancer by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
FX Enhancer (in µl)	11.5	17.5	11.5	17.5	24	36

    b. Using a multichannel pipette, add 5 µl of the FX Enhancer from the above tubes to the 30 µl of gDNA samples (total of 35 µl in each tube of the 4 or 8-tube strips).

- Using a multichannel pipette set to 20 µl, mix gently by pipetting up and down 5 times and briefly spin in a microcentrifuge.

3. Keep on ice until enzymatic fragmentation reaction setup.

4.2.2. Pre-mixes and Reagents Preparation

COMPONENTS AND REAGENTS

- FX Enzyme Mix
- FX Buffer 10x
- DNA Ligase Buffer 5x
- DNA Ligase
- HiFi PCR Master Mix 2x
- Primer Mix Illumina® Library Amp
- Nuclease-free water
- AMPure® XP beads
- Ethanol

PREPARATION

- Remove the SOPHiA GENETICS™ DNA Library Prep Kit I components from -20°C storage and thaw on ice.
- Remove the Dual Index Adapters Plate from -20°C storage and put it into 4°C refrigerator for later use.
- Remove the AMPure® XP beads from 2-8°C storage and let them equilibrate at room temperature for at least 30 minutes.
- Prepare fresh 80% Ethanol (volume according to the following scheme based on number of reactions):

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

- Once the SOPHiA GENETICS™ DNA Library Prep Kit I components are thawed, mix the reagents by inverting the tube 5-10 times and briefly spin in a microcentrifuge.

PRE-MIXES

1. Prepare the FX reaction pre-mix as follows:

FX REACTION PRE-MIX							
Number of Reactions	4	8	12	16	24	32	48
FX Buffer 10x (in µl)	23.6	47.1	75	95	150	190	300
FX Enzyme Mix (in µl)	47.1	94.2	150	190	300	380	600

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

2. Prepare the Ligation pre-mix as follows:

LIGATION PRE-MIX							
Number of Reactions	4	8	12	16	24	32	48
DNA Ligation Buffer 5x (in µl)	95	190	300	380	600	760	1200
DNA Ligase (in µl)	47.5	95	150	190	300	380	600
Nuclease-free water (in µl)	71.3	142.5	225	285	450	570	900

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

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

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

PCR PRE-MIX							
Number of Reactions	4	8	12	16	24	32	48
HiFi PCR Master Mix 2x (in µl)	115	230	345	460	690	920	1380
Primer Mix Illumina® Library Amp (in µl)	6.9	13.8	20.7	27.6	41.4	55.2	82.8
Nuclease-free water (in µl)	16.1	32.2	48.3	64.4	96.6	128.2	193.2

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

4.2.3. Enzymatic Fragmentation, End Repair and A-Tailing

MATERIALS

- Diluted and conditioned double stranded gDNA in 35 µl
- FX reaction pre-mix
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

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

	TEMPERATURE (°C)	TIME (MINUTES)
Lid	70	-
Step 1	4	1
Step 2	32	5
Step 3	65	30
Hold	4	∞

- Start the FX Fragmentation program. When the block reaches Step 1 (4°C), pause the program.

PROCEDURE

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

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, to facilitate pipetting, create a reservoir of FX Reaction pre-mix by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
FX Reaction pre-mix (in µl)	33	52.5	33	52.5	66	105

2. Assemble the reaction as follows:
    - Using a multichannel pipette if processing 8 or more samples, add 15 µl of FX Reaction pre-mix to each of the 35 µl of gDNA samples (total of 50 µl in 4 or 8-tube strips).
    - Using a pipette set to 40 µl (multichannel if processing 8 or more samples), mix thoroughly by pipetting up and down 5 times and briefly spin in a microcentrifuge.
3. Place in the thermal cycler and continue the FX Fragmentation program.

Proceed immediately to Ligation.

4.2.4. Ligation

MATERIALS

- FX fragmentation reaction products in 50 µl each
- Ligation pre-mix
- Dual Index Adapters
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

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

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

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

PROCEDURE

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, to facilitate pipetting, create a reservoir of Ligation pre-mix in a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
Ligation pre-mix (in µl)	100	160	100	160	200	320

2. Using a multichannel pipette, transfer the 50 µl of each FX fragmentation reaction product to the 4 or 8-tube strips containing 5 µl of Dual Index Adapters.
3. Mix thoroughly by pipetting up and down 10 times and spin briefly.
4. Using a multichannel pipette if processing 8 or more samples, add 45 µl of Ligation pre-mix to each FX 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).

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

Proceed to Post Ligation Clean Up.

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%
- Nuclease-free water
- 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 to collect all liquid.
3. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 170 µl of supernatant using a multichannel pipette.

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

   Remove the tubes from the magnetic rack.
10. Using a multichannel pipette, add 105 µl of nuclease-free water to the beads and wait for a few seconds.

    Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin briefly to collect all liquid.
11. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 5 minutes or until liquid becomes clear.
12. Using a multichannel pipette, carefully transfer 100 µl of the supernatant to new, labeled 4 or 8- tube strips.

Proceed to Dual Size Selection.

4.2.6. Dual Size Selection

MATERIALS

- Ligated 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 60 µl of AMPure XP beads to each of the 100 µl ligated reaction products. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly to collect all liquid.
3. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 5 minutes or until liquid becomes clear.
4. Using a multichannel pipette carefully transfer 140 µl of the supernatant to new, labeled 4 or 8-tube strips containing 20 µl of AMPure XP beads. Mix thoroughly by pipetting up and down 10 times.
5. Incubate at room temperature for 5 minutes and spin briefly to collect all liquid.
6. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 3 minutes or until liquid becomes clear.

7. Carefully discard 150 µl of the supernatant using a multichannel pipette.

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

8. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads.

   Let the tubes stand for 30 seconds to 1 minute.

9. Carefully discard the ethanol using a multichannel pipette.
10. Repeat steps 8 and 9 once.
11. Remove the residual ethanol using a P10 or P20 multichannel pipette.
12. Air-dry the beads at room temperature for 4 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

    Remove the tubes from the magnetic rack.

13. 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.

4.2.7. Library Amplification

MATERIALS

- Dual size selected ligation products and beads resuspended in 20 µl of IDTE each
- PCR pre-mix

PREPARATION

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

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	
Step 1: Initial denaturation	98	120	
Step 2: Denaturation	98	20	
Step 3: Annealing	60	30	8 cycles
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

PROCEDURE

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, proceed as follows:
        a. To facilitate pipetting, create a reservoir of PCR pre-mix by adding the following volumes to a new set of 4 or 8-tube strips, according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	4-tube	4-tube	8-tube	8-tube	8-tube	8-tube
PCR pre-mix (in µl)	65	100	65	100	130	200

2. Assemble the reaction as follows:
    - Using a multichannel pipette if processing 8 or more samples, add 30 µl of PCR pre-mix to the dual size selected ligation products and beads (total volume 50 µl = 30 µl + 20 µl).
    - Mix thoroughly by pipetting up and down 10 times and spin briefly.
3. Place the tubes in the thermal cycler and run the Library Amplification program.

Tip: Safe stopping point overnight at 4°C.

4.2.8. Post-Amplification Clean Up

MATERIALS

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

PROCEDURE

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

4. Carefully discard 90 µl supernatant using a multichannel pipette.

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

5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads.

   Let 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 20 µl of nuclease-free water to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin to collect all liquid.

11. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until liquid becomes clear.

12. Carefully transfer 18 µl of the supernatant (transferring two times 9 µl is recommended at this step) to a new, labeled library storage tube.

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

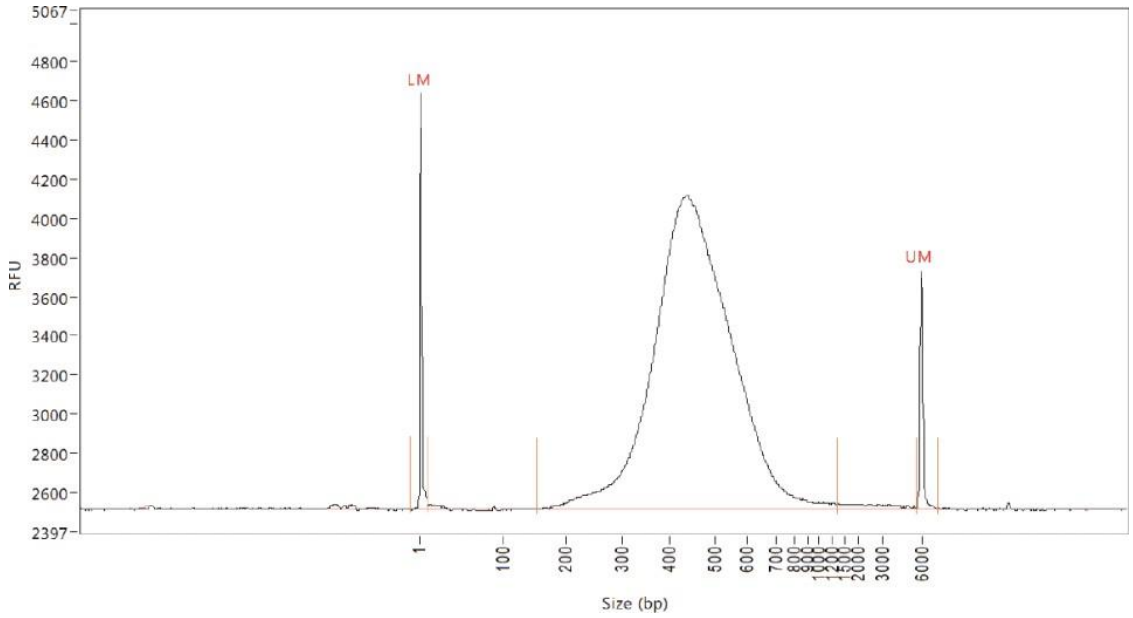
4.2.9. Individual Library Quantification and Quality Control

MATERIALS

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

PROCEDURE

1. Prepare a 4-time dilution of each library with nuclease-free water (e.g., 2 µl of library in 6 µl nuclease-free water).
2. Quantify the libraries with a fluorometric method (e.g., Qubit HS quantification using 2 µl of the 4x library dilution mentioned above).
3. Quality control the libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 300bp and 700bp.



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

4.3. Capture

4.3.1. Library Pooling

MATERIALS

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

PROCEDURE

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

NUMBER OF CAPTURES (Refer to the table in point 3)	1	2	3	4
Human Cot DNA (in µl)	5	11	16.5	22
Universal Blockers - TS Mix (in µl)	2	4.4	6.6	8.8

2. If performing two or more captures, pipette 7 µl of the above pre-mix into individual DNA low- binding tubes.

3. To the individual tubes containing the above pre-mix, add a pool of individual libraries according to the kit format:

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

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

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

4.3.2. Hybridization

MATERIALS

- Lyophilized libraries
- 2x Hybridization Buffer
- Hybridization Buffer Enhancer
- Hereditary Cancer Solution probes
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips
- 1.5 ml tubes
- 10x Wash Buffer I
- 10x Wash Buffer II
- 10x Wash Buffer IIIŸ
- 10x Stringent Wash Buffer
- 2x Beads Wash Buffer

PREPARATION

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

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

PROCEDURE

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

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

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

Important: Do not let the tube temperature drop below 65°C from step 3 to 5 as this can lead to incorrect probe annealing.

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

WASH BUFFER PREPARATION FOR 1 REACTION

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

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

WASH BUFFER PREPARATION FOR 2 REACTIONS

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

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

WASH BUFFER PREPARATION FOR 3 REACTIONS

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

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

WASH BUFFER PREPARATION FOR 3 REACTIONS

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

Important: Pre-warm 1x Stringent Buffer and aliquot 440 µl of 1x Wash Buffer I at 65°C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

4.3.3. Streptavidin Beads Preparation

MATERIALS

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

PROCEDURE

Perform these steps just before the end of the 4-hour hybridization incubation.

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

Important: Do not allow the beads to dry.

Proceed immediately to Binding of Hybridized Targets to the Beads.

4.3.4. Binding of Hybridized Targets to the Beads

MATERIALS

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

PROCEDURE

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

1. Remove the hybridization reaction(s) from the thermal cycler and briefly spin down the tube(s) and place them back on the thermocycler.
2. Place the washed Streptavidin bead tubes in the thermocycler (no more than two tubes at a time to avoid drying of beads).
3. For each hybridization reaction, transfer 17 µl of the hybridization reaction solution to one PCR tube containing cleaned beads. Resuspend the beads by pipetting up and down until the solution is homogeneous.

4. Bind the DNA to the beads by placing the tube(s) into a thermal cycler set at 65°C (lid at 75°C). Incubate for 45 minutes.
5. During the incubation, gently pipette up and down the tube(s) every 15 minutes to ensure that the beads remain in suspension.

Proceed directly to Wash Streptavidin Beads to Remove Unbound DNA.

4.3.5. Wash Streptavidin Beads to Remove Unbound DNA

MATERIALS

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

PROCEDURE

Important: Work to ensure that the temperature remains close to 65°C for steps 1 to 7.

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

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

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

   Gently resuspend the beads by pipetting up and down.

   Strong mixing of beads with the stringent wash buffer could decrease the quality of the capture.
5. Incubate at 65°C for 5 minutes.

6. Place the tube on a magnetic rack and let it stand until solution becomes clear. Carefully remove and discard the supernatant.
7. Repeat steps 4 to 6 once.

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

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

4.3.6. Post-capture Amplification

MATERIALS

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

PREPARATION

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

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	
Step 1: Initial Denaturation	98	45	
Step 2: Denaturation	98	15	14 Cycles
Step 3: Annealing	60	30	
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

PROCEDURE

1. Prepare the PCR pre-mix as follows:

PCR PRE-MIX				
Number of Reaction(s)	1	2	3	4
2x KAPA™ HiFi HotStart ReadyMix (in µl)	25	55	82.5	110
10x Library Amplification Primer Mix (in µl)	2.5	5.5	8.25	11
Nuclease-free water (in µl)	2.5	5.5	8.25	11

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

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

4.3.7. Post-capture Amplification Clean Up

MATERIALS

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

PROCEDURE

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

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

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

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

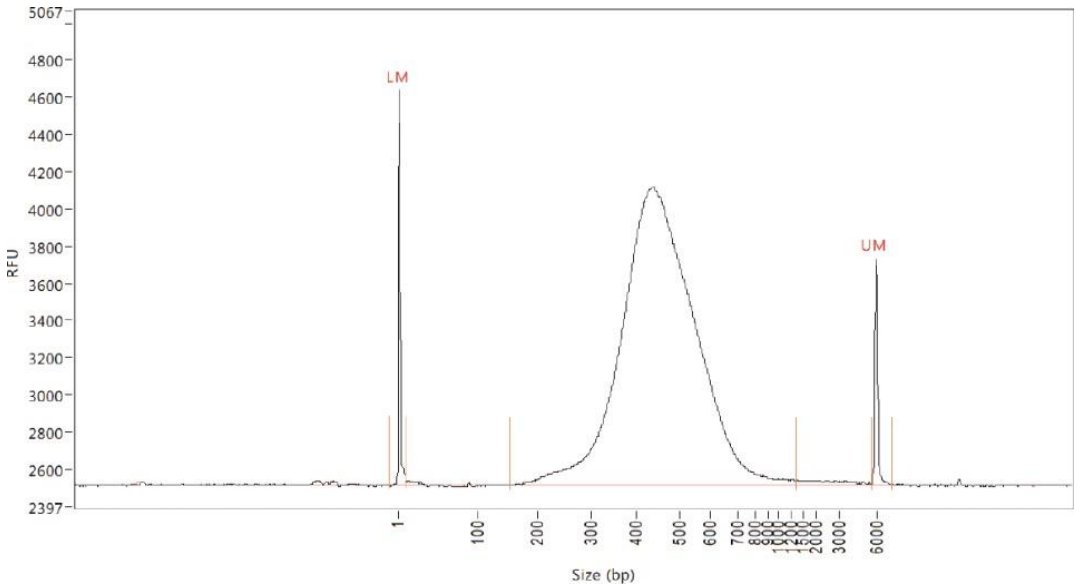
4.3.8. Final Library Quantification and Quality Control

MATERIALS

- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system

PROCEDURE

1. Quantify each captured library pool with a fluorometric method (e.g., Qubit HS quantification using 2 µl of the library).
2. Control the quality of the captured pool of libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 300bp and 700bp.



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

4.4. Sequencing

5.4.1. Library Preparation for Sequencing

MATERIALS

- Illumina MiSeq® Reagent Kit v3
- Final captured libraries
- EBT Buffer or similar

PROCEDURE

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

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

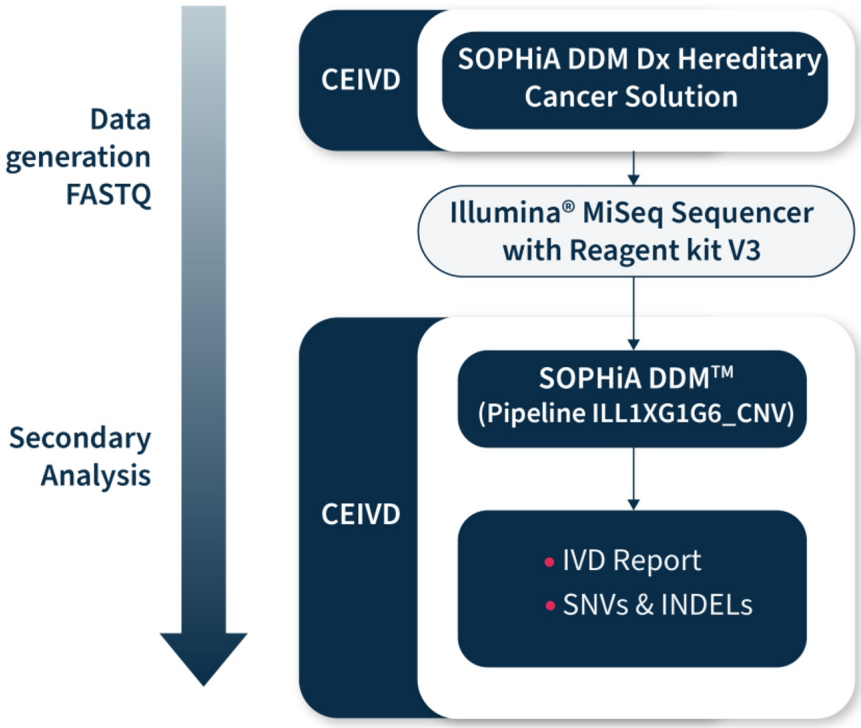
2. Dilute each pool to 4 nM and mix them in equal amount (e.g., 5 µl of each). Mix it well and use this dilution according to Illumina® standard denaturation recommendation.
3. Load a 10 pM dilution of the denatured libraries on MiSeq®.
4. The recommended minimum reads are 1.0 million reads per sample, with a read length 300 bp.

5. ANALYSIS PROCEDURE

5.1. SOPHiA DDM™ Dx Mode Installation Instructions

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

5.2. Analysis Workflow Description for IVD Results Generation



Analysis workflow description for the SOPHiA DDM™ Dx Hereditary Cancer Solution

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

6. LIMITATIONS, WARNINGS AND PRECAUTIONS

GENERAL WARNINGS

- For detailed instructions on the software, refer to the SOPHiA DDM™ Dx mode user manual.
- If any part of the handling, protocol, sequencer, multiplexing etc. is changed, the analyzes are not covered by the described instructions for use.
- The data provided in the Quality Report (available for download from the SOPHiA DDM™ Dx mode Platform is for information only and is not intended to be used for diagnosis.
- The accuracy of the results of the analysis cannot be guaranteed. Sequencing laboratories need to fulfill quality checks of the samples and flag the unqualified samples. Unqualified samples (e.g. insufficient biopsy sample) could lead to compromised results. SOPHiA GENETICS is not liable for the results and consequent decisions taken on the basis of these results.
- Good laboratory practice standards and procedures in addition to strictly following the IFU is required in order to obtain proper performance of the product. For specific safety information, please refer to the corresponding Material Safety Data Sheets (MSDS) provided with each component of the product.
- Physically separated pre- and post- PCR rooms should be defined to prevent DNA sample contamination. Always use fresh reagents, correctly extracted and stored DNA. For details on DNA quality and integrity see IFU Section 5. Kit Materials and Methods - Section 5.2.1 Genomic DNA Preparation.
- Correctly calibrated pipettes and proper lab equipment should be used to perform the experiment.
- Different lot numbers of reagents should not be mixed.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.

GENERAL LIMITATIONS

- Poor quality of the data due to issues in the sample preparation or sequencing step can confound the data analysis and cause False Positives and/or False Negatives.
- The absence of a variant in the report does not rule out the presence of a variant below the limits of detection of the assay.
- Sample crosstalk due to index-hopping can lead to False Positive variant calls. In the majority of cases, these will be detected with a low variant fraction (0.1-2%) and therefore filtered as low confidence variants. The following factors can aggravate sample crosstalk due to index-hopping and lead to higher variant fractions:
    - Very high coverage in one or few samples due to problems during the sample quantification/ normalization or high-level gene amplifications.

SNV / INDELS

- Variant detection in this product has been optimized for SNVs and short INDELs (up to 2/3 of the read length) detection. Please note, that any other type of alteration can be missed by the algorithm.
    1. Gene fusions or inversions cannot be detected.
    2. Deletions or insertions with a breakpoint outside of the target region might not be detected.
    3. Large deletions that are longer than approximately 5,000 bp may not map correctly and might be missed.
    4. Large insertions that are longer than approximately 2/3 of the read length might be missed due to insufficient anchor length for the identification of the insertion site.
    5. Duplications that are not fully covered by complete reads (including the reference sequence that is duplicated) might be missed.
    6. In the event of a tandem duplication of length more than 1/3 of the read length, the exact number of tandem repeats may not be determined.
    7. Alu insertions longer than 2/3 of the read length may be detected if they match at least one sequence in a database of known Alu-sequences. Otherwise, they will be missed.
    8. Quantification of insertions longer than read length can increase the risk of overestimating variant fractions.
- Variant detection in this product has been optimized on the regions defined as “target regions”. Please note that regions outside this definition might present false negatives and/or false positives.
- For a stable performance of the data analysis, we recommend coverage of at least 200x. A minimum coverage of 50x is recommended for reliable variant calling. Variants may be missed in regions that display lower coverage.
- Variants can be missed or wrongly called due to limitations in the kit design
- Variants in the regions for which the relevant fragment of the DNA is not pulled out by the probes cannot be detected by the product. Capturing variants can be limited by:
    1. INDELs that affect the hybridization of probes. This can lead to no detection or incorrect variant fraction estimation.
    2. The presence of additional mutation(s) in the target region of the probe on the same allele.
- SNVs or INDELs in homopolymers of length ten or higher cannot be called confidently as their detection is confounded by high background noise.
- Complex Delins may be reported as multiple variants in case it is represented in the alignment as multiple smaller variants that are separated by more than 2 nucleotides.
- Problematic regions in Appendix 4 are certain intronic regions within the target and could cause some uncertainty in variant calling. It could be related to the sequencing technology. A variant detected in this region will be designated as "Low" in the Confidence Table in SOPHiA DDM™.
- Flagged regions in Appendix 4 present characteristics which render variant detection unreliable. They are reported with warnings in the SOPHiA DDM™ interface, and variants detected in these regions are reported with a flag.

- Regions with high sequence homology can cause uncertainty of mapping and risk of missing or calling wrong variants.
- Variants may be represented in different forms in a given region. If a variant is represented in two different ways within the aligned reads or partially unaligned in some reads, the variant fraction may be underestimated, or the variant may be missed due to low variant fraction.
- In the case of multiple insertions / duplications present within the same region, not all might be reported correctly. Especially, where a smaller insertion is completely contained within a larger insertion, there may be no reads uniquely identifying the smaller insertion and it might be missed and only the larger insertion reported.
- In the case of multiple insertions / duplications present within the same region, not all might be quantified correctly. Especially, where a smaller insertion is completely contained within a larger insertion, there may be no reads uniquely identifying the smaller insertion and the variant fraction reported for the smaller insertion might be under- and the variant fraction of the larger insertion over-estimated.
- Presence of genetic mosaicism in the patient could lead to inaccurate interpretation of the results.

7. NON-CLINICAL PERFORMANCE EVALUATION

7.1. ANALYTICAL PERFORMANCE CHARACTERISTICS

The following study is divided into two parts. The first part describes the Performance evaluation study made for the SOPHiA DDM™ Dx HCS v1.0 and the KAPA™ Library Amplification Kit. The second part contains the non-regression study between SOPHiA DDM™ Dx HCS v1.0 and v1.1 using the KAPA™ Library Amplification Kit or the SOPHiA GENETICS DNA Library Prep I Kit. This last part includes the information about the gene APC included in the SOPHiA DDM™ Dx HCS v1.1.

7.2. METHODS

General

In this study, the performance of the SOPHiA DDM™ Dx HCS kit and the SOPHiA DDM™ Dx pipeline ILL1XG1G6_CNV was evaluated with data generated on an Illumina MiSeq® instrument using the SOPHiA DDM™ Dx HCS assay. The default filters related to low coverage (50X cut-off), variant fraction (20% cut-off for SNV, 15% cut-off for INDEL) and homopolymer region length (10 bp cut-off) were applied. For each sample, the variants detected by the pipeline were compared to the 'gold standard' confirmed variants provided by each sequencing center. Any variants detected outside of the target regions were not considered.

As the SOPHiA DDM™ Dx HCS panel concerns germline variant detection, limit of detection (LOD) evaluations were not necessary as the variant fractions expected are significantly higher than possible sequencing noise levels.

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

Each position that was analyzed by both the reference method and the method combining the use of the MiSeq® instrument and the SOPHiA DDM™ Dx HCS panel was taken into consideration to calculate analytical performance parameters such as sensitivity, specificity, accuracy, precision, and consistency.

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

Target region of the SOPHiA DDM™ Dx HCS panel: CDS ± 25bp (see Appendix 4 for the full list of the target regions as well as applicable problematic and flagged regions).

Undefined regions: Regions with coverage <50x

Identified problematic regions or regions with pseudogenes as specified in our documentation for the SOPHiA DDM™ Dx HCS pipeline (Appendix 4).

Additionally, the INDELs located in homopolymer regions greater than 9 bp were excluded from the calculations.

All the parameters were calculated with the following formulas:

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

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

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

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

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

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

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

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

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

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

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

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

$$Variant\ Repeatability = \frac{\sum_{ie[SP_A \cap SP_B]^\delta SP_A[i]-SP_B[i]}}{\sum_{ie[SP_A \cap SP_B]^1}}$$

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

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

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

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

To determine the confidence intervals in case of 100% sensitivity or other measures, the methods described by Mattocks et al (2010) were used (Mattocks CJ et al, EuroGentest Validation Group, 2010). In cases where the measured criterion was

less than 100%, the exact method (Clopper, et al 1934) was used to obtain the confidence interval on the binomial probability for sensitivity, specificity, accuracy, and precision (Clopper C et al, Biometrika, 1934). To reflect the true diversity of variants only unique TP, FP, FN and TN were used in confidence interval calculations.

7.3. DATA (SOPHiA DDM™ Dx HCS v1.0)

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

The second category of samples consists of reference and clinical samples where wide portions of the SOPHiA DDM™ Dx HCS target regions have been analyzed with either a CE-IVD marked diagnostics pipeline, SOPHiA DDM™ Dx—BRCA—MASTR Dx—GL—MiSeq, or at least two separate NGS experiments where only consensus positions are considered.

7.4. GENERAL CONCLUSIONS (SOPHiA DDM™ Dx HCS v1.0)

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

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

Run	# Samples	TP	FN
SiteA_01	22	343	0
SiteA_02	10	11	0
SiteA_03	11	124	0
SiteA_04	5	5	0
SiteB_01	12	27	0
SiteB_02	8	9	0
SiteC_01	12	115	0
SiteC_02	12	72	0
SiteD	13	28	0
SiteE	9	57	0
SiteF	18	286	0
SiteG	27	180	0
Total	**159**	**1257**	**0**

The following Table 3 compiles the complete summaries of detected variants compared to the list of confirmed variants (TP/FN/FP) and base positions with no variants (TN) for each run of fully characterized samples.

Table 3. Variant summaries for each run of fully characterized samples

Run	# Samples	TP	FN	FP	TN
Run A	24	665	0	0	1515530
Run B	24	329	0	2	583462
Run C	11	439	0	0	980668
Total	**59**	**1433**	**0**	**2**	**3079660**

The table below displays the unique TN base positions and variants detected in this performance evaluation study. The total number of base positions in the analyzed regions was 90754 bases. Three hundred and seventy-three (373) unique variants were detected in the study. True negatives were merged across all samples where high confidence reference data was available and covered a total of 90623 positions.

Table 4. Variant Summaries across all runs

TP	373
FP	2
FN	0
TN	90623

7.5. RESULTS (SOPHiA DDM™ Dx HCS v1.0)

The combination of the Illumina MiSeq® instrument, SOPHiA DDM™ Dx HCS assay and SOPHiA DDM™ lead to an observed performance of 100% sensitivity, 99.99% specificity, 99.99% accuracy, 99.86% precision, 99.983% repeatability and 99.93% reproducibility.

Table 5. Performance summary

N°	Performance Measurement	Mean	5th Percentile
A	On-target Rate	79.39%	[59.23%]
B	Uniformity	99.72%	[98.24%]

N°	Performance Measurement	Observed	[lower 95% CI]*
1	Sensitivity	100%	[99.20%]
2	Specificity	99.99%	[99.99%]
3	Accuracy	99.99%	[99.99%]

N°	Performance Measurement	Observed	[lower 95% CI]*
4	Precision	99.86%	[96.42%]
5	Repeatability	99.98%	[99.98%]
6	Reproducibility	99.93%	[99.92%]

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

** The 95% CI were calculated on all positions for all samples. Repeatability and reproducibility are based on replicas.

7.6. BRIDGING STUDY (SOPHiA DDM™ Dx HCS v1.1)

SOPHiA GENETICS HCS v1.0 has previously been thoroughly tested on a large scope of confirmed variants and using well characterized samples. We processed the same samples using different kits (SOPHiA GENETICS HCS v1.0 and SOPHiA DDM™ Dx HCS v1.1) and library preparation reagents KAPA™ Library Preparation Kit (KAPA™) or Qiaseq® FX DNA Library Kit (Qiagen®) to establish that:

- Performances using the SOPHiA DDM™ Dx HCS v1.1 kit and KAPA™ library preparation is on par with performance using the SOPHiA GENETICS HCS v1.0 kit and KAPA™ library preparation.
- Performance using the SOPHiA DDM™ Dx HCS v1.1 kit and Qiagen library preparation is also on par with SOPHiA GENETICS HCS v1.0 and KAPA™ library preparation.
- Analytical performance within the new regions targeted by SOPHiA DDM™ Dx HCS v1.1 (APC CDS+- 25bp) is high.

Table 6: Performance summary kit comparison

N°	Performance Measurement	Observed	[lower 95% CI]**
1	Reproducibility KAPA™ v1.1 versus KAPA™ v1.0 (3 runs on 59 samples)	99.99%	[99.99%]
2	Reproducibility Qiagen® v1.1 versus KAPA™ v1.0 (3 runs on 23 samples)	99.99%	[99.99%]
3	Reproducibility KAPA™ (2 runs on 24 samples)	99.95%	[99.95%]
4	Reproducibility Qiagen® (2 runs on 23 samples)	99.99%	[99.99%]
5	Reproducibility KAPA™-Qiagen® (2 runs on 23 samples)	99.99%	[99.99%]

** The 95% CI were calculated on all positions for all samples. Reproducibility is based on replicas.

7.7. CONCLUSION

Based on the data analyzed internally, the SOPHiA DDM™ Dx HCS v1.1 kit attained similar performance as previous SOPHiA DDM™ Dx HCS v1.0 in terms of on-target rates, covered regions, uniformity, and variant detection, with both KAPA™ and Qiagen® library preparation kits. For the new regions targeting the APC gene, the combination of Illumina MiSeq® instrument, SOPHiA DDM™ Dx HCS assay and SOPHiA DDM™ leads to an observed performance of 100% sensitivity, 100% specificity, 100% accuracy, 100% precision. Overall reproducibility using either KAPA™ or Qiagen® library preparation kits was greater than 99.9%.

8. SYMBOLS

Symbol	Title
	Consult instructions for use
	Catalog number
	Batch code (Lot Number)
	Caution
	Manufacturer
	Temperature Limit
	Use-by date
	European Conformity
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Contains sufficient for <n> tests
	Importer
	Date of manufacture
	Refer to **Warnings and Precautions** in "Section 5. Kit Materials and Methods"
	Refer to **Warnings and Precautions** in "Section 5. Kit Materials and Methods"

9. SUPPORT

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

APPENDIX 1. DUAL INDEX ADAPTER PLATES

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

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

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

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

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

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

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

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

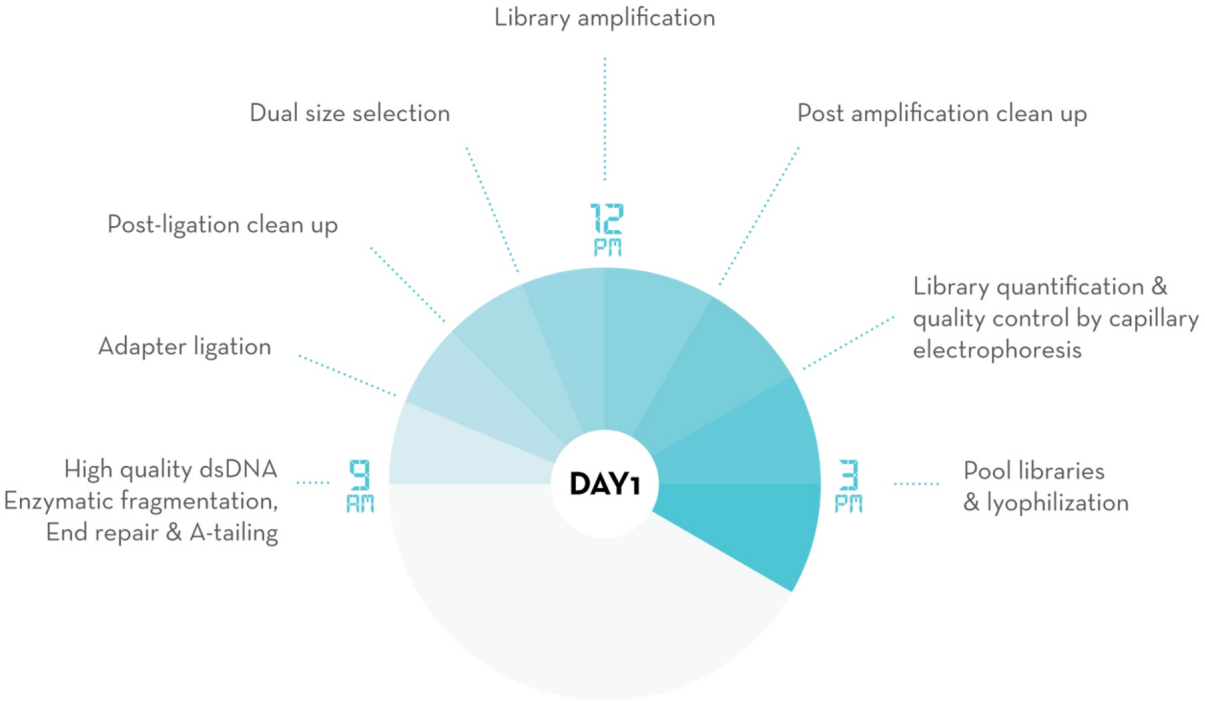
APPENDIX 2. LABORATORY EQUIPMENT USED IN SOPHIA GENETICS LABORATORY

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

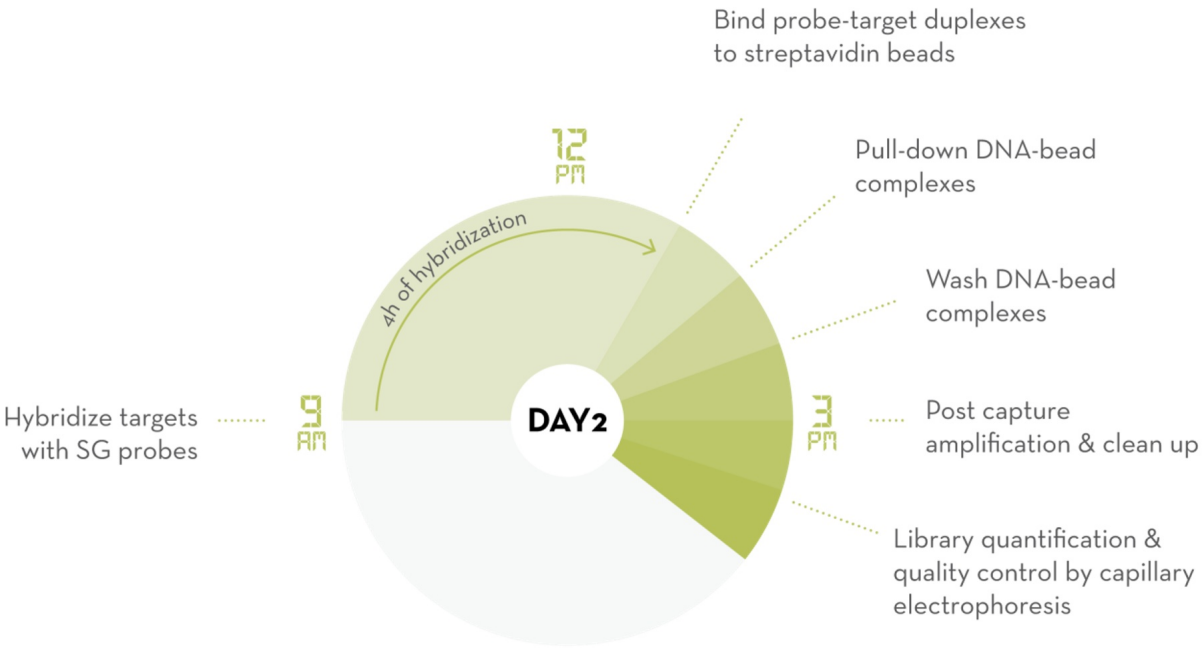
PRE-PCR ZONE	SUPPLIER	PRODUCT NAME	CATALOG N°
Vortex mixer	Scientific Industries	Vortex Genie 2	SI-0236
Table top microcentrifuge (8-tube strips compatible)	Starlab	Mini Centrifuge	N2631-0007
Magnetic separation rack 96-well type	Alpaqua	96S Super Magnet Plate	A001322
Magnetic separation rack 96-well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

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

APPENDIX 3. GENERAL WORKFLOW – SOPHIA DDM™ CAPTURE SOLUTIONS



Library Preparation
With SOPHiA GENETICS™ DNA Library Prep Kit I



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

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

TARGET REGIONS*

Target Regions

CHROMOSOME	START	END
1	45794953	45795134
1	45796163	45796254
1	45796829	45797031
1	45797067	45797253
1	45797308	45797546
1	45797670	45797783
1	45797813	45798007
1	45798038	45798185
1	45798221	45798384
1	45798410	45798531
1	45798565	45798656
1	45798744	45798867
1	45798932	45799021
1	45799060	45799300
1	45800038	45800208
1	45805866	45805951
2	47596620	47596745
2	47600577	47600734
2	47600922	47601212
2	47602348	47602463
2	47604128	47604241
2	47606067	47606218
2	47606883	47607069
2	47612280	47612374
2	47613686	47613777

CHROMOSOME	START	END
2	47630306	47630566
2	47635515	47635719
2	47637208	47637536
2	47639528	47639724
2	47641383	47641582
2	47643410	47643593
2	47656871	47657105
2	47672662	47672821
2	47690145	47690318
2	47693772	47693972
2	47698079	47698226
2	47702139	47702434
2	47703481	47703735
2	47705386	47705683
2	47707810	47708035
2	47709893	47710113
2	48010348	48010657
2	48018041	48018287
2	48023008	48023227
2	48025725	48028319
2	48030534	48030849
2	48032024	48032191
2	48032732	48032871
2	48033318	48033522
2	48033566	48033815
2	48033893	48034024
2	215593375	215593757
2	215595110	215595257
2	215609766	215609908
2	215610421	215610603
2	215617146	215617304
2	215632181	215632403

CHROMOSOME	START	END
2	215633931	215634061
2	215645259	215646258
2	215656996	215657194
2	215661760	215661866
2	215674111	215674318
3	37035014	37035179
3	37038085	37038225
3	37042421	37042569
3	37045867	37045990
3	37048457	37048579
3	37050280	37050421
3	37053286	37053378
3	37053477	37053615
3	37055898	37056060
3	37058972	37059115
3	37061776	37061979
3	37067103	37067523
3	37070250	37070448
3	37081652	37081810
3	37083734	37083847
3	37088985	37089199
3	37089983	37090125
3	37090370	37090533
3	37091952	37092169
3	178916589	178916990
3	178917453	178917712
3	178919053	178919353
3	178921307	178921602
3	178922266	178922401
3	178927358	178927513
3	178927949	178928151
3	178928194	178928378

CHROMOSOME	START	END
3	178935973	178936147
3	178936959	178937090
3	178937334	178937548
3	178937712	178937865
3	178938749	178938970
3	178941844	178942000
3	178942463	178942634
3	178943725	178943853
3	178947035	178947255
3	178947767	178947934
3	178947988	178948189
3	178951857	178952177
4	84383597	84384080
4	84384622	84384786
4	84388582	84388716
4	84390160	84390329
4	84391331	84391574
4	84393350	84393466
4	84397771	84397857
4	84403282	84403422
4	84406114	84406250
5	112043390	112043604
5	112090563	112090747
5	112101998	112102132
5	112102861	112103112
5	112111301	112111459
5	112116462	112116625
5	112128118	112128251
5	112136951	112137105
5	112151167	112151315
5	112154638	112155066
5	112157568	112157713

CHROMOSOME	START	END
5	112162780	112162969
5	112163601	112163728
5	112164528	112164694
5	112170623	112170887
5	112173225	112179848
5	131892992	131893170
5	131894951	131895084
5	131911444	131911645
5	131914984	131915219
5	131915529	131915783
5	131923229	131923407
5	131923591	131923806
5	131924354	131924597
5	131925298	131925554
5	131926891	131927123
5	131927544	131927751
5	131930536	131930761
5	131931240	131931527
5	131938967	131939206
5	131939587	131939763
5	131940473	131940716
5	131944282	131944442
5	131944784	131944926
5	131944950	131945113
5	131951670	131951751
5	131951754	131951847
5	131953737	131954011
5	131972782	131972917
5	131973748	131973940
5	131976339	131976522
5	131977845	131978081
7	6013005	6013198

CHROMOSOME	START	END
7	6017194	6017413
7	6018202	6018352
7	6022430	6022647
7	6026365	6027276
7	6029406	6029611
7	6031579	6031713
7	6035140	6035289
7	6036932	6037079
7	6038714	6038931
7	6042059	6042292
7	6043296	6043448
7	6043578	6043714
7	6045498	6045687
7	6048603	6048675
7	6776682	6777592
7	6781291	6781508
7	6785694	6785844
7	6786642	6786861
7	6790855	6791257
7	152345702	152346473
7	152357761	152357892
7	152373101	152373189
8	90947785	90947865
8	90949229	90949328
8	90955456	90955619
8	90958343	90958548
8	90960027	90960145
8	90965447	90965944
8	90967486	90967808
8	90970928	90971107
8	90976613	90976760
8	90982567	90982810

CHROMOSOME	START	END
8	90983376	90983543
8	90990423	90990576
8	90992937	90993146
8	90993578	90993776
8	90994925	90995108
8	90996728	90996814
10	89624202	89624330
10	89653757	89653891
10	89685245	89685339
10	89690778	89690871
10	89692745	89693033
10	89711850	89712041
10	89717585	89717801
10	89720626	89720900
10	89725019	89725254
11	94153266	94153372
11	94163052	94163177
11	94168973	94169090
11	94170318	94170426
11	94178951	94179084
11	94180360	94180629
11	94189417	94189529
11	94192549	94192772
11	94194077	94194227
11	94197254	94197430
11	94200954	94201084
11	94203612	94203833
11	94204715	94204950
11	94209430	94209594
11	94211876	94212067
11	94212815	94212952
11	94219065	94219275

CHROMOSOME	START	END
11	94223974	94224156
11	94225923	94225992
11	108098327	108098448
11	108098478	108098640
11	108099880	108100075
11	108106372	108106586
11	108114655	108114870
11	108115490	108115778
11	108117666	108117879
11	108119635	108119854
11	108121403	108121824
11	108122539	108122783
11	108123519	108123664
11	108124516	108124791
11	108126917	108127092
11	108128183	108128358
11	108129688	108129827
11	108137873	108138094
11	108139112	108139361
11	108141766	108141898
11	108141953	108142158
11	108143234	108143359
11	108143424	108143604
11	108150193	108150360
11	108151697	108151920
11	108153412	108153631
11	108154929	108155225
11	108158302	108158467
11	108159679	108159855
11	108160304	108160553
11	108163321	108163545
11	108164015	108164229

CHROMOSOME	START	END
11	108165629	108165811
11	108167989	108168134
11	108170416	108170637
11	108172350	108172541
11	108173555	108173781
11	108175377	108175604
11	108178599	108178736
11	108180862	108181067
11	108183113	108183250
11	108186525	108186663
11	108186713	108186865
11	108188075	108188273
11	108190656	108190810
11	108192003	108192172
11	108196012	108196296
11	108196760	108196977
11	108198347	108198510
11	108199723	108199990
11	108200916	108201173
11	108202146	108202309
11	108202581	108202789
11	108203464	108203652
11	108204588	108204720
11	108205671	108205861
11	108206547	108206713
11	108213924	108214123
11	108216445	108216660
11	108217981	108218117
11	108224468	108224632
11	108225513	108225626
11	108235784	108235970
11	108236027	108236260

CHROMOSOME	START	END
13	32890573	32890689
13	32893189	32893487
13	32899188	32899346
13	32900213	32900312
13	32900354	32900444
13	32900611	32900775
13	32903555	32903654
13	32905031	32905192
13	32906384	32907549
13	32910377	32915358
13	32918670	32918815
13	32920939	32921058
13	32928973	32929450
13	32930540	32930771
13	32931854	32932091
13	32936635	32936855
13	32937291	32937695
13	32944514	32944719
13	32945068	32945262
13	32950782	32950953
13	32953429	32953677
13	32953862	32954075
13	32954119	32954307
13	32968801	32969095
13	32971010	32971206
13	32972274	32972932
16	23614755	23615015
16	23619160	23619358
16	23625300	23625437
16	23632667	23632824
16	23634265	23634476
16	23635305	23635440

CHROMOSOME	START	END
16	23637532	23637743
16	23640500	23640621
16	23640936	23641815
16	23646158	23647680
16	23649146	23649298
16	23649366	23649475
16	23652406	23652503
16	68771294	68771391
16	68772175	68772339
16	68835548	68835821
16	68842302	68842495
16	68842571	68842776
16	68844075	68844269
16	68845562	68845787
16	68846013	68846191
16	68847191	68847423
16	68849393	68849687
16	68853158	68853353
16	68855879	68856153
16	68857277	68857554
16	68862052	68862232
16	68863532	68863725
16	68867168	68867427
17	7572902	7573033
17	7573902	7574058
17	7576568	7576682
17	7576828	7576951
17	7576994	7577180
17	7577474	7577633
17	7578152	7578314
17	7578346	7578579
17	7579287	7579615

CHROMOSOME	START	END
17	7579675	7579746
17	7579814	7579937
17	33427947	33428080
17	33428195	33428409
17	33430248	33430368
17	33430448	33430588
17	33433380	33433525
17	33433982	33434166
17	33434360	33434491
17	33443909	33444081
17	33445495	33445663
17	33446105	33446216
17	33446526	33446657
17	41197670	41197844
17	41199635	41199745
17	41201113	41201236
17	41203055	41203159
17	41209044	41209177
17	41215325	41215415
17	41215866	41215993
17	41219600	41219737
17	41222920	41223280
17	41226323	41226563
17	41228480	41228656
17	41231326	41231441
17	41234396	41234617
17	41242936	41243074
17	41243427	41246902
17	41247838	41247964
17	41249236	41249331
17	41251767	41251922
17	41256114	41256303

CHROMOSOME	START	END
17	41256860	41256998
17	41258448	41258575
17	41267718	41267821
17	41276009	41276138
17	56769980	56770174
17	56772267	56772579
17	56774029	56774245
17	56780532	56780715
17	56787195	56787376
17	56798082	56798198
17	56801376	56801486
17	56809820	56809930
17	56811454	56811608
17	59760632	59761526
17	59763172	59763551
17	59770766	59770898
17	59793287	59793449
17	59820349	59820520
17	59821768	59821977
17	59853737	59853948
17	59857597	59857787
17	59858176	59858391
17	59861606	59861810
17	59870933	59871115
17	59876436	59876685
17	59878589	59878860
17	59885803	59886143
17	59924437	59924606
17	59926465	59926642
17	59934394	59934617
17	59937132	59937293
17	59938783	59938925

CHROMOSOME	START	END
19	1206888	1207227
19	1218391	1218524
19	1219298	1219431
19	1220347	1220529
19	1220555	1220741
19	1221187	1221364
19	1221923	1222030
19	1222959	1223196
19	1226428	1226671
22	29083860	29083999
22	29085098	29085228
22	29089995	29090130
22	29091090	29091255
22	29091673	29091886
22	29092864	29093000
22	29095801	29095950
22	29099468	29099579
22	29105969	29106072
22	29107872	29108030
22	29115358	29115498
22	29120940	29121137
22	29121206	29121380
22	29130366	29130734

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

APPLICABLE PROBLEMATIC REGIONS**

CHROMOSOME	START	END
chr2	48018282	48018315
chr3	37067075	37067120
chr5	112111309	112111311
chr11	94152620	94152662
chr17	59757835	59757859

**A problematic region is a certain intronic region within the target and could cause some uncertainty in variant calling. It could be sequencing technology related. A variant detected in this region will be put Low in the Confidence Table in SOPHiA DDM™. The corresponding variant's filter column in the final full variant table will be marked as "problematic regions". Coordinates are 1-based and the end coordinate is included in the region.

APPLICABLE FLAGGED REGIONS***

Applicable Flagged Regions

CHROMOSOME	START	END	DESCRIPTION
2	47635515	47635560	variants in this region may be confounded by sequencing artifacts
2	48032732	48032820	variants in this region may be confounded by sequencing artifacts
3	37067103	37067240	variants in this region may be confounded by sequencing artifacts
7	6036932	6037079	variants in this region may be confounded by sequencing artifacts
3	178937334	178937548	region is very similar to another genomic region (the region with highest similarity is chr22:17053913- 17054324, hg19/GRCh37) - variants must be interpreted with care
3	178937712	178937865	region is very similar to another genomic region (the region with highest similarity is chr22:17054288- 17054442, hg19/GRCh37) - variants must be interpreted with care
7	6012845	6013198	region is very similar to another genomic region (the region with highest similarity is in pseudogene PMS2CL, chr7:6790854-6791209, hg19/GRCh37) - variants must be interpreted with care
7	6017194	6017413	region is very similar to another genomic region (the region with highest similarity is in pseudogene PMS2CL, chr7:6786641-6786861, hg19/GRCh37) - variants must be interpreted with care

CHROMOSOME	START	END	DESCRIPTION
7	6018202	6018352	region is very similar to another genomic region (the region with highest similarity is in pseudogene PMS2CL, chr7:6785693-6785844, hg19/GRCh37) - variants must be interpreted with care
7	6022430	6022647	region is very similar to another genomic region (the region with highest similarity is in pseudogene PMS2CL, chr7:6781290-6781508, hg19/GRCh37) - variants must be interpreted with care
7	6026365	6027276	region is very similar to another genomic region (the region with highest similarity is in pseudogene PMS2CL, chr7:6776681-6777592, hg19/GRCh37) - variants must be interpreted with care
7	6776682	6777592	region is very similar to another genomic region (the region with highest similarity is in gene PMS2, chr7:6026364-6027276, hg19/GRCh37) - variants must be interpreted with care
7	6781291	6781508	region is very similar to another genomic region (the region with highest similarity is in gene PMS2, chr7:6022429-6022647, hg19/GRCh37) - variants must be interpreted with care
7	6785694	6785844	region is very similar to another genomic region (the region with highest similarity is in gene PMS2, chr7:6018201-6018352, hg19/GRCh37) - variants must be interpreted with care
7	6786642	6786861	region is very similar to another genomic region (the region with highest similarity is in gene PMS2, chr7:6017193-6017413, hg19/GRCh37) - variants must be interpreted with care
7	6790855	6791257	region is very similar to another genomic region (the region with highest similarity is in gene PMS2, chr7:6012796-6013198, hg19/GRCh37) - variants must be interpreted with care
22	29085098	29085228	region is very similar to another genomic region (the region with highest similarity is chr16:33366704-33366835, hg19/GRCh37) - variants must be interpreted with care

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

Document Approvals

Approved Date: 28 Jan 2026

Approval Verdict: Approve	Coleman Spence, (cspence@sophiagenetics.com) Regulatory Approval 28-Jan-2026 01:09:47 GMT+0000

Approval Verdict: Approve	Tamara Maas, (tmaas@sophiagenetics.com) Technical Approval 28-Jan-2026 07:07:12 GMT+0000

QA Approval Verdict: Approve	Claire Mullane, (cmullane@sophiagenetics.com) Quality Assurance Approval 28-Jan-2026 08:57:04 GMT+0000