INSTRUCTIONS FOR USE

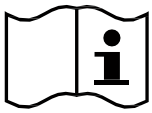
16, 32, 48 and 96 Samples

SOPHiA GENETICS™ Dx Universal Library Prep

SOPHiA GENETICS™

SUMMARY INFORMATION

Product Name	SOPHiA GENETICS™ Dx Universal Library Prep
Product Type	Library Preparation Kit
Product Family	Molecular kit
Product Version	v1.0
Sample Type	Genomic DNA (gDNA) isolated from blood, bone marrow, fresh frozen (FF) tissue, formalin-fixed paraffin-embedded (FFPE) tissue
Document ID	SG-08540
Document Version	v3.0
Revision Date	January 2026



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

BX6002UNICMDL30-16
BX6002UNICMDL30-32
BX6002UNICMDL30-48
BX6002UNICMDL30-96
BX6002UNICMDL31-16
BX6002UNICMDL31-32
BX6002UNICMDL31-48
BX6002UNICMDL31-96

For in vitro diagnostic use

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PRODUCT CODES

Product codes for SOPHiA GENETICS™ Dx Universal Library Prep – Stubby Adapters configuration

FULL PRODUCT CODE	PRODUCT VERSION
BX6002UNICMDL30-16	SOPHiA GENETICS™ Dx Universal Library Prep Stubby - Y30 - 16rx
BX6002UNICMDL30-32	SOPHiA GENETICS™ Dx Universal Library Prep Stubby - Y30 - 32rx
BX6002UNICMDL30-48	SOPHiA GENETICS™ Dx Universal Library Prep Stubby - Y30 - 48rx
BX6002UNICMDL30-96	SOPHiA GENETICS™ Dx Universal Library Prep Stubby - Y30 - 96rx

Product codes for SOPHiA GENETICS™ Dx Universal Library Prep – CUMIN™ Adapters configuration

FULL PRODUCT CODE	PRODUCT VERSION
BX6002UNICMDL31-16	SOPHiA GENETICS™ Dx Universal Library Prep CUMIN - Y31 - 16rx
BX6002UNICMDL31-32	SOPHiA GENETICS™ Dx Universal Library Prep CUMIN - Y31 - 32rx
BX6002UNICMDL31-48	SOPHiA GENETICS™ Dx Universal Library Prep CUMIN - Y31 - 48rx
BX6002UNICMDL31-96	SOPHiA GENETICS™ Dx Universal Library Prep CUMIN - Y31 - 96rx

COMPONENT ID	COMPONENT NAME
LIBRARY PREPARATION KIT BOX	SOPHiA GENETICS™ Dx Modular Universal Library Prep
BOX 1	SOPHiA GENETICS™ Dx Modular Library Prep - Box1
BOX 2	SOPHiA GENETICS™ Dx Modular Library Prep - Box2
CUMIN BOX*	SOPHiA GENETICS™ Dx Modular CUMIN Adapters

*Component provided only with CUMIN™ Adapters configurations

Component codes for SOPHiA GENETICS™ Dx Universal Library Prep – Stubby Adapters configuration

FULL PRODUCT CODE	LIBRARY PREPARATION KIT BOX	BOX 1	BOX 2
BX6002UNICMDL30-16	4C0232	B1.G1.6002.C-16	B2.6002.C-16
BX6002UNICMDL30-32	2 x 4C0232	B1.G1.6002.C-32	B2.6002.C-32
BX6002UNICMDL30-48	4C0234	B1.G1.6002.C-48	B2.6002.C-48
BX6002UNICMDL30-96	2 x 4C0234	B1.G1.6002.C-96	2 x B2.6002.C-48

Component codes for SOPHiA GENETICS™ Dx Universal Library Prep – CUMIN™ Adapters configuration

FULL PRODUCT CODE	LIBRARY PREPARATION KIT BOX	BOX 1	BOX 2	CUMIN BOX
BX6002UNICMDL31-16	4C0232	B1.F1.6002.C-96	B2.6002.C-16	905C1648
BX6002UNICMDL31-32	2 x 4C0232	B1.F1.6002.C-96	B2.6002.C-32	905C1648
BX6002UNICMDL31-48	4C0234	B1.F1.6002.C-96	B2.6002.C-48	905C1648
BX6002UNICMDL31-96	2 x 4C0234	B1.F1.6002.C-96	2 x B2.6002.C-48	2 x 905C1648

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REVISION HISTORY

DOCUMENT ID / VERSION	DATE	DESCRIPTION OF CHANGE
SG-08540 v3.0	January 2026	• Change to version numbering system, no additional changes between 1.1 and 3.0.
SG-08540 v1.1	January 2026	• Modification of Authorised Representative and Manufacturer address
SG-08540 v1.0	December 2025	• Initial release

Table Of Contents

1. Intended Purpose

SOPHiA GENETICS™ Dx Universal Library Prep is a reagent kit used for preparation of Next-Generation Sequencing (NGS) libraries from genomic DNA isolated from human cells and tissue. The generated libraries can be used for downstream sequencing platforms.

SOPHiA GENETICS™ Dx Universal Library Prep is non-automated reagent set intended to be used as part of user developed in vitro diagnostic examination by trained health care professionals.

2. General Statement Of The Test Principles And Procedure

SOPHiA GENETICS™ Dx Universal Library Prep contains all enzymes, associated buffers as well as index plates and ligation adapters necessary to perform the user workflow from extracted DNA to a non-enriched whole genome library suitable for enrichment and sequencing (**Figure 1**).

The SOPHiA GENETICS™ Dx Universal Library Prep product allows to perform the following steps:

1. Fragmentation, end-repair, and A-tailing: enzymatic fragmentation of DNA and preparation of DNA ends for adapter ligation (Frag/ERAT in **Figure 1**).
2. Stubby Adapter or SOPHiA GENETICS™ CUMIN Adapters (abbreviated to CUMIN™ Adapters) ligation: ligation of Stubby Adapters or CUMIN™ Adapters to DNA fragments to prepare them for indexing and amplification (Ligation in **Figure 1**).
3. Indexing and amplification: amplification of adapter-ligated DNA fragments with barcoded PCR primers to prepare DNA for sequencing (PCR in **Figure 1**).

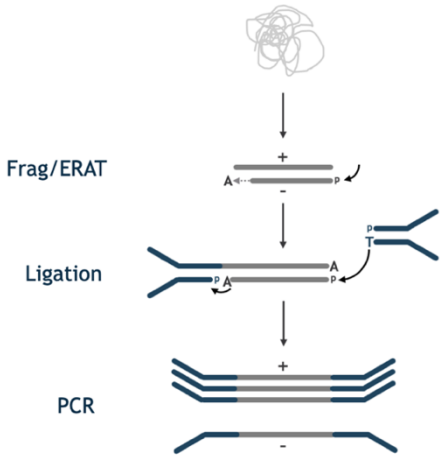


Figure 1: Workflow description for the SOPHiA GENETICS™ Dx Universal Library Prep.

3. Materials And Methods

3.1 Materials

3.1.1 Initial Considerations

Upon receipt, please ensure that all tubes are physically intact and stored at recommended temperatures for optimum kit performance. Inappropriate handling and storage of the kit components under other conditions may adversely affect the kit's performance. Contact manufacturer (see section 8 SUPPORT for details) if kit components arrived damaged or the dry ice was depleted.

The shipping package contains dry ice for cooling. Dry ice releases carbon dioxide gas. Ensure adequate ventilation during unpacking. Avoid direct contact with dry ice. Use protective equipment including lab coat, insulated gloves and eye protection when handling.

3.1.2 Kit Content (16, 32, 48 and 96 reactions)

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

Depending on the kit format (number of reactions intended to be covered by the kit) and version (kit configuration with STUBBY or CUMIN adapters), the following components are provided:

COMPONENT	NUMBER OF ITEMS DEPENDING ON KIT FORMAT			
	16 reactions	32 reactions	48 reactions	96 reactions
SOPHiA GENETICS™ Dx Modular Universal Library Prep	1 x 16 reactions box	2 x 16 reactions box	1 x 48 reactions box	2 x 48 reactions box
*Stubby Adapters configuration:** SOPHiA GENETICS™ Dx Modular Library Prep – Box 1	1 x 16 reactions box	1 x 32 reactions box	1 x 48 reactions box	1 x 96 reactions box
CUMIN™ Adapters configuration: SOPHiA GENETICS™ Dx Modular Library Prep – Box 1	1 x 96 reactions box	1 x 96 reactions box	1 x 96 reactions box	1 x 96 reactions box
SOPHiA GENETICS™ Dx Modular Library Prep – Box 2	1 x 16 reactions box	1 x 32 reactions box	1 x 48 reactions box	2 x 48 reactions box
CUMIN™ Adapters configuration only: SOPHiA GENETICS™ Dx Modular CUMIN Adapters	1 x 48 reactions box	1 x 48 reactions box	1 x 48 reactions box	2 x 48 reactions box

* SOPHiA GENETICS™ Dx Modular Library Prep – Box 1 in Stubby Adapters configuration contains 96A Unique Dual Index Primer Plate that support 96 reactions

SOPHiA GENETICS™ Dx Modular Universal Library Prep (Store at -25 °C to -15 °C)*

REAGENT (CAP COLOR)	KIT FORMAT				ACTIVE INGREDIENTS
	16 reactions	32 reactions*	48 reactions	96 reactions*	
PCR Master Mix 2x (white cap)	520 µl	2 x 520 µl	1560 µl	2 x 1560 µl	The Master Mix includes DNA polymerase, reaction buffer, dNTPs and MgCl2
Fragmentation Buffer (brown cap)	77 µl	2 x 77 µl	231 µl	2 x 231 µl	Buffered aqueous solution containing Tetramethyl-ammonium chloride
Fragmentation Enzyme Mix (brown cap)	116 µl	2 x 116 µl	346 µl	2 x 346 µl	Mixture of fragmentase enzymes and buffering agents
Ligation Enzyme (purple cap)	192 µl	2 x 192 µl	576 µl	2 x 576 µl	Mixture of ligation enzymes and buffering agents
Ligation Buffer 5x (purple cap)	384 µl	2 x 384 µl	1153 µl	2 x 1153 µl	Pre-mixed buffer for DNA ligation reaction

* For 32 reactions kit format (32rx), two 16 reactions boxes are provided.
* For 96 reactions kit format (96rx), two 48-reactions boxes are provided.

Refer to 3.1.3 Hazardous Substance section for details on hazardous substances included in the reagents.

SOPHiA GENETICS™ Dx Modular Library Prep – Box 1 (Store at +2 °C to +8 °C)

SOPHiA GENETICS™ Dx Universal Library Prep – Stubby Adapters configuration:

REAGENT (CAP COLOR)	KIT FORMAT				ACTIVE INGREDIENTS
	16 reactions	32 reactions	48 reactions	96 reactions	
SU-Adapter (blue cap)	110 µl	220 µl	320 µl	650 µl	Stubby Universal adapters - Oligonucleotide pool
96A Unique Dual Index Primer Plate [Plate A]	1x96-well plate format (7 µl of primer per well)				Oligonucleotide pool, please refer to Appendix I for information on plate layouts and primer sequences

SOPHiA GENETICS™ Dx Universal Library Prep – CUMIN Adapters configuration:

REAGENT*	KIT FORMAT				ACTIVE INGREDIENTS
	16 reactions	32 reactions	48 reactions	96 reactions	
96A Unique Dual Index Primer Plate [Plate A]	1x96-well plate format (7 µl of primer per well)				Oligonucleotide pool, please refer to Appendix I for information on plate layouts and primer sequences

*In case of configuration with CUMIN™ Adapters the adapters are provided in separate box

SOPHiA GENETICS™ Dx Modular Library Prep – Box 2 (Store at +2 °C to +8 °C)

REAGENT (CAP COLOR)	KIT FORMAT				ACTIVE INGREDIENTS
	16 reactions	32 reactions	48 reactions	96 reactions*	
Agencourt® AMPure® XP (transparent cap)	3 x 1.5 ml	7.2 ml	9.2 ml	2 x 9.2 ml	Paramagnetic beads suspended in a buffered solution
IDTE Buffer pH 8.0 (transparent cap)	10 ml	10 ml	10 ml	2 x 10 ml	10 mM Tris-HCl, 0.1 mM EDTA solution
Nuclease Free Water (transparent cap)	20 ml	20 ml	20 ml	2 x 20 ml	Nuclease-free water

* For 96 reactions kit format (96rx), two 48-reactions kits are provided.

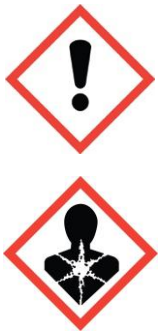
SOPHiA GENETICS™ Dx Modular CUMIN Adapters (Store at -25 °C to -15 °C)

SOPHiA GENETICS™ Dx Universal Library Prep – CUMIN Adapters configuration:

REAGENT (CAP COLOR)	KIT FORMAT				ACTIVE INGREDIENTS
	16 reactions	32 reactions	48 reactions	96 reactions	
CUMIN family "A" (orange cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool
CUMIN family "B" (pink cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool
CUMIN family "C" (red cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool
CUMIN family "D" (yellow cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool
CUMIN family "E" (brown cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool
CUMIN family "F" (green cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool
CUMIN family "G" (blue cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool
CUMIN family "H" (purple cap)	36 µl	36 µl	36 µl	2x36 µl	Oligonucleotide pool

3.1.3 Hazardous substances

Safety data sheet available on request and online on the following website: www.sophiagenetics.com/docs/

REAGENT NAME	GHS PICTOGRAM	H&P STATEMENTS	SIGNAL WORD	HAZARDOUS SUBSTANCE
Fragmentation Buffer and PCR Master Mix 2x		H302 Harmful if swallowed. H370 Causes damage to organs. H412 Harmful to aquatic life with long lasting effects. P273 Avoid release to the environment. P260 Do not breathe vapor. P270 Do not eat, drink or smoke when using this product. P264 Wash thoroughly after handling. P308 + P311 IF exposed: Call a POISON CENTER or doctor. P301 + P312, P330 IF SWALLOWED: Call a POISON CENTER or doctor if you feel unwell. Rinse mouth. P405 Store locked up. P501 Dispose of contents and container in accordance with all local, regional, national and international regulations.	Warning	Tetramethyl-ammonium chloride [CAS 75-57-0] 1-3%

Wear protective equipment, including eye protection, gloves, and laboratory coat to reduce risk of exposure

3.1.4 Materials Required (Not Provided)

User-Supplied Materials (To Be Purchased Separately)

Lab-related plasticware

- DNA low binding 1.5 ml tubes
- 50 ml conical tubes
- Filter tips
- 1.5 ml tubes
- RNase/DNase-free 0.2 ml 8-tube strips

Reagents

- Ethanol (molecular biology grade)

Other

- Ice to ensure reagents and pre-mixes are kept at 4 °C
- Illumina® sequencing reagents and flowcells

Laboratory Equipment

To avoid sample contamination, separate the workspace into:

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

3.2 Sample recommendation

3.2.1 Specimen collection and storage

Follow the specimen collection, transport, and storage requirements applicable to the specimen type and DNA extraction method of choice. Any validated extraction method that allows to obtain DNA samples matching requirements indicated in input recommendation can be used. Purified DNA should be transported and stored under appropriate conditions according to the recommendations provided by DNA extraction kit manufacturer. Avoid repeated freeze–thaw cycles.

3.2.2 DNA Input Recommendation

DNA used as input of SOPHiA GENETICS™ Dx Universal Library Prep must be qualified prior to use. DNA integrity, concentration, and purity are critical factors affecting assay performance. Use any validated nucleic acid extraction method that ensures efficient DNA recovery and maintains sample quality.

INPUT QUALITY RECOMMENDATIONS

FRESH-FROZEN TISSUE, BLOOD, OR BONE MARROW EXTRACTED DNA: The purity of the DNA can be assessed using a UV spectrophotometer. Recommended absorbance ratios are between 1.8–2.0 for the 260/280 nm ratio and within 1.6–2.4 for 260/230. Absorbance ratios outside of the above-mentioned ranges may result in library preparation failure or reduced performance.

FFPE TISSUE EXTRACTED DNA: The quality of FFPE-extracted DNA is variable and may negatively affect assay performance. Exposure to formalin damages the integrity of the molecule by generating DNA fragmentation. It also induces sequencing artifacts due to deamination events. We recommend confirmation of the sample integrity by capillary electrophoresis or an equivalent technique.

DNA quality assessment: Run 5–10 ng of genomic DNA on the Agilent Fragment Analyzer™ System using the HS large fragment 50 kb kit (DNF-464- 0500) or equivalent. The quality of genomic DNA is evaluated with the DNA quality number (DQN). Set the threshold for the DQN to 375 bp and extract the DQN for your DNA samples. Processing a sample without measuring DQN or a DQN less than 3 might lead to library preparation failure. A DQN higher or equal to 3 cannot exclude the presence of other quality issues in the DNA sample.

INPUT AMOUNT RECOMMENDATIONS

We recommend 50 ng DNA input material, but the protocol can be used over a range from 10 ng to 100 ng input material by adjusting the number of PCR cycles according to the following table:

CORRELATION BETWEEN DNA INPUT AND REQUIRED PCR CYCLES (SECTION 3.3.6 LIBRARY AMPLIFICATION)			
Amount of DNA (ng)	10 ≤ n < 50	50	50 < n ≤ 100
PCR cycles	10	8	8

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

3.3 Library Preparation

3.3.1 Input Material Preparation

Materials

- Genomic DNA (gDNA) extracted from blood, bone marrow, fresh frozen (FF) tissue, formalin-fixed paraffin-embedded tissue (FFPE DNA)
- IDTE Buffer pH 8.0
- Nuclease Free Water
- RNase/DNase-free 0.2 ml 8-tube strips

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 type	**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 extracted DNA sample into the appropriate number of PCR strips, in the following manner:

DNA DILUTION	
DNA	Amount of DNA (see table of DNA input recommendations above)
IDTE Buffer pH 8.0	Complete to 40 µl

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

Safe stopping point overnight at 4 °C.

3.3.2 Pre-Mixes and Reagents Preparation

Components And Reagents

- Fragmentation Enzyme Mix
- Fragmentation Buffer
- Ligation Enzyme
- Ligation Buffer 5x
- SU-Adapter - Stubby Adapters configuration
- SOPHiA GENETICS™ CUMIN Adapters (refered to as CUMIN™ Adapters) - CUMIN Adapters configuration
- 96A Unique Dual Index Primer Plate in 96-well plate format
- PCR Master Mix 2x
- Nuclease Free Water
- Agencourt® AMPure® XP beads
- Ethanol

Preparation

1. Remove the SOPHiA GENETICS™ Dx Universal Library Prep components from -20 °C storage and thaw on ice.
2. Remove the 96A Unique Dual Index Primer Plate from -20 °C storage and place in 4 °C refrigerator for later use.
3. Remove the Agencourt® AMPure® XP beads from 2–8 °C storage and let them equilibrate at room temperature for at least 30 minutes. Vortex the Agencourt® AMPure® XP beads thoroughly, to ensure proper resuspension of the beads, prior to use in all subsequent steps.
4. Prepare fresh 80% Ethanol (volume according to the following scheme based on number of reactions):

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

5. Ensure that the Fragmentation Buffer from the SOPHiA GENETICS™ Dx Modular Universal Library Prep component is completely thawed.
6. Once the SOPHiA GENETICS™ Dx Universal Library Prep components are thawed, mix the reagents by inverting the tube 5–10 times to ensure that there is no remaining precipitate and briefly spin in a microcentrifuge. Place on ice.

Pre-Mixes

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

FRAGMENTATION PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
Fragmentation Buffer (µl)	19.2	38.4	57.6	76.8	115.2	153.6	230.4
Fragmentation Enzyme Mix (µl)	28.8	57.6	86.4	115.2	172.8	230.4	345.6

- 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**
Ligation Buffer 5x (µl)	96	192	288	384	576	768	1152
Ligation Enzyme (µl)	48	96	144	192	288	384	576
Nuclease Free Water (µl)	72	144	216	288	432	576	864

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

The Ligation Buffer 5x is highly viscous, pipette gently and make sure to obtain a homogeneous Ligation pre-mix.

3.3.3 Enzymatic Fragmentation, End Repair and A-Tailing

Materials

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

Preparation

1. Program the thermal cycler for Fragmentation with the following settings:

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

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

Procedure

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

1. 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 Fragmentation pre-mix by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip type	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Fragmentation pre-mix (µl)	23	35	23	35	46	70

2. Assemble the reaction as follows:
    - Using a multichannel pipette add 10 µl of Fragmentation pre-mix to each of the 40 µl of DNA samples (total of 50 µl in 4 or 8-tube strips).
    - Using a multichannel pipette set to 35 µl, mix thoroughly by pipetting up and down 5 times and briefly spin in a microcentrifuge.
3. Place in the thermal cycler and continue the Fragmentation program.
4. Proceed immediately to Ligation.

3.3.4 Ligation

Materials

- Fragmentation reaction products in 50 µl each
- Ligation pre-mix
- SOPHiA GENETICS™ CUMIN Adapters *OR* SU-Adapter (Stubby Adapters)
- RNase/DNase-free 0.2 ml 8-tube strips

SOPHiA GENETICS™ CUMIN Dx Adapters Recommendations

CUMIN™ Adapters allow for molecular barcoding of DNA input molecules. They (or alternatively the Stubby Adapters) are to be used during the ligation step of the SOPHiA GENETICS™ Dx Universal Library Prep. The SOPHiA GENETICS™ Dx Modular CUMIN Adapters box contains 8 adapter families in individual tubes. These families have to be used in a library-specific fashion, i.e. only one family of CUMIN™ Adapters must be ligated to each sample. The schematic in Figure 1 below illustrates the recommended usage of CUMIN™ Adapters.

Figure 2: Schematic illustrating the strategy of CUMIN™ Adapters usage depending on the number of reactions required for the 48 reactions kit. Pink squares represent the CUMIN families which are recommended depending on the number of samples being processed.

When using CUMIN™ Adapters in a bundle solution product, sample-specific statistics will include the percentage of reads supporting each CUMIN™ family.

It is essential that within one sequencing run, all 8 CUMIN™ families are present at least once in the library pool. Deviations from this will lead to a failure of the sequencing run. Processing of lower sample numbers with CUMIN™ Adapters requires addition of 20% PhiX library to the sequencing run to ensure nucleotide diversity required for successful cluster formation. Thus, when setting up 12, 24 or 48 library preps, equal use of CUMIN™ Adapters across experiments must be considered to avoid depletion of the individual stocks. Cluster density yield and base quality were verified with a NextSeq® 2000 sequencer. Other sequencers were tested successfully during product development (see 4.2 Limitation section).

Preparation

1. During the Frag/ERAT reaction, prepare new PCR strips with 5 µl of Stubby Adapters OR CUMIN™ Adapters per tube according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip type	**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. Set up the thermal cycler at 20 °C (open lid).

Procedure

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

1. 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 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 type	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**2 x 8-tube**
Ligation pre-mix (µl)	100	150	100	150	200	150

2. Using a multichannel pipette, transfer the 50 µl of each Fragmentation reaction product to the 4 or 8-tube strips containing 5 µl of Stubby Adapters OR CUMIN™ Adapters according to Figure 2.
3. Mix thoroughly by pipetting up and down 10 times and spin briefly.
4. Using a multichannel pipette, add 45 µl Ligation pre-mix to each Fragmentation reaction product (55 µl in each tube of the 4 or 8-tube strip).
5. Mix thoroughly by pipetting up and down 10 times and spin briefly.
6. Incubate in the thermal cycler at 20 °C for 15 minutes (open lid).

Proceed to Post-Ligation Clean Up.

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

3.3.5 Post-Ligation Clean Up

Materials

- Ligation reaction products in 100 µl each
- Agencourt® AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- IDTE Buffer pH 8.0
- RNase/DNase-free 0.2 ml 8-tube strips

Procedure

1. Using a multichannel pipette, add 80 µl of Agencourt® AMPure® XP beads to each of the 100 µl ligation reaction products. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 170 µl of supernatant using a multichannel pipette.

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

   Remove the tubes from the magnetic rack.
10. Using a multichannel pipette, add 20 µl of IDTE Buffer pH 8.0 to the beads. Mix thoroughly by pipetting up and down 10 times and spin briefly.
11. Proceed to Library Amplification.

3.3.6 Library Amplification

Materials

- Ligation reaction products and beads resuspended in 20 µl IDTE Buffer pH 8.0 each
- PCR Master Mix 2x
- 96A Unique Dual Index Primer Plate

Preparation

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

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

* Follow the table in section 3.2.2 DNA Input Recommendation to determine the number of PCR cycles based on the amount of starting material.

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 PCR Master Mix 2x by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip type	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
PCR Master Mix 2x (µl)	60	85	60	85	120	170

2. Assemble the reaction as follows:
   - Using a multichannel pipette, add 5 µl of different Unique Dual Index Primer per tube to the ligation products and beads, according to your indexing strategy.
   - Mix thoroughly by pipetting up and down 10 times and spin briefly.
   - Using a multichannel pipette, add 25 µl of PCR Master Mix 2x to the ligation products and beads (50 µl in 4 or 8-tube strips).
   - Mix thoroughly by pipetting up and down 10 times and spin briefly.
3. Place the tubes in the thermal cycler and run the Library Amplification program.

Safe stopping point overnight at 4 °C.

3.3.7 Post-Amplification Clean Up

Materials

- PCR reaction products in 50 µl each
- Agencourt® 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 Agencourt® AMPure® XP beads to each 50 µl of the PCR product. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 90 µl supernatant using a multichannel pipette.

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

   Remove the tubes from the magnetic rack.
10. Using a multichannel pipette, add 30 µl of Nuclease Free Water to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin briefly if required.
11. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until liquid becomes clear.
12. Carefully transfer 28 µl of the supernatant to a new and labelled library storage tube.

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

3.3.8 Individual Library Quantification and Quality Control

Materials

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

Procedure

1. Prepare a 4-time dilution of each library with Nuclease Free Water (e.g., 2 µl of library in 6 µl Nuclease Free Water).
2. Quantify the libraries with a fluorometric method (e.g., Qubit HS quantification using 2 µl of the 4x library dilution prepared previously).
3. Quality control the libraries by analysing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 200 bp and 800 bp.

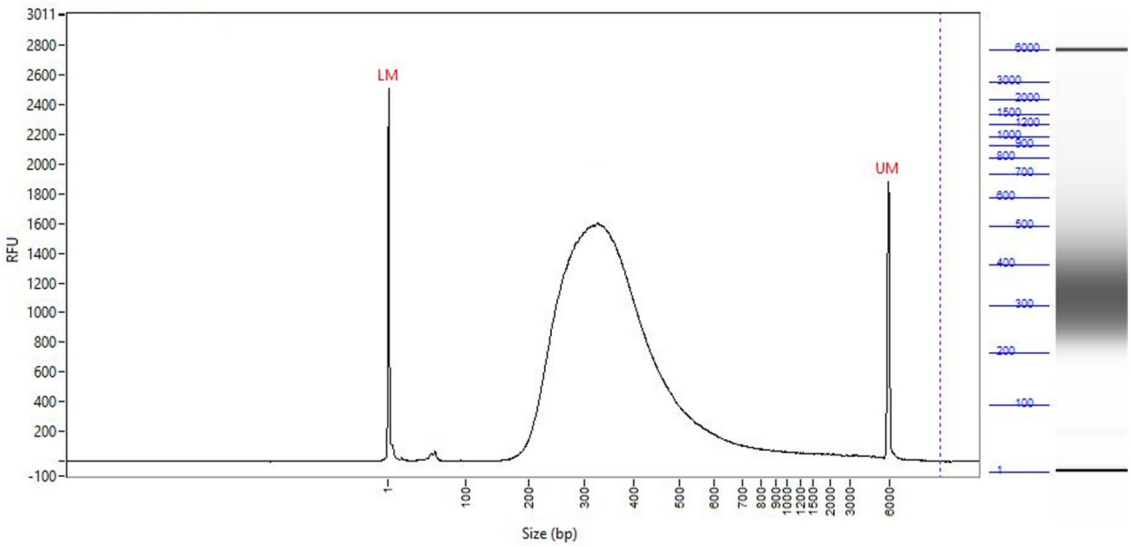


Figure 3. Example of a DNA library distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system for **fresh frozen blood samples**. LM: Lower Marker, UM: Upper Marker.

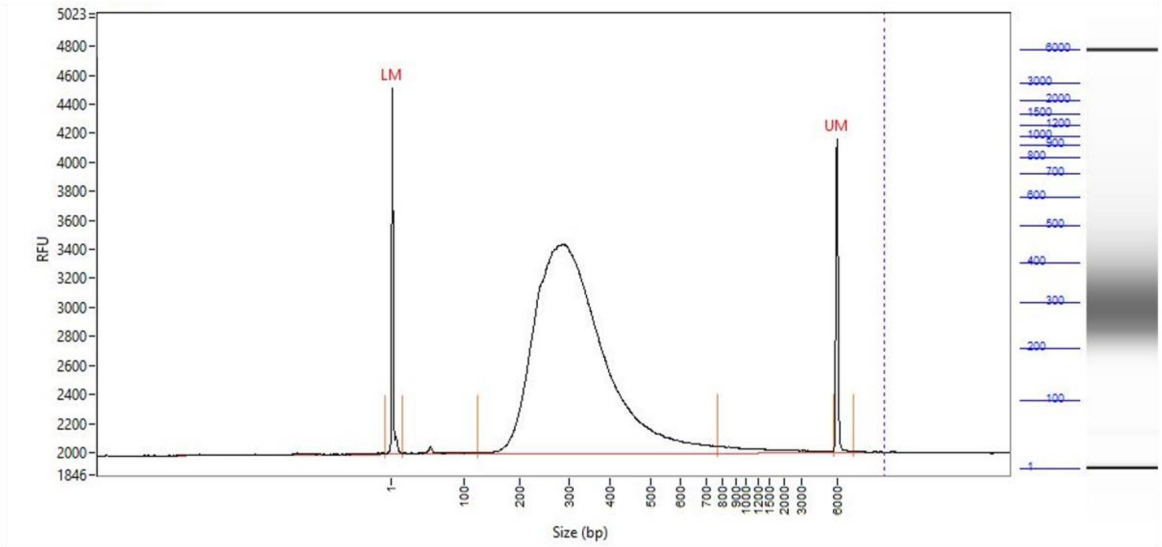


Figure 4. Example of a DNA library distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system for **FFPE samples**. LM: Lower Marker, UM: Upper Marker.

3.4 Sequencing

3.4.1 Sequencing Preparations

Materials

- Illumina® sequencing reagents and flow cell
- Final libraries
- IDTE Buffer pH 8.0 or similar

Procedure

1. Determine the molarity of each library pool with the average size of the library (peak size in base pairs) and concentration (ng/µl) obtained during step 3.3.8 Individual Library Quantification and Quality Control as follows:

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

2. Dilute each pool to 4 nM.
3. If processing multiple sequencing pools, mix them in equal amounts (e.g., 5 µl of each) and use this dilution according to Illumina® standard denaturation recommendations.
4. Load an X pM dilution* of the denatured libraries**.

* For dilution details, please see section 9.1.3 Loading Dilutions For Sequencers.
** Loading concentration and library dilution depend on the sequencing instrument used. Please see Appendix IV for details.

4. Warnings, Limitations, And Precautions

4.1 Warnings

1. All diagnostic procedures developed for use with SOPHiA GENETICS™ Dx Universal Library Prep must undergo full validation covering all performance aspects. The validation should include the chosen DNA extraction kit, any additional reagents, equipment, the selected sequencing platform and analysis software.
2. Devices for high-resolution gel electrophoresis other than the Agilent Fragment Analyzer™ system (e.g., Agilent TapeStation) were observed to lead to biased estimation of DQN on FFPE DNA samples.
3. Users must verify that the quality of their DNA samples meets the minimum criteria for qualification, as listed in Section 3.2.2 DNA Input Recommendation:
    - For FFPE samples, DQN larger or equal to 3 measured using the Agilent Fragment Analyzer™ system
    - For FF samples, absorbance ratios between 1.8–2.0 for the 260/280 nm ratio and between 1.6–2.4 for 260/230
4. A DQN larger or equal to 3 and absorbance ratios within the ranges mentioned in point 3 cannot exclude the presence of other quality issues in the DNA samples.
5. Processing of unqualified samples could lead to insufficient quality of generated sequencing libraries and erroneous results of diagnostic procedure.
6. Any serious incident that has occurred in relation to the SOPHiA GENETICS Dx Universal Library Prep shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

4.2 Limitations

1. Even if sample multiplexing recommendations are followed, the read coverage obtained in the region of interests may be insufficient for selected diagnostic use for various reasons, including: poor sample quality, poor NGS data quality, low quality or incompatible downstream reagents, significant uneven read allocation between samples multiplexed in the same run and skewed read depth allocation across assessed genomic regions (e.g. consistently lower read depth in AT rich regions).
2. The library preparation has been validated for DNA extracted from blood, bone marrow, fresh frozen and FFPE cell pellets, and FFPE tissue samples and sequenced on Illumina® MiSeq®, NextSeq® 550, NextSeq® 2000 and NovaSeq 6000™.
3. Sequencing of NGS libraries generated using SOPHiA GENETICS™ Dx Universal Library Prep should be performed using a 2×150 bp paired-end read configuration to ensure optimal data quality and assay performance.
4. The stability of frozen reagents was verified up to four freeze-thaw cycles

4.3 Precautions

1. Good laboratory practice standards and procedures, in addition to strictly following the IFU, are required for adequate performance of the product. Any deviation from instructions regarding sample handling, laboratory protocol, multiplexing and sequencing may negatively affect raw NGS data quality.
2. Physically separated pre- and post- PCR rooms should be used to prevent DNA sample contamination.
3. Correctly calibrated pipettes and proper lab equipment should be used to perform the experiment.

4. Use pipette tips with filters to reduce risk of contamination of samples and reagents.
5. Avoid microbial contamination of reagents when removing aliquots from reagent vials.
6. Do not mix different lot numbers of reagents.
7. Store and handle reagents according to instructions provided on the kit boxes and in this Instruction For Use. The reagents are stable when stored as indicated until the specified expiration date on the kit labels. Do not use reagents after expiration date.
8. Some reagents require safety precautions. See section hazardous substances section for details. For more specific safety related information, please refer to the corresponding Safety Data Sheets (SDS) for each component of the product available on the following website: www.sophiagenetics.com/docs/
9. Dispose reagents that contain potentially hazardous chemical substances in accordance with guidelines set by your institution as well as local and national regulations.
10. Use, storage, and disposal of kit components and samples must be carried out in accordance with national and local biohazard safety guidelines and regulations. Ensure compliance with all relevant safety procedures and regulatory requirements to protect personnel and the environment.
11. Always handle all human specimens, including whole blood, as potentially infectious to ensure safety.
12. Avoid touching any hot surfaces on heated instruments used during the procedure to prevent burns.
13. Store and use all flammable reagents like ethanol away from open flames and heat sources to avoid fire hazards.
14. Reagents are shipped with dry ice (solid carbon dioxide) to maintain low temperatures. Handle dry ice with care, always using insulated gloves and protective goggles to avoid frostbite upon contact. Do not touch dry ice with bare skin. Ensure proper ventilation when opening containers with dry ice. Do not inhale the gas released from dry ice. Store dry ice only in insulated containers that are not airtight to allow gas to escape safely.
15. Do not use components that are damaged. Please contact support (see details in section 8 Support) upon receiving damaged components.
16. The recommended EDTA concentration in DNA storage buffer is 0.1 mM. An excess of EDTA in sample storage buffers could impair sample processing.

5. Troubleshooting

Please refer to the following table to troubleshoot potential issues that may arise during the workflow.

OBSERVATION	POTENTIAL CAUSE	RECOMMENDED ACTION
Low library yield	Amount of material used lower than recommended Poor quality of DNA sample Reduced performance of the Fragmentation and ERAT reaction Reduced performance of ligation reaction Reduced performance of PCR reaction Inefficient bead clean-up	Use recommended amounts of DNA and follow guidelines for qualification Verify accuracy of DNA quantification methods Verify reagents have been stored and handled as per manufacturer's recommendations Resuspend the paramagnetic beads well before usage Use fresh ethanol Work on ice for the appropriate steps and use multichannel pipettes as indicated
Library size distribution outside of the targeted range (200 bp – 800 bp)	Poor quality of DNA sample DNA input used outside recommended range DNA and/or reagents were not prepared or maintained at 4 °C prior to usage Inadequate mixing of fragmentation reagents with the DNA sample	Follow guidelines for DNA qualification Keep diluted gDNA and reagents on ice until use Verify reagents are well thawed before usage Verify reagents have been stored and handled as per manufacturer's recommendations Work on ice for the appropriate steps and use multichannel pipettes as indicated
Presence of adapter dimers	Amount of DNA material lower than recommended Poor quality of DNA sample Inefficient bead clean-up	Use recommended amounts of DNA and follow guidelines for qualification Avoid resuspending ligation adapters with ligation pre-mix before mixing with Fragmentation reaction product Perform an extra clean-up step (note: this may reduce library yield)

6. Performance Characteristics

6.1.1 Specimen type

To verify that SOPHiA GENETICS™ Dx Universal Library Prep generates high quality sequencing libraries for all intended specimen types, average fragment size, library yield and coverage uniformity were measured on libraries generated using different specimen types. Libraries were prepared from DNA material extracted from blood or various Fresh Frozen tissue (68 specimens), FFPE tissue (83 specimens) and bone marrow tissue (12 specimens). For all sample types utilized, SOPHiA GENETICS™ Dx Universal Library Prep successfully generated libraries that satisfied the predefined acceptance thresholds.

6.1.2 Inter-laboratory reproducibility

Inter-laboratory reproducibility was evaluated using characterized reference samples of either germline or somatic origin processed using the SOPHiA GENETICS™ Dx Universal Library Prep kit by 6 and 3 independent sites respectively. Coverage uniformity and insert size were used as analytical readouts for this test. For both sample types utilized, SOPHiA GENETICS™ Dx Universal Library Prep satisfied the predefined acceptance thresholds.

6.1.3 Amount of input DNA

To verify the performance of SOPHiA GENETICS™ Dx Universal Library Prep when using the lowest recommended input amount, 24 samples were generated using 10 ng input material isolated from an FFPE reference sample. The preparation was performed by 2 operators at a single site and carried out on 2 different days to account for within-laboratory reproducibility. Library yield, size and coverage uniformity were used as analytical readouts for this test. All samples met the performance requirements. No reduction in performance was observed when using the recommended lowest DNA input amount.

6.1.4 Reproducibility using different batches of reagents

To verify the quality and robustness of SOPHiA GENETICS™ Dx Universal Library Prep when using different reagent batches, lot-to-lot reproducibility was assessed. Samples were processed with 5 different lots of library prep reagents. Library yield, size and coverage uniformity were used as analytical readouts for this test. All SOPHiA GENETICS™ Dx Universal Library Prep lots passed the requirements. Variations between lots were found to be below the predefined acceptable thresholds for all QC criteria.

6.1.5 Cross-sample contamination

To evaluate potential cross-contamination in the SOPHiA GENETICS™ Dx Universal Library Prep, female samples were processed in adjacent wells of a 8-tube strip as a baseline configuration. Two additional 8-tube strips, each containing a mix of male and female samples distributed with a checkerboard layout, were processed using the same method as test configurations. To ensure maximum stringency in contamination assessment, the highest recommended input for genomic DNA was used for each sample. Y-chromosome coverage in female samples from the test plates was compared to that of the baseline female-only plate, and a sample was considered passed if its Y-chromosome coverage was within mean * 3 standard deviations (SD) of the baseline average. All samples fulfilled the acceptance criteria and no significant variation in Y-chromosome coverage was detected between female samples in the cross-contamination test set and those in the baseline female-only set.

6.1.6 Interfering substances

The impact of potential interferents was assessed by evaluating the performance of the SOPHiA GENETICS™ Dx Universal Library Prep in the presence of endogenous and exogenous interfering substances using both blood- and FFPE-derived DNA samples. Testing concentrations were selected in accordance with CLSI EP37-ED1:2018, with an additional 20% to ensure an assay safety margin. Library yield, size and coverage uniformity were used as analytical readouts for this test. All specimens met the performance requirements, with no observed interference affecting the assay.

INTERFERENT (CONCENTRATION)	TYPE	SAMPLE TYPE	NUMBER OF REPLICATES	NUMBER OF FAILED REPLICATES	OUTCOME
Albumin (72 mg/ml)	Endogenous	Blood	10	0	No interference
Haemoglobin (12 mg/ml)	Endogenous	Blood	10	0	No interference
Conjugated bilirubin (0.5 mg/ml)	Endogenous	Blood	10	0	No interference
Triglycerides (18 mg/ml)	Endogenous	Blood	10	0	No interference
EDTA (9 mg/ml)	Exogenous	Blood	10	0	No interference
Haemoglobin (0.5 mg/ml)	Endogenous	FFPE	10	0	No interference
Ethanol (5% of total volume)	Exogenous	FFPE	10	0	No interference
Proteinase K (0.6 mg/ml)	Exogenous	FFPE	10	0	No interference
Wash buffer 2 (1.875% of total volume)	Exogenous	FFPE	10	0	No interference

7. Symbols

SYMBOL	TITLE
	Consult Instructions For Use
	Catalog number
	Batch code (Lot Number)
	Caution
	Manufacturer
	Date of manufacture
	Temperature limit
	Use-by date
	CE Marking: European Conformity
	In vitro diagnostic medical device
	Importer
	Authorized representative in the European Community/ European Union
	Contains sufficient for <n> tests
	Wear protective gloves
	Wear eye protection

	Wear laboratory coat
	Refer to Warnings and Precautions section
	Refer to Warnings and Precautions section

8. Support

In case of difficulty using the SOPHiA GENETICS™ Dx Universal Library Prep , please consult the troubleshooting section in the document or contact our support line by telephone at +41 21 694 10 60, e-mail support@sophiagenetics.com or our customer support portal. Please visit www.sophiagenetics.com for further details.

Do not use components that are damaged. Contact our support team if there are any concerns with the kits.

9. Appendices

9.1 Appendix I: Unique Dual Index Primer Plates

9.1.1 96A Unique Dual Index Primer Plate, 96- well Plate Format (7 µl Each) - Plate A (IPdCUdIpA96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	sgUDI-1	sgUDI-9	sgUDI-17	sgUDI-25	sgUDI-33	sgUDI-41	sgUDI-49	sgUDI-57	sgUDI-65	sgUDI-73	sgUDI-81	sgUDI-89
B	sgUDI-2	sgUDI-10	sgUDI-18	sgUDI-26	sgUDI-34	sgUDI-42	sgUDI-50	sgUDI-58	sgUDI-66	sgUDI-74	sgUDI-82	sgUDI-90
C	sgUDI-3	sgUDI-11	sgUDI-19	sgUDI-27	sgUDI-35	sgUDI-43	sgUDI-51	sgUDI-59	sgUDI-67	sgUDI-75	sgUDI-83	sgUDI-91
D	sgUDI-4	sgUDI-12	sgUDI-20	sgUDI-28	sgUDI-36	sgUDI-44	sgUDI-52	sgUDI-60	sgUDI-68	sgUDI-76	sgUDI-84	sgUDI-92
E	sgUDI-5	sgUDI-13	sgUDI-21	sgUDI-29	sgUDI-37	sgUDI-45	sgUDI-53	sgUDI-61	sgUDI-69	sgUDI-77	sgUDI-85	sgUDI-93
F	sgUDI-6	sgUDI-14	sgUDI-22	sgUDI-30	sgUDI-38	sgUDI-46	sgUDI-54	sgUDI-62	sgUDI-70	sgUDI-78	sgUDI-86	sgUDI-94
G	sgUDI-7	sgUDI-15	sgUDI-23	sgUDI-31	sgUDI-39	sgUDI-47	sgUDI-55	sgUDI-63	sgUDI-71	sgUDI-79	sgUDI-87	sgUDI-95
H	sgUDI-8	sgUDI-16	sgUDI-24	sgUDI-32	sgUDI-40	sgUDI-48	sgUDI-56	sgUDI-64	sgUDI-72	sgUDI-80	sgUDI-88	sgUDI-96

9.1.2 Index Sequences For The Unique Dual Index Primers

The table below lists the index sequences. Please note that when using manual Sample Sheets, the following Illumina® instruments require the **reverse complement orientation** of the i5 sequence:

- NextSeq® 500/550
- NextSeq® 1000/2000 in Standalone mode (Sample Sheet v1)
- HiSeq® 3000/4000/X
- NovaSeq™ 6000 with v1.5 reagent kits and NovaSeq™ X/X Plus
- MiniSeq™ (except when used with Rapid Reagent Kits)
- iSeq™ 100

All other Illumina® instruments (e.g., MiSeq®) and instrument configurations require the **forward** i5 orientation.

This information is subject to change. Please always refer to the Illumina® support pages for the latest information on which sequencing systems require the i5 index sequence in forward or reverse complement orientation:

https://support-docs.illumina.com/SHARE/AdapterSequences/Content/SHARE/AdapterSeq/Overview.htm

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE COMPLEMENT ORIENTATION)	i7 SEQUENCE
sgUDI-1	CGGTTCAA	TTGAACCG	GACTTCCT
sgUDI-2	TTCGTGAA	TTCACGAA	AGTCGATG
sgUDI-3	ATACCTGC	GCAGGTAT	CTGTCCAC
sgUDI-4	TGTAACCG	CGGTTACA	GCGAATTG
sgUDI-5	GCTTCGCT	AGCGAAGC	GGTGCTTA
sgUDI-6	GAACGATC	GATCGTTC	TCATAGGT
sgUDI-7	TGGCACCT	AGGTGCCA	ATGTCGCA
sgUDI-8	GACTGGCT	AGCCAGTC	CTCAAGTG
sgUDI-9	TCGGTTAT	ATAACCGA	TGAATCGT
sgUDI-10	ATGATCAC	GTGATCAT	TCGCCTTA
sgUDI-11	CATGACGA	TCGTCATG	GATGGAAC
sgUDI-12	CCAGCATG	CATGCTGG	ACGGTGGA
sgUDI-13	CGAGCATA	TATGCTCG	TCCTTGCG
sgUDI-14	CACTCGCA	TGCGAGTG	CTGCATGT
sgUDI-15	TCTAGTAC	GTACTAGA	AGCGGCAA
sgUDI-16	GTGCACGT	ACGTGCAC	CAAGTCGA
sgUDI-17	GGCATAGA	TCTATGCC	GCTACATC
sgUDI-18	AATCTTGG	CCAAGATT	TGAAGCAA
sgUDI-19	GATGCAAG	CTTGCATC	GGTGGATA
sgUDI-20	GACTACTG	CAGTAGTC	TTGATGGC
sgUDI-21	CACGTGGA	TCCACGTG	GTGCATCT
sgUDI-22	CCGATGAG	CTCATCGG	CACTCAGC
sgUDI-23	ATGCGTCT	AGACGCAT	AGATCTGG

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE COMPLEMENT ORIENTATION)	i7 SEQUENCE
sgUDI-24	TGATGCTC	GAGCATCA	TCTTGCAA
sgUDI-25	GTTAACGG	CCGTTAAC	CTCATTCA
sgUDI-26	ATACGGTA	TACCGTAT	TTCGAGCT
sgUDI-27	TTGCCATC	GATGGCAA	GCTCATAG
sgUDI-28	AGCCGTAC	GTACGGCT	AGAACCTC
sgUDI-29	CCGGTCTT	AAGACCGG	TTGACCGA
sgUDI-30	GAGTAACA	TGTTACTC	GACTGACA
sgUDI-31	CCAGGTTA	TAACCTGG	CACGTATC
sgUDI-32	CGTTAATG	CATTAACG	TCGAGCGA
sgUDI-33	GTTGGAAG	CTTCCAAC	TGCCACTC
sgUDI-34	AGGCTCGT	ACGAGCCT	AGTGCTGG
sgUDI-35	TACTCGAA	TTCGAGTA	GCATAGCT
sgUDI-36	TTCAGACC	GGTCTGAA	CTAGCGAG
sgUDI-37	TGAGTTCT	AGAACTCA	TACCGACC
sgUDI-38	TCATGATG	CATCATGA	ATGCTTGG
sgUDI-39	GAGGTCGA	TCGACCTC	GATCGGTT
sgUDI-40	CTGCATCG	CGATGCAG	CCGACTCA
sgUDI-41	AGTACAAG	CTTGTACT	GGATACAT
sgUDI-42	TACTAGTC	GACTAGTA	ATGGTAGG
sgUDI-43	GTCGTATG	CATACGAC	ACCATAGC
sgUDI-44	CAAGGTAT	ATACCTTG	TGCGTCCA
sgUDI-45	ACGCAGGA	TCCTGCGT	AGACCTAT
sgUDI-46	TCGTGGCT	AGCCACGA	TCTTGCTG
sgUDI-47	ATTATCGC	GCGATAAT	CATCACTC
sgUDI-48	CGTACCAG	CTGGTACG	GTGAAGTA
sgUDI-49	GCTCACTG	CAGTGAGC	ACCAAGGA
sgUDI-50	CAGGATTG	CAATCCTG	CAGACCTG
sgUDI-51	GTCTAGTT	AACTAGAC	CGAGCAAC
sgUDI-52	TGAATGGC	GCCATTCA	TCTTGACT
sgUDI-53	ACGACAAT	ATTGTCGT	GACAATGG
sgUDI-54	GAACGCCA	TGGCGTTC	GTTCTACG

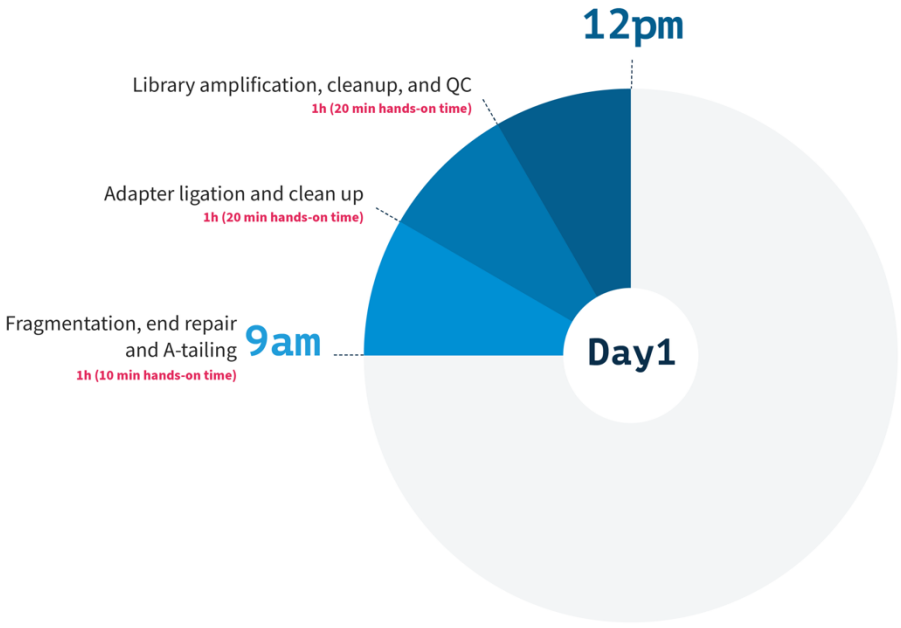
INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE COMPLEMENT ORIENTATION)	i7 SEQUENCE
sgUDI-55	CTGTCCTG	CAGGACAG	AACGCTGC
sgUDI-56	ATAAGGAC	GTCCTTAT	GGACATCA
sgUDI-57	TACATTCC	GGAATGTA	TTGAGCTC
sgUDI-58	GGTTAGCT	AGCTAACC	ACGTTGAG
sgUDI-59	CCTGCTGA	TCAGCAGG	CTTCAGGA
sgUDI-60	CCTCAATC	GATTGAGG	TGCCAACT
sgUDI-61	CGAAGAAT	ATTCTTCG	AGGTCATG
sgUDI-62	TAGTCGAG	CTCGACTA	TACTAGCA
sgUDI-63	ATCCTCAC	GTGAGGAT	GTAACTGT
sgUDI-64	GCTGCAGT	ACTGCAGC	TGAGTTGA
sgUDI-65	GGCAATCG	CGATTGCC	CCTTAGAC
sgUDI-66	GCATCTTA	TAAGATGC	TATCGCCA
sgUDI-67	GTGCTGAA	TTCAGCAC	GCAGAACA
sgUDI-68	ATTGACGC	GCGTCAAT	AGGAATGC
sgUDI-69	GCCGCATT	AATGCGGC	CGTGAGGT
sgUDI-70	TGTAGTCA	TGACTACA	CAATTCAG
sgUDI-71	TCCTCCGA	TCGGAGGA	GGTCCTTC
sgUDI-72	CAATCGAG	CTCGATTG	TTCCGGCA
sgUDI-73	TAAGTCCG	CGGACTTA	ATGCCTGA
sgUDI-74	GGACAGTT	AACTGTCC	TCCAGGAC
sgUDI-75	TTGAGTGA	TCACTCAA	GCTGTCAC
sgUDI-76	CGTAACAT	ATGTTACG	CGACGATT
sgUDI-77	TACGCAGT	ACTGCGTA	TCGCAACG
sgUDI-78	ACCTGACC	GGTCAGGT	GAGTTGTA
sgUDI-79	CCACCTGA	TCAGGTGG	CGCTAAGG
sgUDI-80	TCACCGTG	CACGGTGA	TTGCGTGC
sgUDI-81	GTCTGGAA	TTCCAGAC	CAAGACTA
sgUDI-82	CGAACTCC	GGAGTTCG	CAATTGGT
sgUDI-83	AAGCTCAT	ATGAGCTT	ACTAGCAA
sgUDI-84	CTTGAACG	CGTTCAAG	GATCCACG
sgUDI-85	ATCGTAGA	TCTACGAT	GTCGCTTC

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE COMPLEMENT ORIENTATION)	i7 SEQUENCE
sgUDI-86	TGACGATG	CATCGTCA	CCTAACGA
sgUDI-87	GCTGATAA	TTATCAGC	TGGCCACT
sgUDI-88	CTCATGTC	GACATGAG	ACAAGGTG
sgUDI-89	CGATGCGT	ACGCATCG	GACAGCAA
sgUDI-90	AAGACTCA	TGAGTCTT	AAGTTCGA
sgUDI-91	CGGTTGTC	GACAACCG	ACTCGCTC
sgUDI-92	ATCGCTAC	GTAGCGAT	GACGTAAC
sgUDI-93	GATAGACG	CGTCTATC	TTGCCTGT
sgUDI-94	TGATCCGA	TCGGATCA	GGAGTCGA
sgUDI-95	CCTATTAG	CTAATAGG	CGGAATCG
sgUDI-96	CGCCACTT	AAGTGGCG	ACGTCGGT

9.1.3 Loading Dilutions For Sequencers

TYPE OF SEQUENCER	LOADING DILUTION
Illumina® NextSeq® 500/550	1.3 pM (Mid-Output Kit) 1.4 pM (High-Output Kit) *Note:* Adjust the dilution (1.1 pM to 1.5 pM range) according to the number of clusters obtained in the first run
Illumina® NextSeq® 1000/2000	1000 pM (On-Board Denature/Dilute) 100 pM (Manual Denature/Dilute) *Note:* Manual Denature/Dilute was not internally tested by SOPHiA GENETICS
Illumina® HiSeq®	10 pM
Illumina® MiSeq®	10 pM
Illumina® MiniSeq™	1.4 pM (Standard Kit) 1.6 pM (Rapid Kit)
Illumina® NovaSeq™ 6000	300 pM

9.2 Appendix II: General Workflow of SOPHiA GENETICS™ Dx Universal Library Prep



Library Preparation with SOPHiA GENETICS™ Dx Universal Library Prep

Document Approvals

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Approval Verdict: Approve	Jonathan Day, (jday@sophiagenetics.com) Regulatory Approval 27-Jan-2026 08:26:18 GMT+0000

QA Approval Verdict: Approve	Nora Kormos, Quality Specialist (nkormos@sophiagenetics.com) Quality Assurance Approval 27-Jan-2026 08:51:56 GMT+0000