INSTRUCTIONS FOR USE

16, 32, 48 AND 96 SAMPLES

SOPHiA DDM™ Dx Myeloid Solution

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

SOPHiA GENETICS™

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SUMMARY INFORMATION

Product Name	SOPHiA DDM™ Dx Myeloid Solution
Product Type	Bundle Solution
Product Family	Molecular diagnostic application (kit + analytics)
Algorithm ID	ILL1XG1S9_CNV
Gene Panel ID	MYS_v1
Product Version	1.0
Sample Type	Somatic DNA isolated from blood
Sequencer	Illumina - MiSeq
GMDN Description	Reagent kit IVD / Human genomic analysis interpretive software
Document ID	SG-00659
Document Version	v5.1
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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

BS0103ILLCSML01-016
BS0103ILLCSML01-032
BS0103ILLCSML01-048
BS0103ILLCSML01-96

CE IVD

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PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	LIBRARY PREPARATION KIT
REF	BS0103ILLCSML01-016	B1.01.0003.C-16	B2.0003.C-16	700232
	BS0103ILLCSML01-032	B1.01.0003.C-32	B2.0003.C-32	700232
	BS0103ILLCSML01-048	B1.01.0003.C-48	B2.0003.C-48	700234
	BS0103ILLCSML01-96	B1.01.0003.C-96	B2.0003.C-48	700234

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REVISION HISTORY

DOCUMENT ID/VERSION	DATE	DESCRIPTION OF CHANGE
SG-00659 - 5.1	09.Jan.23	• Correction of formatting errors
SG-00659 – 5.0	14.Sep.22	• SOPHiA DDM and SOPHiA GENETICS trademark symbol added. • Library Prep Kit name amended to SOPHiA GENETICS™ Dx throughout. • Document version upgraded to next full integer without any decimal.
SG-00659 – 4.5	19.Apr.22	• SOPHiA DDM™ Web App instructions added
SG-00158 – 4.4	14.Apr.22	• Limitations and Warnings: Modified • SOPHiA GENETICS™ Office address updated • Wet Lab changes as recommended globally • Minor changes and corrections to typos
ID-60101-17 - 4.3	21.Jul.21	• Page 2 - Document ID corrected. • Page 2, 29 - Cosmetic changes. • Page 17 - Step order changed. Page 26 - "48-sample" PCR pre-mix volume corrected.
ID-60101-17 - 4.2	16.Jun.21	• Page 3 - Trademark modified. • Page 44 - Minor addition in file specifications. • Page 55, 56 - Heading typo 'Unique' removed.
ID-60101-17 - 4.1	28.May.21	• Page 2 - Summary information table modified. • Page 3 - Disclaimer modified. • Page 11, 13, 15, 29 - Table header modified. • Page 16 - Step 1 and 2 sequence exchanged. • Page 17 - Ethanol preparation moved under 'Preparation'. • Page 12, 18, 19, 21, 22, 23, 27, 35, 36, 37, 38, 43 - Cosmetic changes and typos. • Page 20 - Point 1, Bullet point 2 - changed "FX Enhancer" to "FX Reaction pre-mix". • Page 47 - Bullet point 2 and 18 added, point 19 modified.
ID-60101-17 - 4.0	30.Mar.21	• Title page, Company logo, Header, Footer, Last page. • Reorganized the topics. • Included SOPHiA DDM™ Web App installation, upload and naming convention instructions. • Combined four kit size "Instructions For Use" together to include different sample numbers. Included tables and made appropriate changes as and when necessary for this purpose. • Following Kit "Instructions For Use" documents were combined: PM_CEIVD_B2.1.1.14_r2en PM_CEIVD_B2.1.1.16_r2en PM_CEIVD_B2.1.1.18_r2en PM_CEIVD_B2.1.1.24_r1en • Minor changes for clarity in the following sections: • Section 5.3.1 Library Pooling • Section 5.3.5 Wash Streptavidin Beads to Remove Unbound DNA

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1. INTENDED USE/PURPOSE

The product is intended to be used to identify variants occurring in 30 genes involved in myeloid neoplasms by targeting specific mutation-prone positions within the genomic sequence. The function of the product is to be an aid to healthcare professionals to make a clinical decision related to myeloid neoplasms, and to provide molecular rationale for appropriate therapy.

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

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2. SUMMARY AND EXPLANATION OF THE TEST

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

LEUKAEMIA

Acute myeloid leukaemia (AML) is the most common acute leukaemia in adults, with around 20,000 new cases annually of AML in the US. AML is still fairly rare overall, accounting for only about 1% of all cancers. AML is caused by defective regulation of the differentiation and self-renewing programs of multipotent hematopoietic stem cells (HSCs), resulting in the bone marrow expansion of myeloid precursors with limited or abnormal differentiation capacity, which disseminate to the periphery. On average, there are 13 genes mutated per AML case with the top mutated genes being FLT3, NPM1, DNMT3A, IDH1, IDH2, TET2, RUNX1, TP53, NRAS, CEBPa and WT1. NPM1-mutated AML is the largest patient group with more than 70% of patients also carrying mutations in other genes, such as DNMT3A, IDH1, IDH2(R140), and TET2. The second largest group contains patients with mutations in genes regulating RNA splicing (SRSF2, SF3B1, U2AF1, ZRSR), chromatin structure (ASXL1, STAG2, BCOR, MLL-PTD, EZH2, PHF6), or transcription (RUNX1) (Papaemmanuil et al. 2016). Germline disorders include telomere biology disorders involving the TERC or TERT genes, familial AML with mutated CEBPa, familial myelodysplastic syndromes or familial AML (GATA2), and familial platelet disorders with propensity to myeloid malignancy.

MYELOPROLIFERATIVE NEOPLASMS (MPN)

Myeloproliferative Neoplasms (MPNs) are characterized by an excessive production of terminally differentiated myeloid cells clonally derived from a single mutated hematopoietic stem cell (HSC). The most frequent MPNs comprise of chronic myeloid leukemia (CML), essential thrombocytemia (ET), Polycytemia Vera (PV) and primary myelofibrosis (PMF). Aside from these malignancies, MPNs include other rare entities such as chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), not otherwise specified (NOS) and unclassifiable MPNs. These four MPNs are further divided into CML and BCR-ABL1 negative or

Philadelphia negative MPNs. In MPNs a single somatic mutation might be associated with different phenotypes which include the driver genes JAK2, CALR, MPL. In contrast, mutations associated with Philadelphia negative MPNs are not specific to a single MPN entity and therefore cannot be used alone for MPN diagnosis. However, specific recurrent mutations can be found in a very high proportion of MPN patients in genes such as JAK2, CALR or MPL for essential thrombocytemia, ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2 or SF3B1 for primary myelofibrosis and CSF3R for chronic myeloid leukemia.

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS (MDS/MPN)

Myelodysplastic diseases/myeloproliferative neoplasms (MDS/MPN) represent a distinct category of myeloid disorders that possess both dysplastic and proliferative features. These disorders are not properly classified as either MDS or MPN because they have some features of both MDS and MPNs. MDS/MPN share with MPNs an excessive production of myeloid cells and signs of myeloid cell dysplasia and/or cytopenia with MDS. The MDS/MPN category includes three major myeloid disorders: chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (jMML) and atypical chronic

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myeloid leukemia (aCML). The diagnosis may sometimes be difficult, and exclusion of other chronic myeloid malignancies is often necessary.

Most mutations identified in MDS/MPN are not specific to a single entity. However, the frequencies at which individual genes are mutated vary significantly across the different subtypes. SF3B1 mutations are associated with MDS criteria; JAK2, CALR and MPL mutations with MPNs; CSF3R with CNL; any clonal evidence with CMML; and SETBP1 or ETNK1 mutations with aCML.

The following table shows genes that are targeted by the SOPHiA DDM™ Dx Myeloid Solution (MYS) and the diseases that are associated with those genes.

Table 1. Genes targeted by the SOPHiA DDM™ Dx Myeloid Solution (MYS) and their associated diseases

Leukemia	MPN	MDS/MPN
ASXL1	ABL1	ABL1
BRAF	ASXL1	AXSL1
CBL	CALR	CALR
CEBP*a*	CBL	CBL
DNMT3A	DNMT3A	CSF3R
EZH2	ETV6	DNMT3A
FLT3	EZH2	EZH2
HRAS	FLT3	JAK2
IDH1	IDH1	KRAS
IDH2	IDH2	MPL
KIT	JAK2	NRAS
KRAS	MPL	PTPN11
NPM1	NRAS	RUNX1
NRAS	RUNX1	SETBP1
PTPN11	SF3B1	SF3B1
RUNX1	SRSF2	SRSF2
SF3B1	TET2	TET2
SRSF2	TP53	ZRSR2
TET2	U2AF1	-
U2AF1	-	-
WT1	-	-
ZRSR2	-	-

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

The validated function of the SOPHiA DDM™ Dx Myeloid Solution (MYS) analytics is to analyze raw NGS data generated by an Illumina MiSeq® instrument with MiSeq® Reagent Kit v3, on somatic samples isolated from blood with the KAPA® Library Amplification Kit and SOPHiA GENETICS™ DNA Library Prep Kit I (QIAseq® FX DNA Library Kit OEM-licensed from QIAGEN).

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

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

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4. PRODUCT COMPONENTS

The SOPHiA DDM™ DxMYS is composed of three major components: the NGS kit, the bioinformatics pipeline and the SOPHiA DDM™ for IVD platform.

- 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 (“ MYS pipeline”) processes the raw NGS data via algorithms capable of assessing genomic integrity.
- The SOPHiA DDM™ platform (“SDDM”), is a front-end web-based application available as a “software-as- a-service” (SaaS) used to generate a downloadable report for genes mentioned in Table 1 for SNVs and INDELs. Limitations apply - please see section 7 - Limitations, Warnings and Precautions.

The SOPHiA DDM™ DxMYS also offers an additional component via the SOPHiA DDM™ Desktop App platform that allows users to visualize, interpret and report Clinical Decision Support (CDS) results computed by the bioinformatics pipeline. The key CDS features supported by the SOPHiA DDM™ platform allows users to: visualize and interpret SNVs and INDELs from 30 genes as well as CNVs. Limitations associated with the CDS functions are provided in a subsection within the section 7 - Limitations, Warnings and Precautions.

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5. KIT MATERIALS AND METHODS

5.1. Initial Considerations

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

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

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

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

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

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BOX 1 (STORE AT -25°C TO -15°C)

- IDT Blocking Oligo xGen® Universal Blockers - TS Mix (12 µl)
- Human Cot DNA (25 µl)
- Myeloid Solution xGen® Lockdown® 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)
- Depending on the kit format: 16, 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.

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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

* SOPHiA GENETICS™ is the exclusive distributor of this library preparation kit.

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

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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
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	Danger	Formamide

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Name of Product	GHS Pictogram	H&P Statements	Signal word	Hazardous Component
		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	Ethylenedi-aminetetra-acetic 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 center/ doctor. • P501 Dispose of contents/ container in accordance with national regulations.	Danger	Sodium dodecyl sulfate
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)

Please use and as personal protective equipment.

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5.1.2. 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

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5.2. Library Preparation

5.2.1. Genomic 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.

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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.

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5.2.2. 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:

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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.

5.2.3. 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

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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.

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5.2.4. 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).

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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.

5.2.5. Post-Ligation Clean Up

MATERIALS

- Ligation reaction products in 100 µl each
- AMPure XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- 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.

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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.

5.2.6. 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.

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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.

5.2.7. 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	∞	

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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.

5.2.8. Post-Amplification Clean Up

MATERIALS

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

PROCEDURE

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

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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.

5.2.9. Individual Library Quantification and Quality Control

MATERIALS

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

PROCEDURE

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

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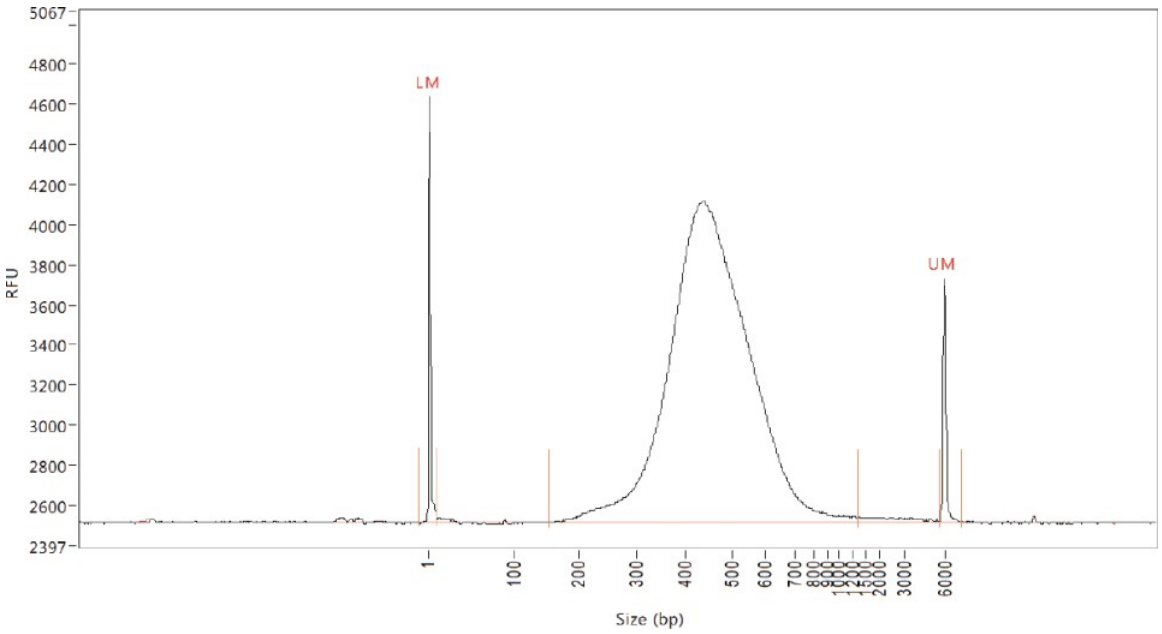

Example of a DNA library distribution obtained with the Agilent Fragment Analyzer capillary electrophoresis system. UM – Upper Marker, LM – Lower Marker

5.3. Capture

5.3.1. Library Pooling

MATERIALS

- Individual libraries
- Human Cot DNA
- Blocking oligos xGen® 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
Blocking oligos xGen 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.

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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.

5.3.2. Hybridization

MATERIALS

- Lyophilized libraries
- 2x Hybridization Buffer
- Hybridization Buffer Enhancer
- Myeloid Solution xGen® Lockdown Probes
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips
- 1.5 ml tubes
- 10x Wash Buffer I
- 10x Wash Buffer II
- 10x Wash Buffer III
- 10x Stringent Wash Buffer
- 2x Beads Wash Buffer

PREPARATION

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

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

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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 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.

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WASH BUFFER PREPARATION FOR 2 REACTIONS

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

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

WASH BUFFER PREPARATION FOR 3 REACTIONS

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

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

WASH BUFFER PREPARATION FOR 4 REACTIONS

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

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

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5.3.3. Streptavidin Beads Preparation

MATERIALS

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

PROCEDURE

Perform these steps just before the end of the 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.

5.3.4. Binding of Hybridized Targets to the Beads

MATERIALS

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

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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.

5.3.5. Wash Streptavidin Beads to Remove Unbound DNA

MATERIALS

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

PROCEDURE

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

Note:

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.

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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 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.

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5.3.6. Post-capture Amplification

MATERIALS

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

PREPARATION

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

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

PROCEDURE

1. Prepare the PCR pre-mix as follows:

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

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

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

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5.3.7. Post-capture Amplification Clean Up

MATERIALS

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

PROCEDURE

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

   Keep tube(s) on the magnetic rack for the following steps.
5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Let 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.

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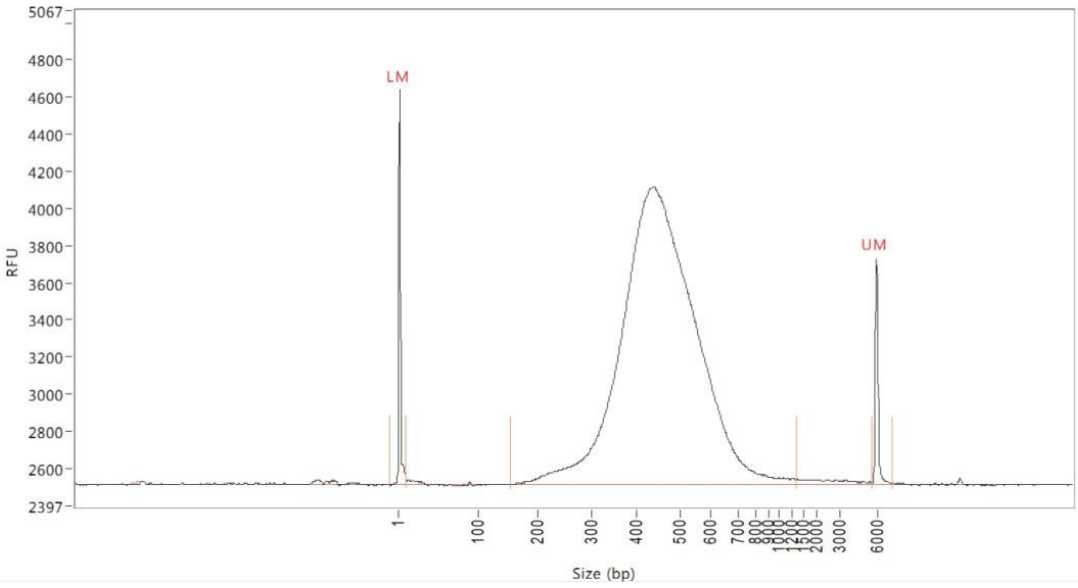
5.3.8. Final Library Quantification and Quality Control

MATERIALS

- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system

PROCEDURE

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

5.4. Sequencing

5.4.1. Library Preparation for Sequencing

MATERIALS

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

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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 2.0 million reads per sample, with a read length 300 bp.

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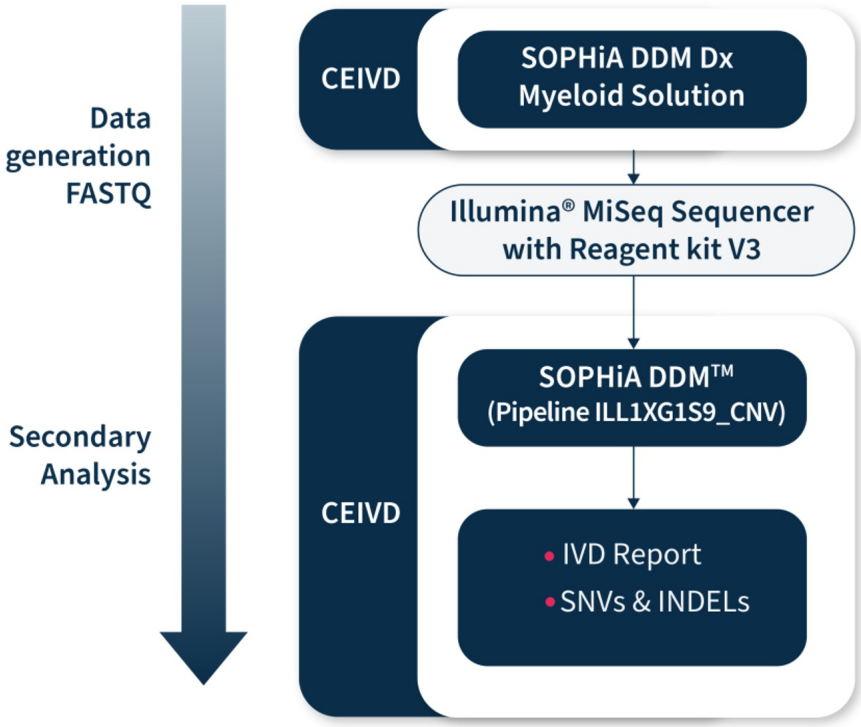
6. ANALYSIS PROCEDURE

6.1. SOPHiA GENETICS™ Web App Installation Instructions

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

Note: To access CDS functions the SOPHiA DDM™ Desktop App is required which can be downloaded and installed separately. Access to the SOPHiA DDM™ Web App will be granted at the same time as access to the SOPHiA DDM™ Web App.

6.2. Analysis Workflow Description for IVD Results Generation



Analysis workflow description for the SOPHiA DDM™ DxMyeloid Solution

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

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7. LIMITATIONS, WARNINGS AND PRECAUTIONS

GENERAL WARNINGS

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

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FOR IN-VITRO DIAGNOSTIC PROCEDURES

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.
- Variant detection in this product has been optimized on the regions defined as “target regions”. Please note that regions outside this definition might present false negatives and/or false positives.
- For robust performance of the algorithm, we recommend a coverage of at least 1,000x to meet statistical significance compared to noise for most positions. Lower coverage will increase the risk of False Negative variant calls significantly.
- Variants can be missed or wrongly called due to limitations in the kit design.
- Variants in the regions for which the relevant fragment of the DNA is not pulled out by the probes cannot be detected by the product. Capturing variants can be limited by:
    1. INDELs that affect the hybridization of probes. This can lead to no detection or incorrect variant fraction estimation.
    2. The presence of additional mutation(s) in the target region of the probe on the same allele.
- SNVs or INDELs in homopolymers with a length of ten base pairs or higher cannot be called confidently as their detection is confounded by high background noise.
- Complex Delins may be reported as multiple variants in case it is represented in the alignment as multiple smaller variants that are separated by more than 2 nucleotides.
- SNVs or INDELs in long repeat or low complexity regions specified in the flagged regions in Appendix 4 may also be missed.
- 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 one portion of the reads reports the variant in a different form or does not capture the variant, the variant fraction may be underestimated, or the variant may be missed due to low variant fraction.
- In the case of multiple insertions / duplications present within the same region, not all might be reported correctly. Especially, where a smaller insertion is completely contained within a larger insertion, there may be no reads uniquely identifying the smaller insertion and it might be missed and only the larger insertion reported.
- In the case of multiple insertions / duplications present within the same region, not all might be quantified correctly. Especially, where a smaller insertion is completely contained within a larger insertion, there may be no

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reads uniquely identifying the smaller insertion and the variant fraction reported for the smaller insertion might be under- and the variant fraction of the larger insertion over-estimated.

- Sample crosstalk due to index-hoping can be aggravated by the following factors:
    1. Very high coverage in one or few samples due to problems during the sample quantification / normalization or high-level gene amplifications
    2. Overloading of the flow-cell, i.e., high cluster density
    3. Differences in library conversion rate
- Sample crosstalk in the presence of low-level index contamination (<1%) can be aggravated by the following factors:
    1. Very high coverage in one or few samples due to problems during the sample quantification / normalization or high-level gene amplifications
    2. Overloading of the flow-cell, i.e., high cluster density
    3. Differences in library conversion rate
- It is recommended that the user exercise caution in interpreting variants reported below 2.5% and possibly use alternative testing methods to confirm.
- Any issue during NGS processing or issues with sample degradation can cause low signal-to noise ratio and negatively affect variant calling.
- Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. The test does not provide information about susceptibility.

FOR CLINICAL DECISION SUPPORT PROCEDURES

Large INDELs

- Deletions that are longer than approximately 5,000 bp may not map correctly and might be missed.
- Insertions that are longer than approximately 2/3 of the read length may be missed due to insufficient anchor length needed for the identification of the insertion site.
- Duplications that are not fully covered by complete reads (including the reference sequence that is duplicated) might be missed.
- 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.
- Large INDELs, such as FLT3 ITD, below 10% variant fraction may not be detected. The reported Limit of detection of 2.5% does not include FLT3 ITD.
- Quantification of insertions longer than read length can increase the risk of overestimating variant fractions.

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Copy Number Variants

- In order to provide the optimal performance of the CNV module, it is essential that all the samples in the batch are processed under the same laboratory condition. For processing involving PCR amplification, it is strongly recommended that only samples processed together at the PCR amplification step are combined in one batch.
- At the PCR step of library preparation, temperature gradients should be avoided as much as possible (e.g., by not using the outer rows of PCR plates). Temperature gradients may produce false positive CNV calls.
- For a reliable determination of the reference coverage level, a sufficient number of reference samples is needed. For a good performance, it is recommended to have at least 20 samples in a batch (and 8 samples as an absolute minimum).
- The CNV module is optimized to reduce the occurrence of false negatives (missed CNVs). As a consequence, one should expect a certain number of false positives and rejected regions. We recommend re-checking such cases with an independent test (possibly using a different technology).
- Samples may get rejected for two reasons: either because of quality problems in the library preparation (e.g., failed PCR) or because of a different coverage pattern from the rest of the samples (for any reason). If the total number of non-rejected samples in the batch is below 4, the whole batch gets rejected.
- The algorithm is based on the statistical inference of CNVs from comparing samples to each other. Therefore, it assumes that, for each region, the CNV are only present in a small fraction of samples. It may fail to detect a CNV in batches with a large fraction of samples having CNVs at the same position.
- The performance of the CNV module is generally higher for longer CNVs than for shorter CNVs and also higher for deletions than for duplications (insertions).
- The first exons of genes frequently have higher coverage fluctuations due to their higher GC content. As a consequence, the performance of the CNV detection is lower for the starting exons of the genes.
- In case of a target region covered by only one probe, a deletion may be called in case of insertions or deletions (INDELs) in the probe region if they are sufficiently large to suppress probe binding.
- The CNV detection algorithm only reliably detects CNVs with whole (or almost whole) target regions deleted or duplicated. In case of CNVs with breakpoints inside target regions, the boundaries of such CNVs may be determined incorrectly.
- In the presence of a novel pseudogene with sufficient sequence identity to be amplified, i.e., a pseudogene that is not present in the reference genome, reads coming from the pseudogene will be attributed to the parent gene. In this case the CNV module might over-estimate the copy number of the affected gene. In the case the pseudogene is further amplified, this will increase the estimated copy number even further.
- For the SOPHiA DDM™ Dx Myeloid Solution, the algorithm is designed to detect somatic CNVs. Copy numbers may be fractional, with the resolution of 0.1. The precision of the copy-level estimates is also about 0.1. Small deviations from the normal coverage level (typically, 10% or smaller) may be missed.
- Four regions are excluded from the CNV detection due to typically high noise level: CEBPA_ex1_1, RUNX1_ex4, SRSF2_ex1, RUNX1_ex9.
- This panel contains one gene from the X chromosome (ZRSR2). For a reliable CNV detection in this gene, the sex of the patient needs to be determined. The current version of the algorithm can deduce the sex of the patient

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from the ratio of the coverage in the X chromosome and in the autosomes. This method may produce inaccurate results, and it is recommended to check the automatically determined sex value against the actual patient data.

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8. NON-CLINICAL PERFORMANCE EVALUATION

8.1. METHODS

General

In this study, the performance of the SOPHiA DDM™ Dx MYS kit and the SOPHiA DDM™ Dx pipeline ILL1XG1S9_CNV was evaluated with data generated on an Illumina MiSeq® instrument using the SOPHiA DDM™ Dx MYS assay. Variant filtering was set-up according to background noise measured in germline dilution samples and the present claims concern variants above the limit of detection determined by our experiments.

Homopolymers of 10bp or more were excluded, as were regions covered by fewer than 1000 reads. For each sample, the variants detected by the pipeline were compared to the 'gold standard' confirmed variants provided by each sequencing centre. Any variants detected outside of the target regions were not considered (see Appendix 4 for the full list of the target regions and flagged regions). One region, exon 15 of JAK2 padded by 25bp, was consistently lower coverage and therefore was excluded from our analyses.

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

Each position that was analysed by both the reference method and the method combining the use of the MiSeq® instrument and the SOPHiA DDM™ Dx MYS 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 MYS 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 of the SOPHiA DDM™ Dx MYS panel, which is CDS ± 25bp of the genes and exons specified in SOPHiA DDM™ Dx MYS documentation:

- Undefined regions: Regions containing low confidence variants, or confirmed variants under the reporting threshold, and for reproducibility and repeatability, regions under 1000x depth (to assess coverage reproducibility and repeatability)
- Exon 15 of JAK2, which has been identified as a region where coverage is difficult
- Known regions with artefacts (flagged regions)
- Regions where no reference data was available or where it was ambiguous (see also 5.1.3). For example, Sanger sequencing cannot report large INDELs in FLT3, therefore these variants were excluded.

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

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All the parameters were calculated with the following formulas:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

8.2. DATA

Available samples are grouped into two categories depending on the high confidence characterization available for all benchmark comparisons. First, partially characterized samples where known high confidence variants have been identified were processed either by SOPHiA GENETICS™ (Site A) or by external partners (Sites B to E) using recommended experimental conditions.

These results contain a large array of representative clinical cases and enable estimates of sensitivity for variant calling. In addition, replicates were included to estimate repeatability and reproducibility. The second category of samples consists in reference samples with vendor-supplied regions of high confidence, or Genome in a bottle (GIAB) high confidence variants, and clinical samples where wide portions of the SOPHiA DDM™ Dx MYS target regions have been analysed by Sanger sequencing or other high accuracy methods (allowing for the limits of detection of respective methods). This set of well-characterized samples enables determination of specificity, precision, and accuracy as well as sensitivity. The input data comes from several multi-patient runs (one sequence file per patient) executed by four distinct sequencing centres: Site A-E. All files of a run were analysed together as part of the same batch process.

8.3. GENERAL CONCLUSIONS

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

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

Run	# Samples	TP	FN
SiteA_Run01	1	15	0
SiteA_Run02	15	36	0
SiteA_Run03	16	37	0
SiteA_Run04	1	2	0
SiteA_Run06	1	15	0
SiteA_Run07	21	31	0
SiteA_Run08	20	142	0
SiteA_Run09	20	142	0
SiteA_Run10	24	599	0
SiteA_Run11	10	15	0
SiteA_Run14	8	8	0

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

Run	# Samples	TP	FN
SiteA_Run15	14	14	0
SiteB_Run01	22	78	0
SiteB_Run02	18	55	1
SiteC_Run01	11	57	0
SiteC_Run02	10	40	0
SiteD_Run01	10	15	0
SiteE_Run01	15	31	0
Total	**237**	**1332**	**1**

The following table 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
SiteA_Run08	18	132	0	0	469693
SiteA_Run09	18	132	0	0	469231
Other	11	152	0	2	480194
Total	**47**	**416**	**0**	**2**	**1419118**

The table below displays the unique TN base positions and variants detected in this performance evaluation study. The total number of base positions in the analysed regions was 48093 bases. Two hundred and nineteen (219) unique confirmed variants were detected in the study. Unique true negatives where high confidence reference data was available and covered a total of 48092 bp, which cover 99% of the target regions minus the excluded JAK2 exon (48547bp).

Table 4. Variant Summaries across all runs

TP	219
FP	2
FN	1
TN	48092

8.4. RESULTS

The combination of Illumina MiSeq® or HiSeqTM instrument, SOPHiA DDM™ Dx MYS assay and SOPHiA DDM™ Dx leads to an observed performance of 99.92% sensitivity, 99.99% specificity, 99.99% accuracy, 99.52% precision, 98.69% repeatability and 99.30% reproducibility.

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Table 5. Performance summary

N°	Performance Measurement	Mean	5th Percentile
A	On-target Rate	87.41%	[59.59%]
B	Uniformity	99.88%	[99.40%]
C	Limit of Detection	2.5%* (observed)	[1%]

N°	Performance Measurement	Observed	[Lower 95% CI]**
1	Sensitivity	99.92%	[97.49%]
2	Specificity	99.99%	[99.98%]
3	Accuracy	99.99%	[99.98%]
4	Precision	99.52%	[91.47%]

N°	Performance Measurement	Observed	[Lower 95% CI]***
5	Repeatability	98.69%	[98.66%]
6	Reproducibility	99.30%	[99.27%]

* For SNVs and INDELs; FLT3 ITD excepted.

** 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 comparisons described in Dual- Size selection equivalence and Inter-site reproducibility.

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9. SYMBOLS

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

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10. SUPPORT

In case of difficulty using SOPHiA DDM™ Web App please consult the troubleshooting section of the SOPHiA DDM™ Web App User Manual available on the SOPHiA DDM™ Web App 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™ Web App.

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APPENDIX 1. DUAL INDEX ADAPTER PLATES

16 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									
F									
G									
H									

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					

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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

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APPENDIX

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

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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

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APPENDIX

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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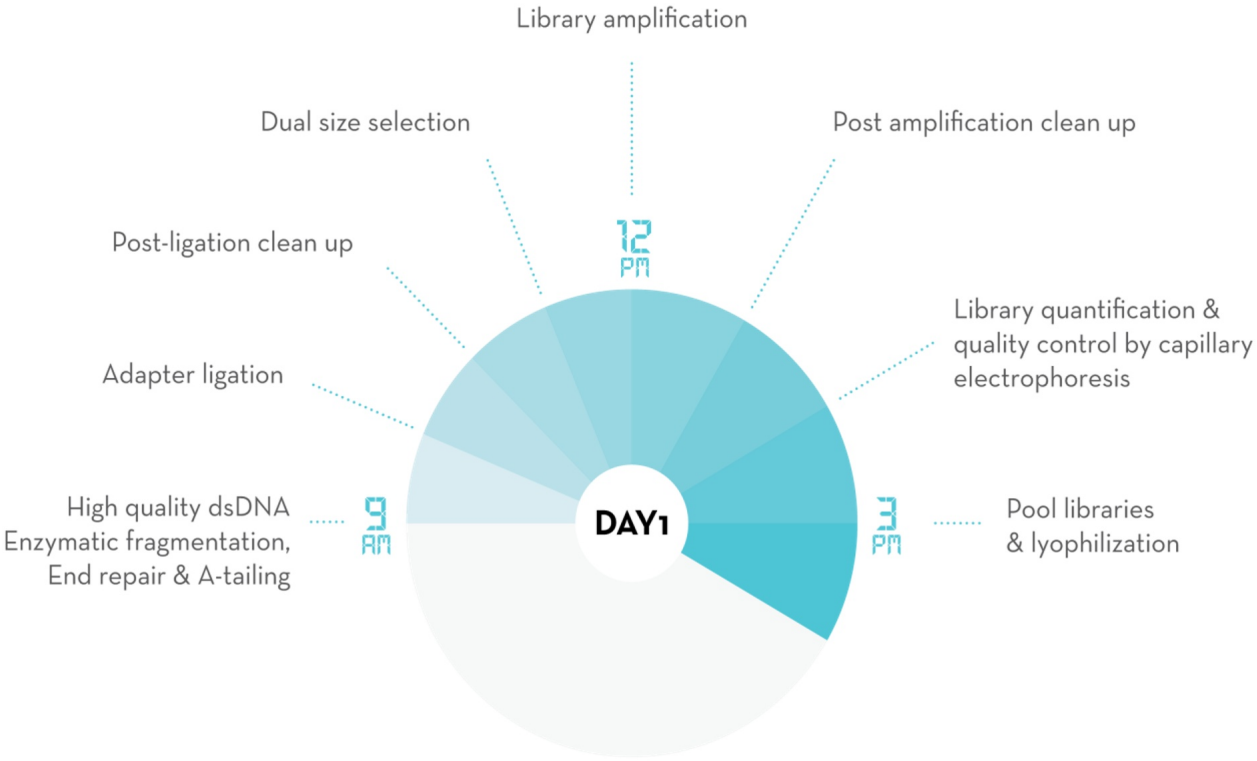
APPENDIX 3. GENERAL WORKFLOW – SOPHiA DDM™ CAPTURE SOLUTIONS



Library Preparation
With SOPHiA GENETICS™ DNA Library Prep Kit I

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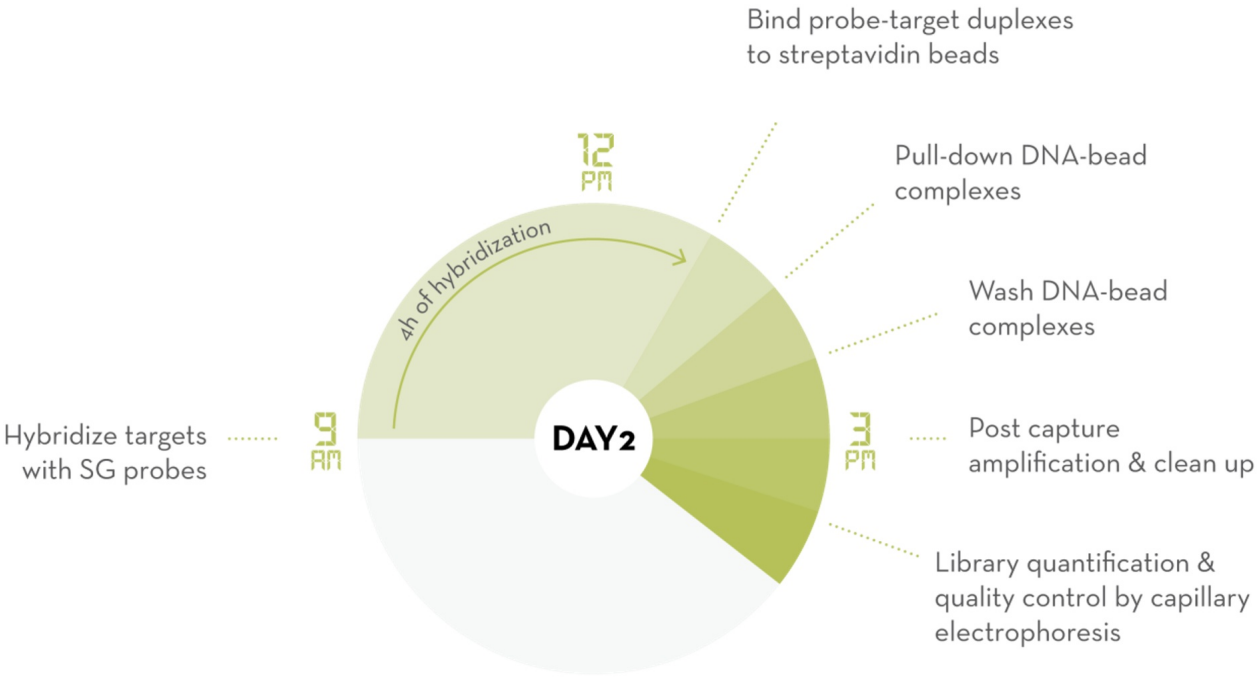

CAPTURE
EASY WORKFLOW
- ONLY 1-4 TUBES TO HANDLE (MULTIPLEX POOLED LIBRARIES)
- ONLY 3 HOURS HANDS-ON TIME

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

TARGET REGIONS*

CHROMOSOME	START	END
1	36931671	36931826
1	36931932	36932534
1	36932805	36932937
1	36933133	36933277
1	36933397	36933588
1	36933650	36933847
1	36934731	36934883
1	36935227	36935466
1	36937008	36937272
1	36937641	36937765
1	36937813	36938017
1	36938092	36938312
1	36939010	36939248
1	36939339	36939513
1	36940952	36941299
1	36945008	36945122
1	43814908	43815055
1	115256395	115256624
1	115258645	115258806
2	25457122	25457314
2	25458550	25458719
2	25459779	25459899
2	25461973	25462109
2	25463145	25463344
2	25463483	25463624
2	25464405	25464601

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CHROMOSOME	START	END
2	25466741	25466876
2	25466998	25467232
2	25467383	25467546
2	25468096	25468226
2	25468863	25468958
2	25469003	25469203
2	25469463	25469670
2	25469894	25470052
2	25470434	25470643
2	25470880	25471146
2	25472500	25472618
2	25475037	25475091
2	25497784	25497981
2	25498343	25498437
2	25505231	25505605
2	25522982	25523137
2	25536756	25536878
2	198266440	198266637
2	198266683	198266879
2	198267254	198267575
2	198267647	198267784
2	198268283	198268513
2	198269774	198269926
2	198269973	198270221
2	209113067	209113409
4	55561652	55561972
4	55589724	55589889
4	55591997	55592241
4	55593358	55593515
4	55593556	55593733

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CHROMOSOME	START	END
4	55594151	55594312
4	55599210	55599383
4	55602638	55602800
4	106155074	106158622
4	106162470	106162611
4	106163965	106164109
4	106164701	106164960
4	106180750	106180951
4	106182890	106183030
4	106190741	106190929
4	106193695	106194100
4	106196179	106197701
5	170834678	170834803
5	170837505	170837594
7	140453049	140453218
7	148504712	148504823
7	148506137	148506272
7	148506376	148506507
7	148507399	148507531
7	148508691	148508837
7	148511025	148511254
7	148511980	148512156
7	148512572	148512663
7	148513750	148513895
7	148514288	148514508
7	148514943	148515234
7	148516662	148516804
7	148523520	148523749
7	148524230	148524383
7	148525806	148525997

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CHROMOSOME	START	END
7	148526794	148526965
7	148529700	148529867
7	148543536	148543715
7	148544248	148544415
9	5021962	5022238
9	5029757	5029931
9	5044377	5044545
9	5050660	5050856
9	5054537	5054909
9	5055643	5055813
9	5064857	5065065
9	5066652	5066814
9	5068996	5069233
9	5069899	5070077
9	5072466	5072651
9	5073672	5073810
9	5077427	5077605
9	5078280	5078469
9	5080203	5080405
9	5080507	5080708
9	5081699	5081886
9	5089648	5089888
9	5090420	5090595
9	5090713	5090936
9	5122978	5123146
9	5126307	5126471
9	5126658	5126816
9	133738124	133738447
9	133747490	133747625
9	133748221	133748449

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CHROMOSOME	START	END
9	133750229	133750464
9	133753776	133753979
9	133755429	133755569
11	533740	533969
11	534186	534347
11	32410578	32410750
11	32413492	32413635
11	32414186	32414326
11	32417777	32417978
11	32421468	32421615
11	119148850	119149032
11	119149194	119149448
12	11803036	11803119
12	11905358	11905538
12	11992048	11992263
12	12006335	12006520
12	12022332	12022928
12	12037353	12037546
12	12038834	12038985
12	12043849	12044005
12	25380142	25380371
12	25398182	25398343
12	112888096	112888341
12	112910722	112910869
12	112915429	112915559
12	112915635	112915844
12	112919852	112920034
12	112924253	112924458
12	112926221	112926339
12	112926802	112927004

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CHROMOSOME	START	END
13	28592578	28592751
13	28607998	28608153
13	28608193	28608376
13	28608412	28608569
15	90631793	90632004
17	7572901	7573033
17	7573901	7574058
17	7576511	7576682
17	7576827	7576951
17	7576993	7577180
17	7577473	7577633
17	7578151	7578314
17	7578345	7578579
17	7579286	7579615
17	7579674	7579746
17	7579813	7579937
17	74732855	74733267
18	42529820	42533330
19	13054501	13054752
19	33792218	33793345
20	31019360	31019507
20	31021061	31021745
20	31022209	31025166
21	36164406	36164932
21	36171572	36171784
21	36193939	36194018
21	36206681	36206923
21	36231745	36231900
21	36252828	36253035
21	36259114	36259434

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CHROMOSOME	START	END
21	36265196	36265285
21	36421113	36421221
21	44514739	44514923
21	44524399	44524537
X	15808593	15808684
X	15809031	15809161
X	15817969	15818101
X	15821785	15821944
X	15822208	15822345
X	15826330	15826419
X	15827297	15827466
X	15833774	15834038
X	15836684	15836790
X	15838304	15838464
X	15840828	15841390

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

APPLICABLE FLAGGED REGIONS**

CHROMOSOME	START	END	DESCRIPTION	GENE_ EXON
1	36933564	36933584	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	CSFR3_ ex14
17	7572977	7573010	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	TP53_ ex11
18	42533111	42533125	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	SETBP1_ ex04
21	36206686	36206787	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	RUNX1_ ex07
			Variants in this region with a variant fraction below	SF3B1_

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CHROMOSOME	START	END	DESCRIPTION	GENE_ EXON
2	198267762	198267783	10% may be confounded by experimental artefacts	ex13
5	170837510	170837526	Variants in this region with a variant fraction below 10% may be confounded by experimental artefacts	NPM1_ ex11
9	5126453	5126462	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	JAK2_ ex24
11	119149355	119149373	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	CBL_ ex09
19	33792754	33792775	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	CEBPA_ ex01
19	33793007	33793041	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	CEBPA_ ex01
20	31022441	31022449	Variants in this region with a variant fraction below 10% may be confounded by sequencing artefacts if Illumina® V2 chemistry is used	ASXL1_ ex12
7	148543693	148543705	Variants in this region with a variant fraction below 10% may be confounded by experimental artefacts	EZH2_ ex41
17	74733013	74733013	Variants in this region with a variant fraction below 10% may be confounded by experimental artefacts	SRSF2_ ex1
20	31022262	31022262	Variants in this region with a variant fraction below 10% may be confounded by experimental artefacts that cause chimeric reads	ASXL1_ ex12
18	42531410	42531410	Variants in this region with a variant fraction below 10% may be confounded by experimental artefacts that cause chimeric reads	SETBP1_ ex04
12	112919878	112920009	This region is highly homologous	PTPN11_ ex10

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

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Document Approvals

Approved Date: 06 Mar 2023

Approval Verdict: Approve	Denisa Dzulova, (ddzulova@sophiagenetics.com) Technical Approval 06-Mar-2023 10:04:37 GMT+0000

Approval Verdict: Approve	Dawn Little, (DLittle@sophiagenetics.com) Regulatory Approval 06-Mar-2023 10:15:53 GMT+0000

QA Approval Verdict: Approve	Kateryna Pyshcholka, (kpyshcholka@sophiagenetics.com) Quality Assurance Approval 06-Mar-2023 10:16:30 GMT+0000

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